SUBCHAPTER E—VIRUSES, SERUMS, TOXINS, AND ANALOGOUS PRODUCTS; ORGANISMS AND VECTORS

PART 101—DEFINITIONS

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AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 38 FR 8426, Apr. 2, 1973, unless otherwise noted.

§ 101.1 Applicability.

When used in parts 101 through 117 of this subchapter, the meaning of the words and phrases listed shall be as defined in this part.

§ 101.2 Administrative terminology.

The following administrative words and phrases shall mean:

Adjacent herd. Adjacent herds are herds physically contiguous to the herd of origin; there are no herds between an adjacent herd and the herd of origin.

Administrator. The Administrator, Animal and Plant Health Inspection Service, or any person authorized to act for the Administrator.

Animal and Plant Health Inspection Service. The agency in the Department of Agriculture responsible for administering the Virus-Serum-Toxin Act.

Biological products. The term *biological products,* also referred to in this subchapter as biologics, biologicals, or products, shall mean all viruses, serums, toxins (excluding substances that are selectively toxic to microorganisms, *e.g.,* antibiotics), or analogous products at any stage of production, shipment, distribution, or sale, which are intended for use in the treatment of animals and which act primarily through the direct stimulation, supplementation, enhancement, or modulation of the immune system or immune response. The term ''biological products'' includes but is not limited to vaccines, bacterins, allergens, antibodies, antitoxins, toxoids, immunostimulants, certain cytokines, antigenic or immunizing components of live organisms, and diagnostic components, that are of natural or synthetic origin, or that are derived from synthesizing or altering various substances or components of substances such as microorganisms, genes or genetic sequences, carbohydrates, proteins, antigens, allergens, or antibodies.

(1) A product's intended use shall be determined through an objective standard and not a subjective one, and would be dependent on factors such as representations, claims (either oral or written), packaging, labeling, or appearance.

(2) The term *analogous products* shall include:

(i) Substances, at any stage of production, shipment, distribution, or sale, which are intended for use in the treatment of animals and which are similar in function to biological products in that they act, or are intended to act, through the stimulation, supplementation, enhancement, or modulation of the immune system or immune response; or

(ii) Substances, at any stage of production, shipment, distribution, or sale, which are intended for use in the treatment of animals through the detection or measurement of antigens, antibodies, nucleic acids, or immunity; or

(iii) Substances, at any stage of production, shipment, distribution, or sale, which resemble or are represented as biological products intended for use in the treatment of animals through appearance, packaging, labeling, claims (either oral or written), representations, or through any other means.

(3) The term *treatment* shall mean the prevention, diagnosis, management, or cure of diseases of animals.

Department. The U.S. Department of Agriculture.

Distributor. A person who sells, distributes, or otherwise places in channels of trade, one or more biological

products he does not produce or import.

Division. A marketing unit established by the licensee which may be named on labels, advertisements and promotional material in addition to the name and address of the producer.

Domestic animals. All animals, other than man, including poultry.

Establishment. One or more premises designated on the establishment license.

Guidelines. Guidelines establish principles or practices related to test procedures, manufacturing practices, product standards, scientific protocols, labeling, and other technical or policy considerations. Guidelines contain procedures or standards of general applicability that are usually not regulatory in nature, but that are related to matters that fall under the Virus-Serum-Toxin Act. Guidelines issued by the agency include Veterinary Biologics Licensing Considerations, Memoranda, Notices, and Supplemental Assay Methods.

Herd. Any group of animals, including birds, fish, and reptiles, maintained at a common location (e.g. lot, farm or ranch) for any purpose. The herd (or flock) includes all animals subsequently housed at the common location. If the principal animals of a group are moved to a different location, the group is still considered the same herd.

Herd of origin. The herd from which the microorganism used as seed for production of an autogenous biologic is isolated. Offspring and excess breeding stock (not the principal animals) moved or sold from one group of animals to another have changed herds and are no longer considered part of the herd they originated from. Groups of animals under the same ownership but at different locations are separate herds.

Inspection. An examination made by an inspector to determine the fitness of animals, establishments, facilities, and procedures used in connection with the preparation, testing, and distribution of biological products and the examination or testing of biological products.

Inspector. Any officer or employee of Animal and Plant Health Inspection Service who is authorized by the Administrator to do inspection work.

Licensed establishment. An establishment operated by a person holding an unexpired, unsuspended, and unrevoked U.S. Veterinary Biologics Establishment License.

Licensee. A person to whom an establishment license and at least one product license has been issued.

Microorganisms. Microscopic or submicroscopic organisms, which are sometimes referred to as organisms, which may introduce or disseminate disease of animals.

Nonadjacent herd. Nonadjacent herds are all herds other than the herd of origin and other than herds adjacent to the herd of origin. Herds adjacent to the herd of origin but in a different State from the herd of origin are also considered nonadjacent herds.

Permittee. A person who resides in the United States or operates a business establishment within the United States, to whom a permit to import biological products has been issued.

Person. Any individual, firm, partnership, corporation, company, association, educational institution, State or local governmental agency, or other organized group of any of the foregoing, or any agent, officer, or employee of any thereof.

Premises. All buildings, appurtenances, and equipment used to produce and store biological products located within a particular land area shown on building plans or drawings furnished by the applicant or the licensee and designated by an address adequate for identification.

Prepare or preparation. Sometimes referred to as manufacture or produce, means the steps and procedures used in the processing, testing, packaging, labeling, and storing of a biological product.

Regulations. The provisions in parts 101 through 118 of this subchapter.

Research investigator or research sponsor. A person who has requested authorization to ship an experimental biological product for the purpose of evaluating such product, or has been granted such authorization.

Secretary. The Secretary of Agriculture of the United States or any officer or employee of the Department to whom authority has heretofore been delegated, or to whom authority may

hereafter be delegated, to act in his stead.

Subsidiary. A corporation in which a corporate licensee owns in excess of 50 percent of the voting stock.

Veterinary Services. Veterinary Services unit of Animal and Plant Health Inspection Service of the Department.

Virus-Serum-Toxin Act. The Act of March 4, 1913, 37 Stat. 832–833; as amended December 23, 1985, Public Law 99–198, 99 Stat. 1654–1655; and as further amended September 28, 1988, Public Law 100–449, 102 Stat. 1868; 21 U.S.C. 151–159.

U.S. Veterinary Biological Product License. A document, sometimes referred to as a product license, which is issued pursuant to part 102 of this subchapter to the holder of an establishment license, as a part of and ancillary to the establishment license, and which authorizes production of a specified biological product in the designated licensed establishment.

U.S. Veterinary Biological Product Permit. A document, sometimes referred to as a permit, issued to a person authorizing the importation of specified biological products subject to restrictions and controls as provided in the regulations.

U.S. Veterinary Biologics Establishment License. A document referred to as an establishment license, which is issued pursuant to part 102 of this subchapter, authorizing the use of designated premises for production of biological products specified in one or more unexpired, unsuspended, and unrevoked product license(s).

[38 FR 8426, Apr. 2, 1973; 38 FR 9221, Apr. 12, 1973, as amended at 40 FR 46093, Oct. 6, 1975; 41 FR 44358, Oct. 8, 1976; 49 FR 22624, May 31, 1984; 52 FR 30131, Aug. 13, 1987; 56 FR 66782, 66783, Dec. 26, 1991; 57 FR 38756, Aug. 27, 1992; 62 FR 31328, June 9, 1997; 64 FR 43044, Aug. 9, 1999]

§ 101.3 Biological products and related terms.

When used in conjunction with or in reference to a biological product, the following terms shall mean:

(a) *Licensed biological product.* A biological product prepared within a licensed establishment by a person holding an unexpired, unsuspended, and unrevoked product license for such product.

(b) *Experimental biological product.* A biological product which is being evaluated to substantiate an application for a product license or permit.

(c) *Completed product.* A biological product in bulk or final container produced in compliance with the regulations to final form and composition.

(d) *Finished product.* A completed product which has been bottled, sealed, packaged, and labeled as required by the regulations.

(e) *Released product.* A finished product released for marketing after all requirements have been satisfactorily complied with.

(f) *Fraction.* A specific antigen, its antibodies, or its antitoxin which constitutes a component of a biological product.

(g) *Diluent.* A liquid used to rehydrate a desiccated product or a liquid used to dilute another substance.

(h) *Serial.* The total quantity of completed product which has been thoroughly mixed in a single container and identified by a serial number: *Provided,* That, when all or part of a serial of liquid biological product is packaged as diluent for all or part of a serial of desiccated product, the resulting combination packages shall be considered a serial of the multiple fraction product.

(i) *Subserial.* Each of two or more properly identified portions of a serial which are further processed at different times or under different conditions such as, but not limited to, being desiccated in different size final containers and/or at different times.

(j) *Outline of production.* A detailed protocol of methods of manufacture to be followed in the preparation of a biological product and which may sometimes be referred to as an outline.

(k) *Product Code Number.* A number assigned by Animal and Plant Health Inspection Service to each type of licensed biological product.

(l) *Harvest date.* Unless otherwise specified in a filed Outline of Production, the harvest date shall be the date blood or tissues are collected for production or the date cultures of living microorganisms are removed from production incubators.

(m) *Bacterin.* An inactivated bacterial product consisting of an antigenic suspension of organisms or particulate parts of organisms, representing a whole culture or a concentrate thereof, with or without the unevaluated growth products, which has been inactivated as demonstrated by acceptable tests written into the filed Outline of Production for the product.

(n) *Toxoid.* An inactivated bacterial product which consists of a sterile, antigenic toxin or toxic growth product, which has resulted from the growth of bacterial organisms in a culture medium from which the bacterial cells have been removed, which has been inactivated without appreciable loss of antigenicity as measured by suitable tests, and which is nontoxic as demonstrated by acceptable tests written into the filed Outline of Production.

(o) *Bacterin-toxoid.* An inactivated bacterial product which is either:

(1) A suspension of organisms, representing a whole culture or a concentrate thereof, with the toxic growth products from the culture which has been inactivated without appreciable loss of antigenicity as measured by suitable tests, the inactivation of organisms and toxins being demonstrated by acceptable tests written into the filed Outline of Production: *Provided,* That it shall contain cellular antigens and shall stimulate the development of antitoxin; or

(2) A combination product in which one or more toxoids or bacterin-toxoids is combined with one or more bacterins or one or more bacterin-toxoids.

(p) *Bacterial extract.* An inactivated bacterial product which consists of the sterile, nontoxic, antigenic derivatives extracted from bacterial organisms or from culture medium in which bacterial organisms have grown.

[38 FR 8426, Apr. 2, 1973, as amended at 42 FR 63770, Dec. 20, 1977; 50 FR 24903, June 14, 1985; 56 FR 66782, Dec. 26, 1991; 60 FR 14354, Mar. 17, 1995]

§ 101.4 Labeling terminology.

Terms pertaining to identification and packaging of biological products shall mean:

(a) *Label.* All written, graphic, or printed matter:

(1) Upon or attached to a final container of a biological product;

(2) Appearing upon any immediate carton or box used to package such final container; and

(3) Appearing on any accompanying enclosures (leaflets, inserts, or circulars) on which required information or directions as to the use of the biological product shall be found.

(b) *Labeling.* All labels and other written, printed, or graphic matter accompanying the final container.

(c) *Final container.* The unit, bottle, vial, ampule, tube, or other receptacle into which any biological product is filled for distribution and sale.

(d) *True name.* The name entered on the product license or permit at the time of issuance to differentiate the biological product from others: *Provided,* That, the principal part of such name shall be emphasized on such license or permit by being more prominently lettered than descriptive terms which may be necessary to complete the differentiation.

(e) *Serial number.* Numbers or numbers and letters used to identify and distinguish one serial from others.

(f) *Expiration date.* A date designating the end of the period during which a biological product, when properly stored and handled, can be expected with reasonable certainty, to be efficacious.

(g) *Label number.* A number assigned by Animal and Plant Health Inspection Service to each label or sketch submitted for review.

(h) *Master label.* The finished carton, container, or enclosure label for the smallest size final container that is authorized for a biological product, that serves as the Master template label applicable to all other size containers or cartons of the same product that is marketed by a licensee, subsidiary, division, or distributor.

[38 FR 8426, Apr. 2, 1973, as amended at 42 FR 63770, Dec. 20, 1977; 56 FR 66782, Dec. 26, 1991; 61 FR 29464, June 11, 1996]

§ 101.5 Testing terminology.

Terms used when evaluating biological products shall mean:

(a) *Standard Requirement.* Test methods, procedures, and criteria established by Animal and Plant Health Inspection Service for evaluating biological products to be pure, safe, potent, and efficacious, and not to be worthless, contaminated, dangerous, or harmful under the Act.

(b) *Log.* Logarithm computed to the base 10.

(c) *Pure or purity.* Quality of a biological product prepared to a final form relatively free of extraneous micro-organisms and extraneous material (organic or inorganic) as determined by test methods or procedures established by Animal and Plant Health Inspection Service in Standard Requirements or in the approved Outline of Production for such product, but free of extraneous microorganisms or material which in the opinion of the Administrator adversely affects the safety, potency, or efficacy of such product.

(d) *Safe or safety.* Freedom from properties causing undue local or systemic reactions when used as recommended or suggested by the manufacturer.

(e) *Sterile or sterility.* Freedom from viable contaminating microorganisms as demonstrated by procedures prescribed in part 113 of this subchapter, Standard Requirements, and approved Outlines of Production.

(f) *Potent or potency.* Relative strength of a biological product as determined by test methods or procedures as established by Animal and Plant Health Inspection Service in Standard Requirements or in the approved Outline of Production for such product.

(g) *Efficacious or efficacy.* Specific ability or capacity of the biological product to effect the result for which it is offered when used under the conditions recommended by the manufacturer.

(h) *Dose.* The amount of a biological product recommended on the label to be given to one animal at one time.

(i) *Vaccinate.* An animal which has been inoculated, injected, or otherwise administered a biological product being evaluated.

(j) *Control animal.* An animal, which may be referred to as a control, used in a test procedure for purposes of comparison or to add validity to the results.

(k) *Day.* Time elapsing between any regular working hour of one day and any regular working hour of the following day.

(l) *No test.* A test which produces inconclusive or invalid results and therefore, cannot be used to evaluate a biological product.

(m) *Healthy.* Apparently normal in all vital functions and free of signs of disease.

(n) *Unfavorable reactions.* Overt adverse changes which occur in healthy test animals subsequent to initiation of a test and manifested during the observation period prescribed in the test protocol which are attributable either to the biological product being tested or to factors unrelated to such product as determined by the responsible individual conducting the test.

(o) *Master reference.* A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity. The Master Reference may be used as the working reference in in vitro tests for relative potency. The Master Reference may also be used to establish the relative potency of a serial of product used in requalification studies and to establish the relative potency of working references. The preparation of a Master Reference as described in a filed Outline of Production may be:

(1) A completed serial of vaccine or bacterin prepared in accordance with a filed Outline of Production;

(2) A purified preparation of a protective immunogen or antigen; or

(3) A nonadjuvanted harvested culture of microorganisms.

(p) *Working reference.* A Working Reference is the reference preparation that is used in the in vitro test for the release of serials of product. Working References may be:

(1) Master References; or

(2) Serials of product that have been prepared and qualified, in a manner acceptable to Animal and Plant Health Inspection Service for use as reference preparations.

(q) *Qualifying serial.* (1) A serial of biological product used to test for immunogenicity when the Master or

Working Reference is a purified antigen or nonadjuvanted harvest material. Qualifying serials shall be produced in accordance with the filed Outline of Production, tested for immunogenicity in accordance with methods deemed appropriate by the Animal and Plant Health Inspection Service, and have a geometric mean relative potency, when compared to the Master Reference, of not greater than 1.0 as established by: independent parallel line assays with five or more replicates; or other valid assay methods for determining relative antigen content which demonstrate linearity, specificity, and reproducibility at least equivalent to the parallel line assay and are acceptable to the Animal and Plant Health Inspection Service.

(2) Qualifying serials used to requalify or extend the dating period of a Master Reference shall be determined to be immunogenic in accordance with methods deemed appropriate by the Animal and Plant Health Inspection Service as provided in paragraph (a)(1) of this section, and, in addition, shall be within their permitted dating period and have been prepared in accordance with the production method described in the currently filed Outline of Production.

(r) *Immunogenicity*. The ability of a biological product to elicit an immune response in animals as determined by test methods or procedures acceptable to the Animal and Plant Health Inspection Service.

[38 FR 8426, Apr. 2, 1973, as amended at 40 FR 45419, Oct. 2, 1975; 41 FR 6751, Feb. 13, 1976; 43 FR 3701, Jan. 27, 1978; 56 FR 66782, 66783 Dec. 26, 1991; 62 FR 19037, Apr. 18, 1997]

§ 101.6 Cell cultures.

When used in conjunction with or in reference to cell cultures, which may be referred to as tissue cultures, the following terms shall mean:

(a) *Batches of primary cells.* A pool of original cells derived from normal tissue up to and including the 10th subculture.

(b) *Cell line.* A pool of cells which are 11 or more subcultures from the tissue of origin.

(c) *Subculture.* Each flask to flask transfer or passage regardless of the number of cell replications.

(d) *Master Cell Stock (MCS).* The supply of cells of a specific passage level from which cells for production of biologics originate.

[38 FR 8426, Apr. 2, 1973, as amended at 40 FR 45419, Oct. 2, 1975; 49 FR 22624, May 31, 1984]

§ 101.7 Seed organisms.

When used in conjunction with or in reference to seed organisms, the following shall mean:

(a) *Master Seed.* An organism at a specific passage level which has been selected and permanently stored by the producer from which all other seed passages are derived within permitted levels.

(b) *Working Seed.* An organism at a passage level between Master Seed and Production Seed.

(c) *Production Seed.* An organism at a specified passage level which is used without further propagation for initiating preparation of a fraction.

[49 FR 22625, May 31, 1984]

PART 102—LICENSES FOR BIOLOGICAL PRODUCTS

Sec.

102.1 Licenses issued by the Administrator.
102.2 Licenses required.
102.3 License applications.
102.4 U.S. Veterinary Biologics Establishment License.
102.5 U.S. Veterinary Biological Product License.
102.6 Conditional licenses.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 102.1 Licenses issued by the Administrator.

Each establishment qualified to prepare biological products under the Virus-Serum-Toxin Act shall hold an unexpired and unrevoked U.S. Veterinary Biologics Establishment License issued by the Administrator and a U.S. Veterinary Biological Product License for each product prepared in such establishment unless the product is subject to the provisions of 9 CFR parts 103 or 106 of this subchapter.

[60 FR 48021, Sept. 18, 1995]

§ 102.2 Licenses required.

(a) Every person who prepares biological products subject to the Virus-Serum-Toxin Act shall hold an unexpired, unsuspended, and unrevoked U.S. Veterinary Biologics Establishment License and at least one unexpired, unsuspended, and unrevoked U.S. Veterinary Biological Product License issued by the Administrator to prepare a biological product.

(b) An applicant who applies for an establishment license must also apply for at least one product license. An establishment license will not be issued without a license authorizing the production of a biological product in the establishment.

[52 FR 11026, Apr. 7, 1987, as amended at 56 FR 66783, Dec. 26, 1991; 61 FR 52873, Oct. 9, 1996]

§ 102.3 License applications.

(a) *U.S. Veterinary Biologics Establishment License.* (1) The operator of each establishment of the kind specified in § 102.2 shall make written application to the Administrator for a license. Blank forms of application will be furnished upon request to Animal and Plant Health Inspection Service.

(2) When a person conducts more than one establishment, a separate application shall be made for each establishment.

(3) Whenever subsidiaries are to operate in an establishment for which license application is made, the applicant shall apply for permission for such subsidiaries to operate in the establishment and furnish therewith a complete statement regarding the relationship between the applicant and the subsidiaries.

(4) Facilities documents, prepared as prescribed in part 108 of this subchapter, shall accompany the application for license unless previously filed with Animal and Plant Health Inspection Service.

(5) Each application for a U.S. Veterinary Biologics Establishment License shall be accompanied by an application for one or more U.S. Veterinary Biological Product Licenses and the supporting documents required by paragraph (b)(2) of this section.

(6) A new application shall be made when a change of ownership, operation, or location of an establishment occurs; or prior to the expiration of a U.S. Veterinary Biologics Establishment License issued for an interim period of time.

(b) *U.S. Veterinary Biological Product License.* (1) The licensee of each establishment or applicant for an establishment license shall make written application to the Administrator for a U.S. Veterinary Biological Product License for each biological product to be prepared in the licensed establishment.

(2) Each application for a U.S. Veterinary Biological Product License shall be supported by:

(i) At least four copies of an Outline of Production prepared in accordance with §§ 114.8 and 114.9 of this subchapter; and

(ii) At least three copies of test reports and research data sufficient to establish purity, safety, potency, and efficacy of the product; and

(iii) Legends prepared as prescribed in § 108.5 of this subchapter designating which facilities are to be used in the preparation of each fraction; and

(iv) Labels in finished form or sketches prepared as prescribed in § 112.5 of this subchapter, together with information regarding all claims to be made on labels and in advertising matter to be used in connection with or related to the biological product.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 37763, Oct. 24, 1974, as amended at 48 FR 57472, Dec. 30, 1983; 49 FR 21043, May 18, 1984; 50 FR 50763, Dec. 12, 1985; 56 FR 66783, Dec. 26, 1991]

§ 102.4 U.S. Veterinary Biologics Establishment License.

(a) Before a U.S. Veterinary Biologics Establishment License will be issued by the Administrator for any establishment, an inspection shall be made to determine whether the condition, equipment, facilities, and the like, of the establishment, and the methods used to prepare biological products are in conformity with the requirements in the regulations.

(b) A license shall not be issued unless:

(1) In the opinion of the Administrator, the condition of the establishment, including its facilities, and the methods of preparation of biological products are such as reasonably to assure that the products shall accomplish the purpose for which they are intended; and

(2) The Administrator is satisfied on the basis of information before him that:

(i) The establishment shall be operated in compliance with the Act and applicable regulations and be under the supervision of person(s) competent in the preparation of biological products; and

(ii) The applicant, or the person having the responsibility for producing biological products in the establishment, or both, is qualified by education and experience, and has demonstrated fitness to produce such products in compliance with the Act and regulations issued pursuant thereto; *Provided*, That, previous violations of the Act, or such regulations or both shall be relevant to the Administrator's determination of fitness.

(3) Written assurance is filed with Animal and Plant Health Inspection Service that the biological products which are licensed to be prepared therein shall not be so advertised as to mislead or deceive the purchasers and that the packages or containers in which the same are to be marketed shall not bear any statement, design, or device which is false or misleading in any particular.

(c) U.S. Veterinary Biologics Establishment Licenses shall be numbered.

(d) Two or more licenses may bear the same number when they are issued for establishments under the same ownership or control, provided a serial letter is added to one or more to identify each license and the product produced thereunder.

(e) When a U.S. Veterinary Biologics Establishment License is issued for an establishment, it shall not apply to more than one person at the same location, except that subsidiaries of the licensee, when named in the license, may operate thereunder at the establishment named. The licensee with its subsidiaries will be held responsible for all operations conducted in the licensed establishment.

(f) When a licensee no longer holds at least one unexpired, unsuspended, or unrevoked product license authorizing the preparation of a biological product, or is in the process of obtaining a product license, the establishment license shall no longer be valid and shall be returned to the Administrator. In the case where an establishment license expires or is suspended or revoked, any product license authorizing preparation of a product at such establishment shall be invalid indefinitely or for as long as the suspension is in effect.

(g) Any license issued under this part to establishments in which biological products are prepared shall be issued on condition that the licensee permit the inspection of such establishments, products, product preparation, and all relevant records as provided in part 115 of this subchapter. Failure to permit inspection may result in the license being suspended or revoked.

(h) The provisions of paragraph (b) of this section shall also be applicable to, and be considered by, the Administrator in connection with each application for an additional product license.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 37762, Oct. 24, 1974; 39 FR 38364, Nov. 1, 1974, as amended at 41 FR 44359, Oct. 8, 1976; 48 FR 57472, Dec. 30, 1983; 52 FR 11026, Apr. 7, 1987; 52 FR 30131, Aug. 13, 1987; 56 FR 66783, Dec. 26, 1991; 60 FR 48021, Sept. 18, 1995; 61 FR 52873, Oct. 9, 1996; 62 FR 13294, Mar. 20, 1997]

§ 102.5 U.S. Veterinary Biological Product License.

(a) Authorization to produce each biological product shall be specified on a U.S. Veterinary Biological Product License, issued by the Administrator, and supplementary to the U.S. Veterinary Biologics Establishment License named therein.

(b) The following shall appear on the U.S. Veterinary Biological Product License:

(1) The U.S. Veterinary Biologics Establishment License Number for the establishment from which the product is released for marketing.

(2) The true name of the product.

(3) The product code number for the product.

(4) The date of issuance.

(5) Any restrictions designated by the Administrator under paragraph (e) of this section.

(6) When necessary to comply with § 102.6 of this part, a termination date and a brief description of requirements to be met for reissuance.

(c) The following provisions shall apply to all licensed biological products:

(1) Licensed biological products shall be prepared as required by the regulations and in accordance with a filed Outline of Production as prescribed in §§ 114.8 and 114.9 of this subchapter. No change shall be made in the preparation of a biological product without prior approval of the Administrator.

(2) In addition to restrictions imposed by the Administrator pursuant to paragraph (e) of this section, biological products may be subject to restrictions which are imposed by any State or other jurisdiction pertaining to the distribution and use of such products, based on local disease conditions.

(3) When requested by the Administrator, a licensee shall submit a list of licensed biological products prepared in the licensed establishment.

(d) Where the Administrator determines that the protection of domestic animals or the public health, interest, or safety, or both, necessitates restrictions on the use of a product, the product shall be subject to such additional restrictions as are prescribed on the license. Such restrictions may include, but are not limited to, limits on distribution of the product or provisions that the biological product is restricted to use by veterinarians, or under the supervision of veterinarians, or both.

(e) Any person may request that the distribution and use of a veterinary biological product be restricted if the restriction pertains to the protection of domestic animals or the public health, interest, or safety, or both. All requests must be sent, in writing, to the Director, Center for Veterinary Biologics, Licensing and Policy Development, 510 South 17th Street, Suite 104, Ames, IA 50010–8197. Requests must specify the restriction(s) being requested and must explain why the restrictions are needed. Copies of any supporting documents, such as scientific literature, published or unpublished articles, or data from tests, should be attached to the request. When a decision is reached regarding the request, the person submitting the request will be sent written notification of such decision.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 37763, Oct. 24, 1974, as amended at 48 FR 57472, Dec. 30, 1983; 50 FR 50764, Dec. 12, 1985; 52 FR 11026, Apr. 7, 1987; 56 FR 66783, Dec. 26, 1991; 57 FR 38760, Aug. 27, 1992; 59 FR 67616, Dec. 30, 1994; 62 FR 13294, Mar. 20, 1997; 64 FR 43044, Aug. 9, 1999]

§ 102.6 Conditional licenses.

In order to meet an emergency condition, limited market, local situation, or other special circumstance, including production solely for intrastate use under a State-operated program, the Administrator may, in response to an application submitted as specified in § 102.3(b) of this part, issue a conditional U.S. Veterinary Biological Product License to an establishment under an expedited procedure which assures purity and safety, and a reasonable expectation of efficacy. Preparation of products under a conditional license shall be in compliance with all applicable regulations and standards and may be restricted as follows:

(a) The preparation may be limited to a predetermined time period which shall be established at the time of issuance and specified on the license. Prior to termination of the license, the licensee may request reissuance. Such requests shall be substantiated with data and information obtained since the license was issued. After considering all data and information available, the Administrator shall either reissue the U.S. Veterinary Biological Product License or allow it to terminate.

(b) Distribution may be limited to the extent necessary to assure that the product will meet the basic criteria for issuance of the conditional license.

(c) Labeling for the product may be required to contain information on the conditional status of the license.

[52 FR 11026, Apr. 7, 1987, as amended at 60 FR 48021; Sept. 18, 1995]

PART 103—EXPERIMENTAL PRODUCTION, DISTRIBUTION, AND EVALUATION OF BIOLOGICAL PRODUCTS PRIOR TO LICENSING

Sec.

103.1 Preparation of experimental biological products.
103.2 Disposition of animals administered experimental biological products or live organisms.
103.3 Shipment of experimental biological products.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 103.1 Preparation of experimental biological products.

Except as otherwise provided in this section, experimental biological products which are neither composed of nor prepared with organisms or antigens used in biologicals already licensed, shall not be prepared in the production facilities of a licensed establishment. Upon application therefor, the Administrator may authorize the preparation of experimental products on the premises of a licensed establishment if he determines that such preparation will not result in contamination of the licensed products. Each request for permission to prepare an experimental biological product on licensed premises shall indicate the nature of the unlicensed product, designate facilities to be used, and specify precautions which will be taken to prevent contamination of licensed products. Such requests shall be submitted to the Administrator. Research facilities that are entirely separate and apart from facilities used for the preparation of licensed biological products will not be considered a part of the licensed premises for purposes of this section.

(Approved by the Office of Management and Budget under control number 0579–0013)

[30 FR 11848, Sept. 16, 1965, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66783, Dec. 26, 1991]

§ 103.2 Disposition of animals administered experimental biological products or live organisms.

Safeguards as herein provided shall be established by the research investigator or research sponsor to control disposition of all animals administered experimental biological products or live organisms.

(a) Surviving test animals (including challenged control animals) shall not be removed from the premises on which the tests are conducted for at least 14 days after administration of an experimental biological product or live organisms: *Provided, however,* That this holding period may be increased or decreased as permitted or requested by the Administrator following review of all relevant information or data available.

(b) All animals administered experimental biological products which are to be slaughtered at establishments subject to the Federal Meat Inspection Act, as amended and extended (21 U.S.C. 601 *et. seq.*) are subject to the applicable requirements of §309.16 of this title (Meat Inspection Regulations).

(c) Except as otherwise provided in this paragraph, the research investigator or research sponsor shall maintain adequate records relative to the disposition of each animal administered experimental biological products. These records shall be maintained for a minimum period of two years from the date that an experimental product was administered to such animal, and shall show the name and address of the owner; number, species, class and location of the animals; and if sold, the name and address of the consignee, buyer, commission, firm or abattoir: *Provided, however,* That a research investigator or research sponsor may be exempted from these recordkeeping requirements by the Administrator on the basis of acceptable data demonstrating that use of the experimental biological product will not result in the presence of any unwholesome condition in the edible parts of animals subsequently presented for slaughter.

(Approved by the Office of Management and Budget under control number 0579–0059)

[30 FR 11848, Sept. 16, 1965, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66783, Dec. 26, 1991; 66 FR 21063, Apr. 27, 2001]

§ 103.3 Shipment of experimental biological products.

Except as provided in this section, no person shall ship or deliver for shipment in or from the United States, the District of Columbia, or any Territory

of the United States any unlicensed biological product for experimental use in animals. For the benefit of license applicants and to permit and encourage research, a person may be authorized by the Administrator to ship unlicensed biological products for the purpose of evaluating such experimental products by treating limited numbers of animals, *Provided*, that, the Administrator determines that the conditions under which the experiment is to be conducted are adequate to prevent the spread of disease and approves the procedures set forth in the request for such authorization. Special restrictions or tests may be imposed, especially in the case of products containing live organisms, when they are deemed necessary or advisable by the Administrator. A request for authorization to ship an unlicensed biological product for experimental study and evaluation shall be accompanied by the following:

(a) One copy of a permit or letter of permission from the proper State or foreign animal health authorities of each State or foreign country involved.

(b) Two copies of a tentative list of the names of the proposed recipients and quantity of experimental product that is to be shipped to each individual. In the event of subsequent changes, additional information shall be furnished when such facts are known;

(c) Two copies of a description of the product, recommendations for use, and results of preliminary research work;

(d) Three copies of labels or label sketches which show the name or identification of the product and bear a statement, ''Notice! For Experimental Use Only—Not For Sale,'' or equivalent. The U.S. Veterinary License legend shall not appear on such labels; and

(e) Two copies of a proposed general plan covering the methods and procedures for evaluating the product and for maintaining records of the quantities of experimental product prepared, shipped and used. At the conclusion of field studies, results shall be obtained, summarized, and submitted to the Animal and Plant Health Inspection Service.

(f) Data acceptable to the Administrator demonstrating that use of the experimental biological product in meat animals is not likely to result in the presence of any unwholesome condition in the edible parts of animals subsequently presented for slaughter.

(g) A statement from the research investigator or research sponsor agreeing to furnish, upon the Administrator's request, additional information concerning each group of meat animals involved prior to movement of these animals from the premises where the test is to be conducted. Such information shall include the owner's name and address; number, species, class and location of animals involved; date shipment is anticipated; along with name and address of consignee, buyer, commission firm or abattoir.

(h) Any information the Administrator may require in order to assess the product's impact on the environment.

[26 FR 7726, Aug. 18, 1961, as amended at 30 FR 11848, Sept. 16, 1965; 52 FR 30131, Aug. 13, 1987; 56 FR 66783, Dec. 26, 1991]

PART 104—PERMITS FOR BIOLOGICAL PRODUCTS

Sec.

104.1 Permit required.
104.2 Permit authorized.
104.3 Permit application.
104.4 Products for research and evaluation.
104.5 Products for distribution and sale.
104.6 Products for transit shipment only.
104.7 Product permit.
104.8 Illegal shipments.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 38 FR 32916, Nov. 29, 1973, unless otherwise noted.

§ 104.1 Permit required.

Unless otherwise authorized or directed by the Administrator, each permit to import a biological product into the United States shall be issued in accordance with the regulations in this part.

(a) No biological product shall be brought into the United States unless a permit has been issued for such product. A separate U.S. Veterinary Biological Product Permit shall be required for each shipment of biological product to be imported: *Provided*, That, a permit shall also be required for each

transit shipment of biological products moved through the United States.

(b) Each person importing biological products shall hold an unexpired, unsuspended, and unrevoked permit issued by Animal and Plant Health Inspection Service. Such person shall reside within the United States, or operate a business establishment within the United States, or both.

[38 FR 32916, Nov. 29, 1973, as amended at 56 FR 66783, Dec. 26, 1991; 56 FR 66783, Dec. 26, 1991]

§ 104.2 Permit authorized.

(a) Animal and Plant Health Inspection Service is authorized to issue three types of permits for importing biological products. They shall be:

(1) U.S. Veterinary Biological Product Permit for Research and Evaluation;

(2) U.S. Veterinary Biological Product Permit for Distribution and Sale; or

(3) U.S. Veterinary Biological Product Permit for Transit Shipment Only.

(b) A permit shall not be issued for a biological product from countries known to have exotic diseases, including but not limited to foot-and-mouth disease, rinderpest, fowl pest (fowl plague), swine vesicular disease, Newcastle disease, and African swine fever, if in the opinion of the Administrator, such products may endanger the livestock or poultry of this country.

(c) A permit shall not be issued until an inspector has determined the condition of the equipment and facilities of the producer, of the applicant, or of both if such a determination is considered necessary by the Administrator.

(d) A permit shall not be issued for a biological product prepared in the United States, exported, and presented for reentry except as provided in § 104.4(d).

[38 FR 32916, Nov. 29, 1973, as amended at 56 FR 66783, Dec. 26, 1991; 56 FR 66783, Dec. 26, 1991]

§ 104.3 Permit application.

(a) Each person desiring to import a biological product shall make written application to Animal and Plant Health Inspection Service for a permit. Blank forms of application shall be furnished upon request.

(b) The application shall specify the type of permit required, the port of entry at which the product shall be cleared through Customs, the estimated quantity involved, and the anticipated date on which the importation shall be made.

(Approved by the Office of Management and Budget under control number 0579–0013)

[38 FR 32916, Nov. 29, 1973, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66783, Dec. 26, 1991]

§ 104.4 Products for research and evaluation.

(a) An application for a U.S. Veterinary Biological Product Permit to import a biological product for research and evaluation shall be accompanied by a brief description of such product, methods of propagating antigens including composition of medium, species of animals or cell cultures involved, degree of inactivation or attenuation, recommendations for use, and the proposed plan of evaluation. The applicant shall also provide any information the Administrator may require in order to assess the product's impact on the environment.

(b)(1) A permit to import a biological product for research and evaluation shall not be issued unless the scientific capabilities of the investigator are determined to be adequate to safeguard domestic animals and protect public health, interest, or safety from any deleterious effects which might result from use of such product. Special restrictions or tests may be specified as part of the permit when they are deemed necessary or advisable by the Administrator.

(2) No person shall ship a product imported under this section for research and evaluation anywhere in or from the United States unless authorized by the Administrator in accordance with the provisions of § 103.3 of this subchapter.

(c) A biological product shall not be imported for Research and Evaluation which is not packaged and labeled in accordance with § 112.9 of this subchapter.

(d) When a licensed product has been exported from the United States, a permit may be issued to the producer for a small quantity of such product for in

vitro Research and Evaluation tests: *Provided*, That, the importation of such product will not endanger the livestock or poultry of this country.

(Approved by the Office of Management and Budget under control number 0579–0013)

[38 FR 32916, Nov. 29, 1973, as amended at 48 FR 57473, Dec. 30, 1983; 52 FR 30131, Aug. 13, 1987; 56 FR 66783, Dec. 26, 1991]

§ 104.5 Products for distribution and sale.

An application for a U.S. Veterinary Biological Product Permit to import a biological product for Distribution and Sale shall be accompanied by supporting material necessary to satisfy the requirements provided in this section.

(a) A permit shall not be issued unless the conditions under which the biological product is to be prepared or the methods to be used are such as to reasonably insure that the product is pure, safe, potent, and efficacious.

(1) Three copies of blueprints of the producing foreign establishment shall be submitted with the application unless satisfactory plans are on file with Animal and Plant Health Inspection Service from a previous application. The production facilities to be used for each product prepared at the establishment shall be designated.

(2) The manufacturer shall submit written authorization for properly accredited inspectors to inspect without previous notification, and at such times as may be demanded by the aforesaid inspectors, all parts of the establishment in which biological products shall be prepared, all processes of preparation, and all records relative to such preparation.

(3) The manufacturer shall furnish written assurance that a biological product to be imported for Distribution and Sale shall be prepared under the supervision of a person competent by education and experience to handle all matters pertaining to the preparation of such product and that each biological product shall be prepared in accordance with the regulations applicable to the product or in a manner acceptable to the Administrator so as to carry out the purposes of the Act.

(4) The methods to be used in the preparation of each biological product shall be written into an approved Outline of Production prepared in accordance with the applicable provisions of part 114 of this subchapter. Four copies of such Outlines of Production shall be submitted to Animal and Plant Health Inspection Service and be approved before the permit is issued.

(5) Data shall be furnished by the applicant which establishes that the product involved complies with the provisions of the Act and the regulations issued pursuant thereto. When deemed necessary to obtain required information, Animal and Plant Health Inspection Service may require that the product be tested under field conditions within or outside the United States as the occasion demands.

(b) The permittee shall furnish the following:

(1) Adequate facilities for storing all imported biological products. An inspection of such facilities shall be made by inspectors before a permit is issued and additional inspections shall be made at any time subsequent to the importation of the biological products if deemed necessary by the Administrator;

(2) Information regarding all claims to be made on labels and advertising matter used in connection with or related to the biological product to be imported;

(3) Mounted copies of final container labels, carton labels, and enclosures to be used with the imported product as provided in part 112 of this subchapter; and

(4) Samples of each serial from each shipment of biological products imported or offered for importation. Such samples shall be collected, examined, and tested in a manner specified by the Administrator. The biological products being sampled shall not be further distributed by the permittee until released by Animal and Plant Health Inspection Service.

(Approved by the Office of Management and Budget under control number 0579–0013)

[38 FR 32916, Nov. 29, 1973, as amended at 48 FR 57473, Dec. 30, 1983; 49 FR 21044, May 18, 1984; 56 FR 66783, Dec. 26, 1991]

§ 104.6 Products for transit shipment only.

An application for a permit for Transit Shipment Only shall be required when a biological product is being shipped from one foreign country to another foreign country by way of the United States. The shipment shall move under a permit subject to the following restrictions:

(a) The shipment shall be confined to the carrier at all times when such shipment is to transit the United States on the same carrier on which it arrived. If the shipment is to be transferred to a carrier other than the one on which it shall arrive into the United States, a schedule of arrival and departure of each shipment shall be furnished by the permittee to Animal and Plant Health Inspection Service prior to arrival in the United States.

(b) The permittee shall be responsible to Animal and Plant Health Inspection Service for handling, storing, and forwarding of the biological product. Animal and Plant Health Inspection Service shall be notified of all shipments received and forwarded by the permittee and an accurate accounting shall be made.

(Approved by the Office of Management and Budget under control number 0579–0013)

[38 FR 32916, Nov. 29, 1973, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991; 61 FR 52873, Oct. 9, 1996]

§ 104.7 Product permit.

(a) A permit shall be numbered and dated.

(b) The purpose for which the product is imported shall be specified on the permit as for Research and Evaluation, Distribution and Sale, or Transit Shipment Only.

(c) A permit shall not be used after the date specified.

[38 FR 32916, Nov. 29, 1973, as amended at 56 FR 66783, Dec. 26, 1991; 62 FR 13294, Mar. 20, 1997]

§ 104.8 Illegal shipments.

(a) Biological products which are presented for importation without a permit having been issued shall be returned to the country of origin at the expense of the importer or in lieu thereof, destroyed by Department personnel.

(b) Biological products for Distribution and Sale presented for importation under a permit and found to be worthless, contaminated, dangerous, or harmful shall, within a period of 30 days after such finding, be returned to the country of origin at the expense of the importer or in lieu thereof, destroyed by Department personnel: *Provided,* That such product shall not be returned to the country of origin while bearing a U.S. permit number on the label.

PART 105—SUSPENSION, REVOCATION, OR TERMINATION OF BIOLOGICAL LICENSES OR PERMITS

Sec.

105.1 Suspension or revocation.
105.2 Notification of infractions.
105.3 Notices re: worthless, contaminated, dangerous, or harmful biological products.
105.4 Termination of licenses and permits for inactivity.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 105.1 Suspension or revocation.

(a) An establishment license, product license, or permit issued under the Virus-Serum-Toxin Act may be formally suspended or revoked after opportunity for hearing has been accorded the licensee or permittee as provided in part 123 of this subchapter if the Secretary is satisfied that the license or permit is being used to facilitate or effect the preparation, sale, barter, exchange, shipment, or importation contrary to said Act of any worthless, contaminated, dangerous, or harmful biological product. Such use may be found to exist if:

(1) The construction of the establishment in which the biological product is prepared is defective, or the establishment is not conducted as required by the regulations in parts 101 through 118 of this subchapter;

(2) The methods of preparation of the product are faulty, or the product contains impurities or lacks potency;

(3) The product is so labeled or advertised as to mislead or deceive the purchaser in any particular;

(4) The licensee, permittee, or the foreign manufacturer has failed to maintain and make available for inspection records in connection with the development and preparation of product, has failed to provide complete and accurate information when requested, or has failed to provide complete and accurate information in the Outline of Production or in reports and records;

(5) The licensee or permittee has violated or failed to comply with any provision of the Virus-Serum-Toxin Act or the regulations in this subchapter;

(6) The license or permit is otherwise used to facilitate or effect the preparation, sale, barter, exchange, shipment, or importation, contrary to the Virus-Serum-Toxin Act, of any worthless, contaminated, dangerous, or harmful biological product.

(b) In case of willfulness or where the public health, interest, or safety so required the Secretary may, without hearing, informally suspend such establishment license, product license, or permit upon the grounds set forth in paragraph (a) of this section pending determination of formal proceedings under part 123 of this subchapter for suspension or revocation of the license or permit.

[38 FR 23512, Aug. 31, 1973, as amended at 41 FR 44359, Oct. 8, 1976; 61 FR 52874, Oct. 9, 1996; 64 FR 43044, Aug. 9, 1999]

§ 105.2 Notification of infractions.

If an infraction of a requirement of a product license is brought to the attention of the licensee by written notification thereof by Animal and Plant Health Inspection Service, a subsequent violation of similar nature occurring with the same licensed biological product within 6 months of the said written notification shall be primafacie evidence of willful violation and the license for the product shall be subject to suspension or revocation under the provisions of § 105.1(b).

[42 FR 31430, June 21, 1977, as amended at 56 FR 66783, Dec. 26, 1991]

§ 105.3 Notices re: worthless, contaminated, dangerous, or harmful biological products.

(a) If at any time it appears that the preparation, sale, barter, exchange, shipment, or importation, as provided in the Virus-Serum-Toxin Act, of any biological product by any person holding a license or permit may be dangerous in the treatment of domestic animals, the Secretary may without hearing notify the licensee or permittee, and pending determination of formal proceedings instituted under part 123 of this subchapter for suspension or revocation of the license or permit insofar as it authorizes the manufacture or importation of the particular product, no person so notified shall thereafter so prepare, sell, barter, exchange, ship, deliver for shipment, or import such product.

(b) If a serial of biological product is found to be unsatisfactory according to applicable Standard Requirements, the Administrator may notify the licensee to stop distribution and sale of the serial.

[38 FR 23512, Aug. 31, 1973, as amended at 56 FR 66783, Dec. 26, 1991]

§ 105.4 Termination of licenses and permits for inactivity.

(a) If a biological product has not been prepared by a licensee, or imported by a permittee for a period of 5 years or more, the Administrator may require the licensee to show intent to resume production, or the permittee to show intent to resume importation, within 6 months of notification. If the licensee does not resume preparation, or the permittee does not resume importation, within 6 months of notification, or within a mutually agreeable period, the product license, or permit, may be terminated by the Administrator.

(b) When a license or permit is terminated, the licensee or permittee shall continue to be subject to the applicable records provisions of § 116.8.

[61 FR 52874, Oct. 9, 1996]

PART 106—EXEMPTION FOR BIOLOGICAL PRODUCTS USED IN DEPARTMENT PROGRAMS OR UNDER DEPARTMENT CONTROL OR SUPERVISION

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 106.1 Biological products; exemption.

The Administrator may exempt any biological product from one or more of the requirements of this subchapter if he determines that such product will be used by the Department or under the supervision or control of the Department in the prevention, control or eradication of animal diseases in connection with (a) an official USDA program; or (b) an emergency animal disease situation, or (c) a USDA experimental use of the product.

[45 FR 65184, Oct. 2, 1980, as amended at 56 FR 66783, Dec. 26, 1991]

PART 107—EXEMPTIONS FROM PREPARATION PURSUANT TO AN UNSUSPENDED AND UNREVOKED LICENSE

Sec.
107.1 Veterinary practitioners and animal owners.
107.2 Products under State license.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 107.1 Veterinary practitioners and animal owners.

Products prepared as provided in paragraphs (a) and (b) of this section and establishments in which such products are prepared, shall be exempt from preparation pursuant to unsuspended and unrevoked establishment and product licenses. Persons exempt from licensure under this part shipping products which contain live organisms shall provide any information the Administrator may require prior to shipment, or at any other time deemed necessary, in order to assess the products' safety and effect on the environment. The shipment or delivery for shipment anywhere in or from the United States of any exempted product which is worthless, contaminated, dangerous, or harmful is prohibited, and any person shipping such product, or delivering such product for shipment, shall be subject to sanctions under the Act.

(a)(1) Products prepared by a veterinary practitioner (veterinarian) solely for administration to animals in the course of a State licensed professional practice of veterinary medicine by such veterinarian under a veterinarian-client-patient relationship and establishments in which such products are prepared shall be exempt from licensing under the Act and regulations. Such a relationship is considered to exist when:

(i) The veterinarian has assumed the responsibility for making medical judgments regarding the health of the animal(s) and the need for medical treatment, and the client (owner or other caretaker) has agreed to follow the instructions of the veterinarian; and when

(ii) There is sufficient knowledge of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s). This means that the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s), and/or by medically appropriate and timely visits to the premises where the animal(s) are kept; and when

(iii) The practicing veterinarian is readily available for followup in case of adverse reactions or failure of the regimen.

(2) Veterinarians preparing products subject to the exemption for products under this section shall maintain and make available for inspection by Animal and Plant Health Inspection Service representatives or other Federal employees designated by the Secretary such records as are necessary to establish that a valid veterinarian-client-patient relationship exists and that there is a valid basis for the exemption under this section.

(b) Products prepared by a person solely for administration to animals owned by that person shall be exempt from the requirement that preparation be pursuant to an unsuspended and unrevoked license.

[52 FR 30131, Aug. 13, 1987, as amended at 56 FR 66783, Dec. 26, 1991]

§ 107.2 Products under State license.

(a) The Administrator shall exempt from the requirement of preparation pursuant to an unsuspended and unrevoked USDA establishment and product license, any biological product prepared solely for distribution within the State of production pursuant to a license granted by such State under a

program determined by the Administrator to be consistent with the intent of the Act to prohibit the preparation, sale, barter, exchange, or shipment of worthless, contaminated, dangerous, or harmful biological products.

(b) A request for exemption under this section must be made by the appropriate State authority and shall include information demonstrating that:

(1) The State has the authority to license viruses, serums, toxins, and analogous products and establishments that produce such products; and

(2) The State has the authority to review the purity, safety, potency, and efficacy of such products prior to release to the market; and

(3) The State has the authority to review product test results to assure compliance with applicable standards of purity, safety, and potency prior to release to the market; and

(4) The State has the authority to deal effectively with violations of State law regulating viruses, serums, toxins, and analogous products; and

(5) The State effectively exercises the authority specified in paragraphs (b)(1) through (4) of this section consistent with the intent of the Act prohibiting the preparation, sale, barter, exchange, or shipment of worthless, contaminated, dangerous, or harmful viruses, serums, toxins, or analogous products.

(c) Each product to be exempted and each establishment preparing such product shall be identified by the State and the State shall give written notification to the Administrator of each such product and establishment. The State shall also give written notice to the Administrator of each new license issued and of each license terminated.

(d) In order to determine whether a State exercises its authority with respect to biological products and establishments and whether its laws and regulations are being achieved, the Administrator, in cooperation with proper State authorities, may conduct an on-site evaluation of the State's program which may include inspection of establishments and/or products to be included under the exemptions in this section.

[52 FR 30131, Aug. 13, 1987, as amended at 56 FR 66783, Dec. 26, 1991]

PART 108—FACILITY REQUIREMENTS FOR LICENSED ESTABLISHMENTS

Sec.

108.1 Applicability.
108.2 Plot plans, blueprints, and legends required.
108.3 Preparation of plot plans.
108.4 Preparation of blueprints.
108.5 Preparation of legends.
108.6 Revision of plot plans, blueprints, and legends.
108.7 Filing of plot plans, blueprints, and legends.
108.8 Construction of buildings.
108.9 Dressing rooms and other facilities.
108.10 Outer premises and stables.
108.11 Water quality requirements.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 39 FR 16854, May 10, 1974, unless otherwise noted.

§ 108.1 Applicability.

Unless otherwise authorized by the Administrator, all buildings, appurtenances, and equipment used in the preparation of biological products shall be in compliance with the regulations in this part. Each land area on which such buildings and appurtenances are located shall be identified by an address which shall appear on the establishment license.

[39 FR 16854, May 10, 1974, as amended at 56 FR 66783, Dec. 26, 1991]

§ 108.2 Plot plans, blueprints, and legends required.

Each applicant for an establishment license shall prepare a plot plan showing all buildings for each particular land area, blueprints for each building used in the preparation of biological products and legends containing a brief description of all activities in each room or area.

§ 108.3 Preparation of plot plans.

Plot plans shall show all of the buildings on a particular land area, whether or not they are all used for the preparation and initial shipping of biological products: *Provided,* That, when a great number of buildings are on the same premises, only those surrounding the buildings used for preparation and initial shipping of biological products

shall be shown. The presence of the remainder of the buildings may be accounted for by a single statement denoting the total number of such buildings not used for the preparation or shipping of biological products.

(a) Reduce the entire premises to any standard scale on one sheet of paper which meets any of the American standard trimmed sizes. Indicate the scale used.

(b) Clearly mark the boundaries of the licensed premises and indicate what marking denotes the boundaries. Such boundaries shall coincide with some readily apparent perimeter line. Identify all fences, walls, or streets.

(c) Show buildings as reduced dimensional drawings in the proper scale distance relationship with each other.

(d) Number, letter, or otherwise identify all buildings so that they may be correlated with the respective blueprints and legends.

(e) Describe on the plot plan the use of immediate adjacent properties such as, residential area, pasture, box factory, or the like.

(f) Show compass points.

(g) Show date of preparation.

(h) Apply signature of responsible official of the firm.

§ 108.4 Preparation of blueprints.

(a) Blueprints, drawn to any suitable scale, on regular blueprint paper or a good grade of white paper of any one of the American standard trimmed sizes shall be acceptable: *Provided*, That the same scale shall be used for future revisions unless the entire blueprint is revised. Indicate the scale used.

(b) Use a single sheet of paper for each floor of all buildings in which biological products are prepared. Illustrate in detail the areas in each building utilized for such preparation.

(c) If only a portion of a floor is used in the preparation of a biological product, the blueprint shall illustrate the entire floor in essentially the same detail throughout. All functions or activities performed in the remainder of the floor shall be indicated.

(d) Identify the floors if the drawing is not for all floors in a multiple-story building and identify activities on each floor.

(e) Identify all rooms by letters or numbers.

(f) Show the location of important stationary equipment by a suitable code which will be further identified on legends.

(g) Explain on the blueprint or on the legend, by a statement or listing, which rooms are equipped with water outlets, drains, and lighting. Show the location of doors and windows.

(h) Show compass points.

(i) Show building number.

(j) Show date of preparation.

(k) Apply signature of responsible official of firm.

§ 108.5 Preparation of legends.

A brief description of the activities performed in each room or area shall be prepared as provided in this section and shall be referred to as a legend. Legends shall be provided for each plot plan and each blueprint or drawing. All pages of the legends shall be numbered, identified with corresponding plot plan or blueprint, and submitted in booklet form either stapled together or clipped into a suitable folder.

(a) Plot plan legends shall show the following:

(1) Number of each building and the functions performed in each: *Provided*, That if it is a multiple-story building in which biological products are prepared or handled, briefly describe functions performed on each floor.

(2) A practical and nontechnical description of construction materials used throughout those buildings used entirely or partially for production and handling of biological products.

(b) Blueprint legends shall show the following:

(1) A listing of all rooms by identifying letters or numbers and the fractions prepared in each. Exceptions may be listed for general purpose areas or rooms. Functions performed in each area and room shall be described, whether the licensed or unlicensed products. In rooms where products are exposed to the surroundings, a description of decontamination procedures and other precautions against cross contamination shall be included.

(2) A listing of the coded stationary equipment.

(3) A general listing of other essential biological equipment such as mills, centrifuges, mixing tanks, bottling and sealing equipment, and the like, which are not regarded as stationary but are maintained in certain rooms.

[39 FR 16854, May 10, 1974, as amended at 40 FR 51413, Nov. 5, 1975; 50 FR 50764, Dec. 12, 1985]

§ 108.6 Revision of plot plans, blueprints, and legends.

Preliminary drawings may be submitted to Animal and Plant Health Inspection Service for comment prior to construction of new facilities or when remodeling is anticipated, old facilities are to be torn down, or other changes affecting the workflow are to be made. The licensee shall:

(a) Prepare revised plot plans, blueprints, or legends and submit to Animal and Plant Health Inspection Service for review and filing when changes have been completed. Also prepare a statement to accompany each revision to identify, by date of the superseded item, what is being superseded.

(b) Prepare a drawing of the revised rooms, unit, or section to the same scale as the blueprint on file which shall be stamped and applied to the existing blueprint. If changes are numerous, prepare a new blueprint.

(c) Drawings of new buildings may be added to existing plot plans. Indicate the distance from surrounding buildings and boundary lines.

(d) Any change prescribed in this section shall necessitate a change in one or more pages of the respective legends. The revised pages shall carry the same numbers as superseded pages.

[39 FR 16854, May 10, 1974, as amended at 56 FR 66783, Dec. 26, 1991]

§ 108.7 Filing of plot plans, blueprints, and legends.

Three copies of all plot plans, blueprints, and legends, including revisions, shall be submitted to Animal and Plant Health Inspection Service for review and filing. When the reviewer takes exception to a submitted item, such item shall be returned with appropriate comments for correction and resubmission. Acceptable submissions shall be stamped as filed and the date noted. One stamped copy shall be returned and two copies retained for Animal and Plant Health Inspection Service files.

[39 FR 16854, May 10, 1974, as amended at 56 FR 66783, Dec. 26, 1991]

§ 108.8 Construction of buildings.

(a) The floors, walls, ceilings, partitions, posts, doors, and all other parts of all structures, rooms, or facilities used for the preparation of biological products or ingredients of biological products at licensed establishments shall be of such material, construction, and finish as may be readily and thoroughly cleaned.

(b) All rooms used in connection with the preparation of biological products shall be so constructed and arranged as to prevent cross-contamination of such biological products. Halls or walkways shall be provided for the movement of personnel or materials to each biological products preparation area without going through another such area.

(c) Rooms or compartments separate from the remainder of the establishment shall be provided at licensed establishments for preparing, handling, and storing virulent or dangerous microorganisms and products.

(d) All rooms and compartments at licensed establishments shall have an adequate air handling system to supply proper ventilation sufficient to insure sanitary and hygienic conditions for the protection of the products and personnel.

(e) The supply of hot and cold water at licensed establishments shall be ample and clean. Adequate facilities shall be provided for the distribution of water in each establishment and for the washing of all containers, machinery, instruments, other equipment, and animals used in the preparation of a biological product.

(f) There shall be an efficient drainage and plumbing system for each licensed establishment and premises thereof, and all drains and gutters shall be properly installed with approved traps and vents.

§ 108.9 Dressing rooms and other facilities.

Each licensed establishment shall have dressing rooms, toilet facilities,

and lavatory accommodations, including hot and cold running water, soap, towels, and the like. They shall be in sufficient number, ample in size, conveniently located, properly ventilated, and meeting all requirements as to sanitary construction and equipment.

(a) These rooms and facilities shall be separate from rooms or compartments in which biological products are prepared, handled, or stored.

(b) These rooms and facilities shall be so located in the establishment as to be readily accessible to all persons without having to enter or pass through biological products preparation areas.

§ 108.10 Outer premises and stables.

(a) The outer premises of licensed establishments, embracing docks, driveways, approaches, yards, pens, chutes, and alleys shall be drained properly and kept in a clean and orderly condition. No nuisance shall be allowed in any licensed establishment or on its premises.

(b) Stables or other premises for animals used in the production or testing of biological products at licensed establishments shall be properly ventilated and lighted, appropriately drained and guttered, and kept in sanitary condition.

(c) Every practical precaution shall be taken to keep licensed establishments free of flies, rats, mice, and other vermin. The accumulation, on the premises of an establishment, of any material in which flies or other vermin may breed is forbidden. Suitable arrangements, in keeping with the local health practices, shall be made for the disposal of all refuse.

§ 108.11 Water quality requirements.

A certification from the appropriate water pollution control agency, that the establishment is in compliance with applicable water quality control standards, pursuant to section 401 of the Federal Water Pollution Control Act, as amended (86 Stat. 877; 33 U.S.C. 1341), shall be filed with Animal and Plant Health Inspection Service for each licensed establishment.

[39 FR 16854, May 10, 1974, as amended at 56 FR 66783, Dec. 26, 1991]

PART 109—STERILIZATION AND PASTEURIZATION AT LICENSED ESTABLISHMENTS

Sec.

109.1 Equipment and the like.
109.2 Sterilizers.
109.3 Pasteurizers.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 109.1 Equipment and the like.

(a) All containers, instruments, and other apparatus and equipment, before being used in preparing, handling, or storing biological products, at a licensed establishment, except as otherwise prescribed herein, shall be thoroughly sterilized by live steam at a temperature of at least 120 °C. for not less than one-half hour, or by dry heat at a temperature of at least 160 °C. for not less than one hour. If for any reason such methods of sterilization are impracticable, then a process known to be equally efficacious in destroying microorganisms and their spores may be substituted after approval by the Administrator.

(b) Instruments which are found to be damaged by exposure to the degree of heat prescribed in this section, after having been thoroughly cleaned, may be sterilized by boiling for not less than 15 minutes.

[23 FR 10051, Dec. 23, 1958, as amended at 34 FR 18119, Nov. 11, 1969; 56 FR 66783, Dec. 26, 1991]

§ 109.2 Sterilizers.

Steam and dry-heat sterilizers used in connection with the processing of biological products at licensed establishments shall be equipped with automatic temperature recording gauges: *Provided,* That other record keeping systems may be used when approved by the Administrator. When gauges are used, they shall be periodically standardized to assure accuracy. Charts and other temperature records made during production shall be available at all times charts and records shall be kept in accordance with part 116 of this chapter.

[35 FR 16039, Oct. 13, 1970, as amended at 56 FR 66783, Dec. 26, 1991]

§ 109.3 Pasteurizers.

All pasteurizing equipment shall meet the requirements in paragraphs (a), (b), and (c) of this section and be acceptable to Animal and Plant Health Inspection Service.

(a) Metal serum containers shall be used in licensed establishments. During the heating process, each container shall be surrounded by a separate water jacket or equivalent so that the entire container, including its lid, is heated to the required temperature. Each serum container shall be equipped with a motor-driven agitator and a separate automatic recording thermometer.

(b) Each water bath shall have an automatic temperature control to limit the temperature of the water to a maximum of 62 °C., an automatic recording thermometer, an indicating thermometer set in a fixed position, and circulating mechanism adequate to insure equal temperatures throughout the bath. The heating unit for the bath shall be separated from the serum container and the water jacket.

(c) Accurate thermometers at licensed establishments shall be used at frequent intervals to check temperatures of the serum as registered by recording thermometers.

[35 FR 16039, Oct. 13, 1970, as amended at 56 FR 66783, Dec. 26, 1991]

PART 112—PACKAGING AND LABELING

Sec.

112.1 General.
112.2 Final container label, carton label, and enclosure.
112.3 Diluent labels.
112.4 Subsidiaries, divisions, distributors, and permittees.
112.5 Review and approval of labeling.
112.6 Packaging biological products.
112.7 Special additional requirements.
112.8 For export only.
112.9 Biological products imported for research and evaluation.
112.10 Special packaging and labeling.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 38 FR 12094, May 9, 1973, unless otherwise noted.

§ 112.1 General.

(a) Unless otherwise authorized or directed by the Administrator, each biological product prepared at a licensed establishment, or imported, shall be packaged and labeled as prescribed in this part before it is removed from the licensed establishment or presented for importation: *Provided,* That biological products to be imported for research and evaluation shall be subject to packaging and labeling requirements in § 112.9. *Provided further,* That, unless otherwise exempted, all preparation, including packaging and labeling, of biological products shall only be performed in a licensed establishment under an approved Outline of Production.

(b) No person shall apply or affix to or include with, or cause to be applied or affixed to or included with, any carton or final container of a biological product, any label, stamp, mark or statement that is false or misleading in any particular, is not in compliance with the regulations, or is not approved by APHIS.

(c) No person shall alter, mark or remove any approved labeling affixed to or included with any biological product prior to selling or otherwise distributing such product. In addition, no person shall mark any carton, other container, or final container of a biological product so as to falsify the labeling, make it misleading, or cause it to be illegible.

(d) Labels that are stamped, printed or glued directly on cartons, other containers, or final containers shall be legible throughout the dating period. Biological products bearing labels, which have been altered, mutilated, destroyed, obliterated or removed, shall be withheld from the market.

[38 FR 12094, May 9, 1973, as amended at 59 FR 43445, Aug. 24, 1994]

§ 112.2 Final container label, carton label, and enclosure.

(a) Unless otherwise provided, final container labels, carton labels, and enclosures (inserts, circulars, or leaflets) shall include the information specified in this section.

(1) The principal part of the true name of the biological product which

name shall be identical with that shown in the product license under which such product is prepared, or the permit under which it is imported, shall be prominently lettered and placed giving equal emphasis to each word composing it. Descriptive terms used in the true name on the product license or permit shall also appear. Abbreviations of the descriptive terms may be used on the final container label if complete descriptive terms appear on a carton label and enclosures;

(2) If the biological product is prepared in the United States, the name and address of the producer (licensee or subsidiary) or if the biological product is prepared in a foreign country, the name and address of the permittee and of the foreign producer.

(3) The license or permit number assigned by the Department which shall be shown only in one of the following forms respectively: ''U.S. Veterinary License No. _____,'' or ''U.S. Vet. License No. _____,'' or ''U.S. Vet Lic. No. _____,'' or ''U.S. Veterinary Permit No. _____,'' or ''U.S. Permit No. _____.''

(4) Storage temperature recommendation for the biological product stated as not over 45 °F. or stated as not over 7 °C. or stated as not over 45 °F. or 7 °C.

(5) Full instructions for the proper use of the product, including vaccination schedules, warnings, cautions, and the like: *Provided,* That in the case of very small final container labels or carton, a statement as to where such information is to be found, such as ''See enclosure for complete directions,'' ''Full directions on carton,'' or comparable statement;

(6) In the case of a multiple-dose final container, a warning to use entire contents when first opened: *Provided,* That a diagnostic or a desensitizing antigen packaged in a multiple-dose final container is exempt;

(7) If the biological product contains viable or dangerous organisms or viruses, a warning to ''Burn this container and all unused contents,'' except that in the case of a small one-dose container, the statement ''Burn this container'' or ''Burn this vial'' may be used.

(8) In the case of a biological product recommended for use in domestic animals, the edible portion of which may be used for food purposes, a withholding statement of not less than 21 days to read: ''Do not vaccinate within (insert number) days before slaughter'' or ''Do not vaccinate food-producing animals within (insert number) days before slaughter'': *Provided,* That longer periods shall be stated when deemed necessary by the Administrator. Very small final container labels are exempted from this requirement.

(9) The following information shall appear on the final container label and carton label, if any, but need not appear on the enclosure:

(i) A permitted expiration date;

(ii) The number of doses where applicable;

(iii) The recoverable quantity of the content of each final container stated in cubic centimeters (cc.) or milliliters (ml.) or units.

(iv) A serial number by which the product can be identified with the manufacturer's records of preparation: *Provided,* That when a liquid antigenic fraction is to be used instead of a water diluent for one or more desiccated antigenic fractions in a combination package, a hyphenated serial number composed of a serial number for the desiccated fraction and the serial number for the liquid fraction shall be used on the carton;

(10) In the case of a product which contains an antibiotic added during the production process, the statement ''Contains _____ as a preservative,'' or an equivalent statement indicating the antibiotic added shall appear on cartons and enclosures if used: *Provided,* That if cartons are not used, such information shall appear on the final container label;

(11) The number of final containers of biological product and the number of doses in each final container shall be stated on each carton label for all cartons containing more than one final container of biological product. The number of final containers of diluent, if any, and the quantity in each shall also be stated on each carton label.

(b) Labels may also include any other statement which is not false or misleading and may include factual statements regarding variable response of

different animals when vaccinated as directed but may not include disclaimers of merchantability, fitness for the purpose offered, or responsibility for the product.

(c) Labels of biological products prepared at licensed establishments or imported shall not include any statement, design, or device, which overshadows the true name of the product as licensed or which is false or misleading in any particular or which may otherwise deceive the purchaser.

(d) Carton labels and enclosures shall be subject to paragraph (d)(1), (d)(2), and (d)(3) of this section.

(1) The statement, ''Restricted to use by or under the direction of a veterinarian'' or ''Restricted to use by a veterinarian,'' shall be used on all carton labels and enclosures when such restriction is prescribed on the product license.

(2) If the licensee states on the carton labels and enclosures of a product that its sales are restricted to veterinarians, then the entire production of that particular product in the licensed establishment shall be so restricted by the licensee.

(3) The statement ''For veterinary use only'' or an equivalent statement may appear on the carton labels and enclosures for a product if such statement is being used to indicate that the product is recommended specifically for animals, and not for humans.

(e) When label requirements of a foreign country conflict with the requirements as prescribed in this part, special labels may be approved for use on biological products to be exported to such country. When laws, regulations, or other requirements of foreign countries require exporters of biological products prepared in a licensed establishment to furnish official certification that such products have been prepared in accordance with the Virus-Serum-Toxin Act and regulations issued pursuant thereto, such certification may be made by Animal and Plant Health Inspection Service upon request of the licensee.

(f) If a carton label or an enclosure is required to complete the labeling for a multiple-dose final container of liquid biological product, only one final container shall be packaged in each carton: *Provided,* That if the multiple-dose final container is fully labeled without a carton label or enclosure, two or more final containers may be packaged in a single carton which shall be considered a shipping box. Labels or stickers for shipping boxes shall not contain false or misleading information but need not be submitted for approval.

(Approved by the Office of Management and Budget under control number 0579–0013)

[38 FR 12094, May 9, 1973, as amended at 39 FR 16856, May 10, 1974; 41 FR 44359, Oct. 8, 1976; 42 FR 11825, Mar. 1, 1977; 42 FR 29854, June 10, 1977; 42 FR 41850, Aug. 19, 1977; 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991]

§ 112.3 Diluent labels.

Each final container of diluent, other than a liquid biological product, packaged with desiccated biological products shall bear a label that includes the following:

(a) The name—Sterile Diluent.

(b) True name of the biological product with which the diluent is packaged, except that when the firm packages all desiccated biological products with the same diluent, or two or more types of diluent are used, and the licensees' methods of identification and storage insure that all products are packaged with the correct type of diluent, labels affixed to the containers of diluent are exempt from this provision.

(c) The recoverable quantity of contents in cubic centimeters (cc) or milliliters (ml).

(d) A serial number by which the diluent can be identified with the manufacturer's records of preparation;

(e) Name and address of the licensee or the permittee;

(f) In the case of a diluent with which a desiccated biological product is to come in contact while the diluent is in its original container; and,

(1) Is in a multiple-dose container, a positive warning that all of the biological product shall be used at the time the container is first opened; and/or

(2) The biological product is composed of viable or dangerous organisms or viruses, the notice, ''Burn this container and all unused contents,'' except that, in the case of a small one-dose container, the statement ''Burn this container'' or ''Burn this vial'' may be used.

(g) The establishment license number or the permit number, as the case may be, in one of the forms provided in §112.2(a)(3).

[38 FR 12094, May 9, 1973; 38 FR 13476, May 22, 1973, and amended at 39 FR 16856, May 10, 1974]

§112.4 Subsidiaries, divisions, distributors, and permittees.

Labels used by subsidiaries, divisions, distributors, and permittees shall be affixed by the licensee in a licensed establishment where the product is produced. Such labels shall comply with requirements for their review, approval, and filing as provided in the regulations.

(a) *Subsidiaries.* Labels to be used on a licensed biological product prepared by a subsidiary operating in a licensed establishment shall be submitted in accordance with §112.5. Only labels approved for use on such product shall be used by the subsidiary.

(b) *Divisions.* Labels to be used on a licensed biological product prepared in a licensed establishment for distribution by a division or marketing unit of the licensee shall be submitted in accordance with §112.5. The name, address, and license number of the licensee shall be prominently placed on such labels. The relationship of the division or marketing unit to the licensee shall appear prominently on the label by use of the term ''division of'' or equivalent.

(c) *Distributors.* The name and address of the distributor or any statement, design, or device shall not be placed on the labels or containers of a licensed biological product in a manner which could be false or misleading or which could indicate that the distributor is the manufacturer of such product or operating under the license number shown on the label. The manufacturer shall be identified by name, address, and license number with the term ''manufactured by,'' ''produced by,'' or an equivalent term prominently placed in connection therewith. The name and address of the distributor may be placed on labels or containers if the term ''distributor,'' or ''distributed by,'' or an equivalent term is prominently placed in connection therewith.

(d) *Permittees.* The name and address of the permittee and any statement, design, or device shall not be placed on the labels or containers of a biological product imported for sale and distribution in accordance with §104.5 in a manner which could be false or misleading or which could falsely indicate that the permittee is the manufacturer of such product. The manufacturer shall be identified by name and address with the term ''manufactured by,'' ''produced by,'' or an equivalent term prominently placed in connection therewith. Reference to the permittee shall be made by name, address, and permit number with the term ''imported by,'' ''produced for,'' or an equivalent term prominently placed in connection therewith.

[50 FR 46417, Nov. 8, 1985, as amended at 59 FR 43445, Aug. 24, 1994]

§112.5 Review and approval of labeling.

Labels used with biological products prepared at licensed establishments or imported for general distribution and sale must be submitted to the Animal and Plant Health Inspection Service for review for compliance with the regulations and approval in writing prior to use, except as provided in paragraph (c) of this section and under the master label system provided in paragraph (d) of this section.

(a) Transmittal forms, furnished by Animal and Plant Health Inspection Service upon request, shall be used with each submission of sketches (including proofs) and labels. Separate forms shall be used for each biological product but only one copy of the form shall be used for all sketches and labels submitted at the same time for the same biological product.

(b) Sketches may be submitted for comment to Animal and Plant Health Inspection Service by the licensee or permittee before preparing the finished label. Such sketches shall be returned to the licensee or permittee with comments, if any. Failure of the reviewer to take exception to a sketch shall not constitute approval of a finished label subsequently prepared.

(c)(1) Labels must be submitted to the Animal and Plant Health Inspection Service for review and written approval. Only labels which are approved as provided in § 112.5(d) may be used. When changes are made in approved labels, the new labels shall be subject to review and approval before use: *Provided,* That certain minor changes may be made in labels for products with approved labels or master labels, and the revised labels may be used prior to review by APHIS, with the provision that a new label or master label bearing these changes is submitted to APHIS for review and written approval within 60 days of label use, and that such minor changes do not render the product mislabeled or the label false and misleading in any particular.

(2) Minor label changes that may be made under the provision for products with approved labels or master labels are:

(i) Changes in the physical dimensions of the label provided that such change does not affect the legibility of the label;

(ii) Change in the color of label print, provided that such change does not affect the legibility of the label;

(iii) The addition or deletion of a Trade Mark (TM) or Registered (R) symbol;

(iv) The correction of typographical errors;

(v) Adding or changing label control numbers of bar codes; and

(vi) Revising or updating logos.

(d) Labels and sketches submitted shall be prepared in the number and manner prescribed in this paragraph.

(1) Copies required:

(i) For label sketches, submit two copies of each sketch of a final container label, carton label, and enclosure. Sketches must be legible, and must include all information specified in § 112.2. One copy of each sketch will be returned with applicable comments, and one copy will be held on file by APHIS for no more than one year after processing, until replaced by a finished label: *Provided,* That sketches submitted in support of an application for a license or permit shall be held as long as the application is considered active.

(ii) For master label sketches, submit for each product two copies of each sketch of an enclosure, label for the smallest size final container, and carton label; *Provided,* That labels for larger size containers and/or cartons that are identical, except for physical dimensions, need not be submitted. One copy of each master label sketch will be returned with applicable comments, and one copy will be held on file by APHIS for one year after processing, until replaced by a finished master label that is submitted according to § 112.5(d)(1)(iii): *Provided,* That master label sketches submitted in support of an application for license or permit shall be held as long as the application is considered active.

(iii) For finished labels, submit three copies of each finished final container label, carton label, and enclosure: *Provided,* That when an enclosure is to be used with more than one product, one extra copy shall be submitted for each additional product. Two copies of each finished label will be retained by APHIS. One copy will be stamped and returned to the licensee. Labels to which exceptions are taken shall be marked as sketches and handled under § 112.5(d)(1)(i).

(iv) For finished master labels, submit for each product three copies each of the enclosure and the labels for the smallest size final container and carton. Labels for larger sizes of containers or cartons of the same product that are identical, except for physical dimensions, need not be submitted. Such labels become eligible for use, concurrent with the approval of the appropriate finished master label: *Provided,* That the marketing of larger sizes of final containers is approved in the filed Outline of Production, and the appropriate larger sizes of containers or cartons are identified on the label mounting sheet. When a master label enclosure is to be used with more than one product, one extra copy for each additional product shall be submitted. Two copies of each finished master label will be retained by APHIS. One copy will be stamped and returned to the licensee. Master labels to which exceptions are taken will be marked as sketches and handled under § 112.5(d)(1)(ii).

(2) Mounting:

(i) Each label or sketch shall be securely fastened to a separate sheet of heavy bond paper (8½″ × 11″) in such a manner that all information is available for review.

(ii) Two-or three-part cartons, including ''sleeves,'' shall be considered as one label. All parts shall be submitted together.

(iii)(A) When two final containers are packaged together in a combination package, the labels for each shall be mounted on the same sheet of paper and shall be treated as one label. For diagnostic test kits, the labels for use on the individual reagent containers to be included in the kit shall be mounted together on a single sheet of paper, if possible; if necessary, a second sheet of paper may be used. The carton label and enclosure shall be mounted on separate individual sheets.

(B) If either final container label is also used alone or in another combination package, sets of separate labels for each biological product with which it is used shall be submitted for review.

(iv) When the same final container label is applied by different methods such as paper or screen printing, one of each shall be mounted on the same sheet of paper as one submission.

(3) To appear on the top of each page:

(i)(A) Name and product code number of the biological product as it appears on the product license or permit.

(B) Extra copies of enclosures to be used with another product shall bear the name and code number of the product affected.

(ii)(A) Designation of the specimen as a label or master label: sketch, final container label, carton label, or enclosure.

(B) If two final container labels or multiple parts are on one sheet, each shall be named, and the label or part being revised shall be designated.

(iii) Size of package (dose, ml., cc., or units) for which the labels or enclosures are to be used.

(4) To appear on the bottom of each page: The reason for and information relevant to the submission shall be stated in the lower left hand corner as:

(i) Master label dose sizes approved for code _____.

(ii) Replacement for label, master label, and/or sketch No. _____.

(iii) Reference to label or master label No. _____.

(iv) Addition to label No. _____.

(v) License Application Pending _____.

(vi) Foreign Language copy of Label No. _____.

(e) Special requirements for foreign language labels:

(1) If true, a statement that the label is a direct translation from a corresponding approved domestic label.

(2) If the foreign language label is not a direct translation of an approved domestic label, an English version shall be submitted with an explanation for the difference in texts.

(3) Foreign language portion of a bilingual label shall be a true translation of the English portion. Reference to additional information on the enclosure shall not be made unless that enclosure is also bilingual.

(f) When a request is received from Animal and Plant Health Inspection Service, the licensee or permittee shall submit a list of all approved labels currently being used. Each label listed shall be identified as to:

(1) Name and product code number as it appears on the product license or permit for the product; and

(2) Where applicable, the size of the package (doses, ml., cc., or units) on which the label shall be used; and

(3) Label number and date assigned; and

(4) Name of licensee or subsidiary appearing on the label as the producer.

(g) At the time of an inspection, or when requested by APHIS, licensees or permittees shall make all labels and master labels, including labels approved for use but exempted from filing under the master label system, available for review by authorized inspectors. Such labels shall be identical to the approved label or master label except for physical dimensions, reference to recoverable volume or doses and/or certain minor differences permitted in accordance with § 112.5(c).

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[38 FR 12094, May 9, 1973, as amended at 48 FR 57473, Dec. 30, 1983; 49 FR 21044, May 18, 1984; 56 FR 66783, Dec. 26, 1991; 59 FR 43445, Aug. 24, 1994; 61 FR 29464, June 11, 1996; 61 FR 33175, June 26, 1996; 64 FR 43044, Aug. 9, 1999]

§ 112.6 Packaging biological products.

(a) Each multiple-dose final container of a biological product which requires a diluent for administration shall be packaged in an individual carton with a container of the proper volume of diluent for that dose as specified in the filed Outline of Production. Each multiple-dose final container of a product which does not require a diluent for administration need not be packaged in an individual carton unless the final container labeling does not contain all information required by the regulations. Such information must be included in or on a carton. Exceptions are provided in paragraphs (c) and (d) of this section and § 112.8.

(b) Single-dose final containers of a product need not be packaged one per carton. For single-dose products which require a diluent for administration, the number of containers of the proper amount of diluent specified in the filed Outline of Production for the number of doses contained in the carton shall be included in each carton.

(c) Poultry products for mass administration (including but not limited to administration through drinking water and spray) and products used in automatic vaccinating systems (including but not limited to pneumatic beak injectors and automated needle injectors) may be packaged in multiple-dose final containers as specified in the filed Outline of Production. Poultry products for manual administration to individual birds shall not exceed 1,000 doses in each final container. Diluent need not be packaged with the final container(s) of the product, but the licensee shall provide the required number of containers of diluent as specified in the filed Outline of Production. The following requirements apply to cartons containing more than one final container of poultry product:

(1) They shall be sealed prior to leaving the licensed establishment.

(2) The contents may not be repackaged.

(3) The contents of such cartons may not be sold in fractional units.

(4) The following statement must appear in a prominent place on the carton label: ''Federal regulations prohibit the repackaging or sale of the contents of this carton in fractional units. Do not accept if seal is broken.''

(d) Diluent for the following products need not be packaged with the final container(s) of the product, but the licensee shall provide the consumer with the required number of containers of the proper amount of diluent as specified in the filed Outline of Production:

(1) Marek's Disease Vaccine.

(2) Poultry vaccines administered to individual birds using automatic vaccinating equipment.

(e) Final containers of biological product prepared at a licensed establishment, or imported, in cartons or other containers shall not be removed from such cartons or containers for sale or distribution, unless each final container bears, or is packaged in a carton with, complete and approved labeling which is affixed to or included with each container by the licensed establishment producing the product or by the producer in the case of imported product: *Provided,* That this paragraph is not intended to apply to licensed veterinary practitioners administering or dispensing biological products in the course of their practice under a veterinary-client-patient-relationship as that term is used in § 107.1.

(f) Labels which are affixed to or included with a biological product shall not be removed or altered in any manner.

[47 FR 8761, Mar. 2, 1982, as amended at 48 FR 12691, Mar. 28, 1983; 59 FR 43445, Aug. 24, 1994; 64 FR 43044, Aug. 9, 1999]

§ 112.7 Special additional requirements.

The label requirements in this section are additional to those prescribed elsewhere in this part.

(a) In the case of biological products containing live Newcastle Disease virus, a caution statement indicating that Newcastle Disease can cause inflammation of the eyelids of humans, and a warning to the user to avoid infecting his eyes shall be included on the enclosure.

(b) In the case of a biological product containing infectious bronchitis virus, all labels shall show the infectious bronchitis virus type or types used in the product. Abbreviation is permitted.

(c) In the case of a biological product containing inactivated rabies virus, carton labels, enclosures, and all but very small final container labels shall include a warning against freezing and the recommendations provided in this paragraph.

(1) That vaccine be administered to animals at 3 months of age or older, with a repeat dose 1 year later.

(2) Subsequent revaccination as determined from the results of duration of immunity studies conducted as prescribed in §113.209, paragraph (b) or (c), or both.

(d) In the case of a biological product containing modified live rabies virus, the carton labels, enclosures, and all but very small final container labels shall include the recommendations provided in this paragraph.

(1) For low egg-passage (below the 180th egg-passage level) the statement ''For Use in Dogs Only! Not For Use in Any Other Animal!''

(2) For other vaccines containing modified live rabies virus, the statement ''For Use In (designate animal(s)) Only! Not For Use In Any Other Animal!''

(3) Intramuscular injection at one site in the thigh shall be recommended.

(4) The statement ''In event of accidental exposure to the vaccine virus, the possible hazard to human health should be considered and State Public Health Officials should be consulted for specific recommendations'' shall be prominently placed on all carton labels and on enclosures, if used.

(5) That vaccine be administered to animals at 3 months of age or older, with a repeat dose 1 year later.

(6) Subsequent revaccination as determined from the results of duration of immunity studies conducted as prescribed in §113.312, paragraph (b) or (c), or both.

(e) In the case of bovine rhinotracheitis vaccine containing modified live virus, all labeling except small final container labels shall bear the following statement: ''Do not use in pregnant cows or in calves nursing pregnant cows.'': *Provided,* That such vaccines which have been shown to be safe for use in pregnant cows may be excepted from this label requirement by the Administrator.

(f) Unless otherwise authorized in a filed Outline of Production, labels for inactivated bacterial products shall contain an unqualified recommendation for a repeat dose to accomplish primary immunization to be given at an appropriate time interval: *Provided,* That, repeat dose recommendations prescribed in paragraphs (f)(1) through (3) of this section are required for products containing the fractions listed.

(1) *Clostridium haemolyticum.* ''Repeat the dose every 5 or 6 months in animals subject to reexposure.''

(2) *Erysipelothrix rhusiopathiae.* ''Swine: For breeding animals, repeat after 21 days and annually. Turkeys: Repeat dose every 3 months.''

(3) *Clostridium botulinum Type C.* ''Revaccinate breeders 1 month before breeding.''

(g) In the case of a liquid product authorized in a filed Outline of Production to be used as a diluent in a combination package, the carton labels and enclosures used for serials which are either not tested for bactericidal or viricidal activity or have been found unsatisfactory by such test shall contain the statement: ''CAUTION: DO NOT USE AS DILUENT FOR LIVE VACCINES.''

(h) In the case of wart vaccine, recommendations shall be limited to use in cattle. Indications for use shall be for prophylactic use only, as an aid in the control of viral papillomas (warts). All labels shall include a dosage recommendation of at least 10 ml to be given subcutaneously and the dose repeated in 3 to 5 weeks.

(i) Unless otherwise authorized in an Outline of Production filed subsequent to the effective date of these amendments, all but very small final container labels for Feline Panleukopenia Vaccines shall contain the following recommendations for use:

(1) *Killed virus vaccines.* Vaccinate healthy cats of any age with one dose except that if the animal is less than 12 weeks of age, a second dose should be given at 12 to 16 weeks of age. Annual revaccination with a single dose is recommended.

(2) *Modified live virus vaccines.* Vaccinate healthy cats of any age with one dose except that if the animal is less than 12 weeks of age, a second dose

should be given at 12 to 16 weeks of age. Annual revaccination with a single dose is recommended. Do not vaccinate pregnant cats.

(j) In the case of normal serum, antiserum, or antiserum derivatives, the type of preservative used shall be indicated on all labels.

(k) Unless acceptable data has been filed with Animal and Plant Health Inspection Service, to show that development of corneal opacity is not associated with the product, carton labels and enclosures used with biological products containing modified live canine hepatitis virus or modified live canine adenovirus Type 2 shall bear the following statement: ''Occasionally, transient corneal opacity may occur following the administration of this product.''

(l) All labels for autogenous biologics shall bear the following statement: ''Potency and efficacy of autogenous biologics have not been established. This product is prepared for use only by or under the direction of a veterinarian or approved specialist.''

(m) In the case of biological products containing Marek's disease virus, all labels shall specify the Marek's disease virus serotype(s) used in the product.

(Approved by the Office of Management and Budget under control number 0579–0013)

[38 FR 12094, May 9, 1973]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting § 112.7, see the List of CFR Sections Affected, which appears in the Finding Aids section of the printed volume and on GPO Access.

§ 112.8 For export only.

The applicable regulations for packaging and labeling a biological product produced in the United States shall apply to such biological product if exported from the United States except as otherwise provided in this section. Only labels approved as provided in § 112.5 shall be used.

(a) Biological products which have been packaged and labeled for export or which have been exported, shall be subject to the applicable provisions in this paragraph.

(1) After leaving the licensed establishment, a biological product shall not be bottled, repackaged, relabeled, or otherwise altered in any way while in the United States; and

(2) An exported biological product shall not be returned to the United States: *Provided*, That, in the case of a biological product exported in labeled final containers, the Administrator may authorize by permit the importation of a limited number for research and evaluation by the producing licensee; and

(3) An exported biological product which is bottled, rebottled, or altered in any way in a foreign country shall not bear a label which indicates by establishment license number that it has been prepared in the United States.

(b) Desiccated and frozen liquid products, packaged and labeled as for domestic use, may be exported without the diluent required for rehydration or dilution, as the case may be, if the labeling includes adequate instructions for preparing the product for use and the words ''For Export Only''.

(c) Final containers of products, labeled or unlabeled, may be exported in sealed shipping boxes, adequately identified as to contents with an approved label, and plainly marked ''For Export Only'': *Provided*, That such products shall not be diverted to domestic use.

(d) Completed inactivated liquid products, antiserums, and antitoxins, may be exported in large multiple-dose containers identified with an approved label that contains the words ''For Export Only'' prominently displayed.

(e) Concentrated inactivated liquid product, completed except for dilution to the proper strength for use, may be exported in large multiple-dose containers identified with an approved label that contains the words ''For Export Only'' prominently displayed.

[38 FR 12094, May 9, 1973, as amended at 39 FR 19202, May 31, 1974; 40 FR 46093, Oct. 6, 1975; 43 FR 11145, Mar. 17, 1978; 56 FR 66784, Dec. 26, 1991]

§ 112.9 Biological products imported for research and evaluation.

A biological product imported for research and evaluation under a permit issued in accordance with § 104.4, with the exception of products imported under § 104.4(d), shall be labeled as provided in this section.

(a) The label shall identify the product and the name and address of the manufacturer and shall provide instructions for proper use of the product, including all warnings and cautions needed by the permittee to safely use the product.

(b) Labels on each product to be further distributed in accordance with §103.3 shall bear the statement ''Notice! For Experimental Use Only—Not for Sale!''

(c) The labeling shall contain any other information deemed necessary by the Administrator and specified on the permit.

[50 FR 46417, Nov. 8, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

§ 112.10 Special packaging and labeling.

A biological product, which requires special packaging and/or labeling not provided for in this part, shall be packaged and/or labeled in accordance with requirements written into the approved outline for such product.

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AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 34 FR 18004, Nov. 7, 1969, unless otherwise noted.

APPLICABILITY

§ 113.1 Compliance.

The regulations in this part apply to each serial or subserial of a licensed biological product manufactured in a licensed establishment and to each serial or subserial of a biological product in each shipment imported for distribution and sale.

§ 113.2 Testing aids.

To better ensure consistent and reproducible test results when Standard

Requirement tests prescribed in the regulations are conducted, National Veterinary Services Laboratories, U.S. Department of Agriculture, may provide testing aids, when available, to licensees, permittees, and applicants for licenses and permits. Such aids shall be as follows:

(a) Supplemental Assay Method (SAM) is a technical bulletin containing detailed instructions for conducting a test. Such instructions shall be in accordance with the procedures currently being followed at National Veterinary Services Laboratories and as improved, proven procedures are developed, shall be revised and reissued prior to application.

(b) Standard Reference Preparation is a serum, virus, bacterial culture, or antigen to be used in test systems for direct comparison with serials of biological products under test.

(c) Standard Test Reagent is a serum, antitoxin, fluorescent antibody conjugate, toxin, virus, bacterial cultural, or antigen to be used in test systems but not for direct comparison with serials of biological products under test.

(d) Seed cultures are small quantities of standard organisms to be propagated by the recipient to establish a supply for use.

(e) Test Code Number is a number assigned by Animal and Plant Health Inspection Service to each test procedure specified in the Standard Requirements and in each filed Outline of Production where such test is conducted to support a request for release of a serial or subserial.

[39 FR 21041, June 18, 1974, as amended at 40 FR 758, Jan. 3, 1975; 50 FR 21799, May 29, 1985; 56 FR 66784, Dec. 26, 1991]

§ 113.3 Sampling of biological products.

Each licensee and permittee shall furnish representative samples of each serial or subserial of a biological product manufactured in the United States or imported into the United States as prescribed in this section. Additional samples may be purchased in the open market by a Animal and Plant Health Inspection Service representative.

(a) Either an employee of the Department of Agriculture, of the licensee, or of the permittee, as designated by the Administrator shall select prerelease samples of biological product in the number prescribed in paragraph (b) of this section. Each sample shall be marked for identification by the person making the selection after which they shall be packaged by the licensee or permittee, as the case may be, and forwarded to National Veterinary Services Laboratories; except that an employee of the Department may forward or deliver the samples to National Veterinary Services Laboratories if such action deemed advisable by the Administrator.

(1) Selection shall be made as follows:

(i) Nonviable liquid biological products—either bulk or final container samples of completed product shall be selected for purity, safety, or potency tests. Biological product in final container shall be selected to test for viable bacteria and fungi.

(ii) Viable liquid biological products; samples shall be in final containers and shall be randomly selected at the end of the filling operation. Bulk containers of completed product may be sampled when authorized by the Administrator.

(iii) Desiccated biological products; samples shall be in final containers and shall be randomly selected if desiccated in the final container. Biological products desiccated in bulk shall be sampled at the end of the filling operation.

(iv) Representative samples of each serial or subserial in each shipment of imported biological products shall be selected.

(2) Comparable samples shall be used by Animal and Plant Health Inspection Service, the licensee, and the permittee for similar tests.

(3) When bulk samples of completed product in liquid form are to be tested as prescribed in paragraph (a)(1) of this section, the number of such samples from each serial and the minimum quantity of product to be provided in each sample shall be stated in the filed Outline of Production.

(b) Unless otherwise prescribed by the Administrator, the number of final container samples to be selected from each serial and subserial shall be:

(1) *Vaccines:*

(i) Six multiple-dose samples of Brucella Abortus Vaccine;

(ii) Twelve samples of all other live bacterial vaccines;

(iii) Two samples of Coccidiosis Vaccine;

(iv) Eighteen samples of Rabies Vaccine, Modified Live Virus;

(v) Sixteen samples of all other vaccines consisting of live microorganisms;

(vi) Thirty single-dose or 14 multiple-dose samples of Equine Encephalomyelitis Vaccine, Killed Virus;

(vii) Twenty-two single-dose or 14 multiple-dose samples of Rabies Vaccine, Killed Virus;

(viii) Sixteen single-dose or 12 multiple-dose samples of all other vaccines consisting of killed microorganisms.

(2) *Bacterins and bacterin-toxoids:*

(i) Twelve samples of single-fraction products;

(ii) Thirteen samples of two-fraction products;

(iii) Fourteen samples of products consisting of 3 or more fractions.

(3) *Antiserums:* Twelve samples of antiserum recommended for large animals or 14 samples of antiserum recommended for small animals or the number of reagent serum samples prescribed in the filed Outline of Production for the product.

(4) *Antitoxins:*

(i) Fourteen single-dose or 12 multiple dose samples of Tetanus Antitoxin;

(ii) Twelve samples of all other antitoxins.

(5) *Toxoids:*

(i) Eighteen single-dose or 12 multiple dose samples of all toxoids.

(6) *Antigens:* Twelve samples of poultry antigens or 20 samples of tuberculin or four samples of all other diagnostic antigens.

(7) *Diagnostic test kits:* Two samples of diagnostic test kits. The licensee or permittee will hold one of these selected samples at the storage temperature recommended on the label while awaiting a request by the animal and Plant Health Inspection Service to submit the additional sample. If submission is not requested by the Animal and Plant Health Inspection Service, the additional sample may be returned to the serial inventory after the serial is released. In the case of diagnostic test kits in which final packaging consists of multiple microtiter test plates or strips, the licensee or permittee may submit a specified number of test plates or strips along with all other test reagents as prescribed in a filed Outline of Production and retain a similar amount as a second sample for submission upon request. When the initial sample is not representative of final packaging by the licensee of permittee, e.g., does not consist of all the microtiter test plates or strips, the second sample is not eligible to be returned to serial inventory after the serial is released.

(8) *Autogenous biologics:* With the exception of the first serial or subserial, 10 samples must be selected and submitted to the Animal and Plant Health Inspection Service from each serial or subserial of an autogenous biologic eligible to be shipped that consists of more than 50 containers. For first serials or subserials eligible for shipment consisting of more than 50 containers, 10 samples from each serial or subserial must be selected and held for submission to the Animal and Plant Health Inspection Service upon request in accordance with paragraph (e)(4) of this section. For serials or subserials of autogenous biologic with 50 or fewer containers, no samples, other than those required by paragraph (e) of this section, are required.

(9) *Miscellaneous:* The number of samples from products not in the categories provided for in paragraphs (b)(1) through (b)(8) of this section shall be prescribed in the filed Outline of Production for the product.

(c) Prelicensing and Outline of Production changes: Samples needed to support a license application or a change in the Outline of Production for a licensed product shall be submitted only upon request from the animal and Plant Health Inspection Service. Except for miscellaneous products specified in paragraph (b)(9) of this section, the number of such samples shall be at least one and one-half times the number prescribed for such product in paragraph (b) of this section. Samples of Master Seeds and Master Cell Stocks

with a minimum individual volume of 1 ml shall be submitted as follows:

(1) Ten samples of Bacterial Master Seeds.

(2) Thirteen samples of viral Master Seeds or nonviral Master Seeds requiring cell culture propagation. For Master Seeds isolated or passed in a cell line different from the species of intended use, an additional 2 samples are required for each additional species. For Master Seeds grown in cell culture and intended for use in more than one species, an additional 2 samples are required for each additional species.

(3) Thirty-six samples of at least 1 ml each or six samples of at least 1 ml each, one sample of at least 20 ml, and one sample of at least 10 ml of Master Cell Stocks. In the case of Master Cell Stocks which are persistently infected with a virus, an additional four samples of at least 1 ml each are required. If these persistently infected cell stocks are intended for use in more than one species, an additional two samples of at least 1 ml each are required for each additional species.

(4) Four samples of the Master Cell Stock + n (highest passage) cells.

(d) Sterile diluent: A sample of Sterile Diluent shall accompany each sample of product, other than Marek's Disease Vaccine, if such diluent is required to rehydrate or dilute the product before use. The volume of diluent shall be an appropriate amount to rehydrate or dilute the product. Samples of Sterile Diluent prepared for use with Marek's Disease Vaccine shall be submitted upon request from the Animal and Plant Health Inspection Service.

(e) Reserve samples shall be selected from each serial and subserial of biological product. Such samples shall be selected at random from final containers of completed product by an employee of the Department, of the licensee, or of the permittee, as designated by the administrator. Each sample shall:

(1) Consist of 5 single-dose packages, 2 multiple-dose packages, or 2 diagnostic test kits, except that, in the case of diagnostic test kits in which final packaging consists of multiple microtiter test plates or strips, a sample may consist of a specified number of test plates or strips along with all other test reagents as prescribed in a filed Outline of Production;

(2) Be adequate in quantity for appropriate examination and testing;

(3) Be truly representative and in final containers;

(4) Be held in a special compartment set aside by the licensee or permittee for holding these samples under refrigeration at the storage temperature recommended on the labels for 6 months after the expiration date stated on the labels. The samples that are stored in this manner shall be delivered to the Animal and Plant Health Inspection Service upon request.

(Approved by the Office of Management and Budget under control number 0579–0013)

[38 FR 29886, Oct. 30, 1973, as amended at 40 FR 758, Jan. 3, 1975; 40 FR 49768, Oct. 24, 1975; 41 FR 56627, Dec. 29, 1976; 48 FR 9506, Mar. 7, 1983; 48 FR 57473, Dec. 30, 1983; 50 FR 21799, May 29, 1985; 56 FR 66784, Dec. 26, 1991; 60 FR 14356, Mar. 17, 1995; 67 FR 15713, Apr. 3, 2002]

§ 113.4 Exemptions to tests.

(a) The test methods and procedures contained in all applicable Standard Requirements shall be complied with unless otherwise exempted by the Administrator and provided that such exemption is noted in the filed Outline of Production for the product.

(b) Test methods and procedures by which the biological products shall be evaluated shall be designated in the Outline of Production for such products.

[38 FR 29887, Oct. 30, 1973, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.5 General testing.

(a) No biological product shall be released prior to the completion of tests prescribed in a filed Outline of Production or Standard Requirements for the product to establish the product to be pure, safe, potent, and efficacious.

(b) Tests of biological products shall be observed by a competent employee of the manufacturer during all critical periods. A critical period shall be the time when certain specified reactions must occur in required tests to properly evaluate the results.

(c) Records of all tests shall be kept in accordance with part 116 of this chapter. Results of all required tests

prescribed in the filed Outline of Production or the Standard Requirements for the product shall be submitted to Animal and Plant Health Inspection Service. Blank forms shall be furnished upon request to Animal and Plant Health Inspection Service.

(d) When the initial or any subsequent test is declared a ''No test,'' the reasons shall be reported in the test records, the results shall not be considered as final, and the test may be repeated.

(e) When new test methods are developed and approved by Animal and Plant Health Inspection Service, biological products tested thereafter shall be evaluated by such methods, and if not found to be satisfactory when so tested shall not be released.

(Approved by the Office of Management and Budget under control number 0579–0059)

[34 FR 18004, Nov. 4, 1969, as amended at 39 FR 25463, July 11, 1974; 40 FR 45420, Oct. 2, 1975; 40 FR 46093, Oct. 6, 1975; 41 FR 6751, Feb. 13, 1976; 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991]

§ 113.6 Animal and Plant Health Inspection Service testing.

A biological product shall with reasonable certainty yield the results intended when used as recommended or suggested in its labeling or proposed labeling prior to the expiration date.

(a) The Administrator is authorized to cause a biological product, manufactured in the United States or imported into the United States, to be examined and tested for purity, safety, potency, or efficacy; in which case, the licensee or permittee shall withhold such product from the market until a determination has been made.

(b) The final results of each test conducted by the licensee and Animal and Plant Health Inspection Service shall be considered in evaluating a biological product. A serial or subserial which has been found unsatisfactory by a required test prescribed in a filed Outline of Production or Standard Requirement is not in compliance with the regulations and shall not be released for market.

[34 FR 18004, Nov. 7, 1969, as amended at 40 FR 45420, Oct. 2, 1975; 40 FR 53378, Nov. 18, 1975; 41 FR 6751, Feb. 13, 1976; 56 FR 66784, Dec. 26, 1991]

§ 113.7 Multiple fractions.

(a) When a biological product contains more than one immunogenic fraction, the completed product shall be evaluated by tests applicable to each fraction.

(b) When similar potency tests are required for more than one fraction of a combination biological product, different animals must be used to evaluate each fraction except when written Standard Requirements or outlines of production make provisions and set forth conditions for use of the same animals for testing different fractions.

(c) When the same safety test is required for more than one fraction, requirements are fulfilled by satisfactory results from one test of the completed product.

(d) When an inactivated fraction(s) is used as a diluent for a live virus fraction(s), the inactivated fraction(s) may be tested separately and the live virus fraction(s) may be tested separately: *Provided,* That, the viricidal test requirements prescribed in § 113.100 are complied with.

(e) Virus titrations for a multivirus product shall be conducted by methods which will quantitate each virus.

[34 FR 18004, Nov. 7, 1969, as amended at 40 FR 46093, Oct. 6, 1975; 56 FR 66785, Dec. 26, 1991]

§ 113.8 In vitro tests for serial release.

(a) Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seed for production as specified in the Standard Requirements or in the filed Outline of Production. The Administrator may exempt a product from a required animal potency test for release when an evaluation can, with reasonable certainty, be made by:

(1) Subjecting the master seed to the applicable requirements prescribed in §§ 113.64, 113.100, 113.200, and 113.300;

(2) Testing the Master Seed for immunogenicity in a manner acceptable to the Animal and Plant Health Inspection Service (APHIS);

(3) Establishing satisfactory potency for the product in accordance with the following provisions:

(i) Potency for live products may be determined by $\log_{10}$ virus titer or determining the live bacterial count based on the protective dose used in the Master Seed immunogenicity test plus an adequate overage for adverse conditions and test error; and

(ii) Potency for inactivated products may be determined using tests for relative antigen content by comparing the antigen content of the test serial to a reference preparation using a parallel line immunoassay or equivalent method which measures linearity, specificity, and reproducibility in a manner acceptable to APHIS.

(b) In the case of live products, each serial and subserial of desiccated product derived from an approved Master Seed and bulk or final container samples of each serial of completed liquid product derived from an approved Master Seed shall be evaluated by a test procedure acceptable to APHIS. On the basis of the results of the test, as compared with the required minimum potency, each serial and subserial shall either be released to the firm for marketing or withheld from the market. The evaluation of such products shall be made in accordance with the following criteria:

(1) If the initial test shows the count or titer to equal or exceed the required minimum, the serial or subserial is satisfactory without additional testing.

(2) If the initial test shows the count or titer to be lower than the required minimum, the serial or subserial may be retested, using double the number of samples. The average counts or titers obtained in the retests shall be determined. If the average is less than the required minimum, the serial or subserial is unsatisfactory without further consideration.

(3) If the average is equal to or greater than the required minimum, the following shall apply to live virus vaccines:

(i) If the difference between the average titer obtained in the retests and the titer obtained in the initial test is $10^{0.7}$ or greater, the initial titer may be considered a result of test system error and the serial or subserial considered satisfactory for virus titer.

(ii) If the difference between the average titer obtained in the retests and the titer obtained in the initial test is less than $10^{0.7}$, a new average shall be determined using the titers obtained in all tests. If the new average is below the required minimum, the serial or subserial is unsatisfactory.

(4) If the average is equal to or greater than the required minimum, the following shall apply to bacterial vaccines:

(i) If the average count obtained in the retests is at least three times the count obtained in the initial test, the initial count may be considered a result of test system error and the serial or subserial considered satisfactory for bacterial count.

(ii) If the average count obtained in the retests is less than three times the count obtained in the initial test, a new average shall be determined using the counts obtained in all tests. If the new average count is below the required minimum, the serial or subserial is unsatisfactory.

(5) *Exceptions.* When a product is evaluated in terms other than $\log_{10}$ virus titer or organism count, an appropriate difference between the average potency value obtained in the retests and the potency value obtained in the initial test shall be established for use in paragraphs (b)(3) and (b)(4) of this section to evaluate such products and shall be specified in the product Standard Requirement or filed Outline of Production.

(c) In the case of inactivated products, bulk or final container samples of completed product from each serial derived from an approved Master Seed, shall be evaluated for relative antigen content (potency) as compared with an unexpired reference by a parallel line immunoassay or other procedure acceptable to APHIS.[1] Firms currently using immunoassays which do not satisfy this requirement shall have 2 years from the effective date of the final rule

[1] A method for evaluating relative antigen content, Supplemental Assay Method 318, and relative potency calculation software are available from the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, Center for Veterinary Biologics—Laboratory, 1800 Dayton Road, P. O. Box 844, Ames, Iowa 50010.

to update their filed Outlines of Production to be in compliance with this requirement unless granted an extension by the Administrator based on a showing by the firm seeking the extension that they have made a good faith effort with due diligence to achieve compliance. On the basis of the results of such test procedures, each serial that meets the required minimum potency shall be released to the firm for marketing; each serial not meeting the required minimum potency shall be withheld from the market. The evaluation of such products shall be made in accordance with the following criteria:

(1) A test that results in no valid lines is considered a ''no test'' and may be repeated.

(2) An initial test (test 1) that results in valid lines that are not parallel is considered a valid equivocal test. Release of the serial may not be based on such test since the result cannot be termed ''satisfactory'' or ''unsatisfactory.''

(3) If the initial test (test 1) shows that potency equals or exceeds the required minimum potency, the serial is satisfactory without additional testing.

(4) If the initial test (test 1) is an equivocal test due to lack of parallelism, the serial may be retested up to three times (tests 2, 3, and 4) with disposition to be as specified in paragraphs (c)(4)(i) and (ii) of this section; *Provided,* That, if the serial is not retested or the other provisions of this section are not satisfied, the serial shall be deemed unsatisfactory.

(i) If: The first retest (test 2) following an initial equivocal test; the second retest (test 3) following two consecutive equivocal tests (tests 1 and 2); or the third retest (test 4) following three consecutive equivocal tests (tests 1, 2, and 3) shows that the potency equals or exceeds the required minimum potency, the serial is satisfactory.

(ii) If the first retest (test 2) following an initial equivocal test shows that potency is less than the required minimum potency, disposition of the serial will be based on the outcome of retests 2 and 3 (tests 3 and 4) as follows: if either retest (test 3 or 4) shows that potency is less than the required minimum potency, the serial is unsatisfactory. If either retest 2 or retest 3 (tests 3 or 4) is an equivocal test, or in the event that each retest (tests 2, 3, and 4) following an initial equivocal test is also an equivocal test, the accumulated test results shall be considered indicative of a lack of potency and release of the serial withheld. In which case, the licensee may submit data confirming the continued validity of the test system to APHIS for review and approval. If the data are acceptable to APHIS, the potency test may be repeated by the firm, subject to the provisions specified in paragraphs (i) and (ii) and confirmatory testing by APHIS.

(5) If the initial test (test 1) shows that potency is less than the required minimum potency, the serial may be retested a minimum of two times (tests 2 and 3) but not more than three times (tests 2, 3, and 4) with disposition as specified in paragraphs (c)(5) (i) and (ii) of this section; *Provided,* That, if the serial is not retested or the other provisions of this section are not satisfied, the serial shall be deemed unsatisfactory.

(i) If two consecutive retests (tests 2 and 3) show that potency of the serial equals or exceeds the required minimum potency, the serial is satisfactory. If one of the two retests (test 2 or 3) shows that the potency is less than the required minimum potency, the serial is unsatisfactory.

(ii) If one of the retests (tests 2 or 3) shows that the potency equals or exceeds the required minimum potency and the other retest (test 2 or 3) is an equivocal test, a third retest (test 4) may be performed. If the third retest (test 4) shows that the potency of the serial equals or exceeds the required minimum potency, the serial is deemed satisfactory. If both retests (tests 2 and 3) or if the third retest (test 4) is an equivocal test, the accumulated test results shall be considered indicative of a lack of potency and release of the serial withheld, in which case the licensee may submit data confirming the continued validity of the test system to APHIS for review and approval. If the data are acceptable to APHIS, the potency test may be repeated by the firm, subject to the provisions specified

in paragraphs (c)(4) (i) and (ii) and (c)(5) (i) and (ii) of this section, and confirmatory testing by APHIS.

(d) *Repeat immunogenicity tests.* (1) The accuracy of the protective dose established for live products in the Master Seed immunogenicity test and defined as live virus titer or live bacterial count shall be confirmed in 3 years in a manner acceptable to APHIS, unless use of the lot of Master Seed previously tested is discontinued.

(2) All determinations of relative antigen content using parallel line immunoassays or equivalent methods shall be conducted with an unexpired reference. The lot of reference used to determine antigenic content shall have an initial dating period equal to the dating of the product or as supported by data acceptable to APHIS, except that frozen references may have an initial dating of up to 5 years, *Provided,* That the request for dating of the frozen references beyond the dating of the product is supported by preliminary data acceptable to APHIS and includes provisions for monitoring the stability of the reference to determine when the potency starts to decline and for taking the appropriate steps to requalify a reference with declining potency either by testing a Qualifying Serial in host animals or by providing other evidence of immunogenicity, e.g., antibody titers or laboratory animal test data previously correlated to host animal protection in a manner acceptable to APHIS. Prior to the expiration date, such reference may be granted an extension of dating, *Provided,* That its immunogenicity has been confirmed using a Qualifying Serial of product in a manner acceptable to APHIS. The dating period of the Master Reference and Working Reference may be extended by data acceptable to APHIS if the minimum potency of the Master Reference is determined to be adequately above the minimum level needed to provide protection in the host animal. If a new Master Reference is established, it shall be allowed an initial dating period equal to the dating of the product or as supported by data acceptable to APHIS, except that frozen references may have an initial dating period of 5 years, or as supported by data acceptable to APHIS. Prior to the expiration date, such reference may be granted an extension of dating by confirming its immunogenicity using a Qualifying Serial of product.

(e) Final container samples of completed product derived from Master Seed found immunogenic in accordance with paragraph (a) of this section and found satisfactory in accordance with paragraphs (b) and (c) of this section may also be subjected to an animal potency test by Animal and Plant Health Inspection Service as provided in this paragraph. Products shall be used according to label directions including dose(s) and route of administration.

(1) A one stage test using 20 vaccinates and 5 controls or a two stage test using 10 vaccinates and 5 controls for each stage shall be used. The criteria used for judging the specific response in the controls and vaccinates shall be in accordance with the test protocol used in the Master Seed immunogenicity test.

(2) If at least 80 percent of the controls do not show specific responses to challenge, the test is inconclusive and may be repeated. If a vaccinate shows the specific responses to challenge expected in the controls, the vaccinate shall be listed as a failure.

(3) The results of the testing shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of animals	Failures for satisfactory serials	Failures for unsatisfactory serials
1	10	1 or less	3 or more.
2 (or 1)	20	4 or less	5 or more.

(4) When a serial has been found unsatisfactory for potency by the test provided in paragraphs (e)(1), (2), and (3) of this section, the serial shall be withheld from the market and the following actions taken:

(i) The Administrator shall require that at least two additional serials prepared with the same Master Seed be subjected to similar animal potency tests by Animal and Plant Health Inspection Service or the licensee or both.

(ii) If another serial is found unsatisfactory for potency, the product shall be removed from the market while a

reevaluation of the product is made and the problem is resolved.

[49 FR 22625, May 31, 1984, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 62 FR 19038, Apr. 18, 1997]

§ 113.9 New potency test.

A potency test written into the filed Outline of Production for a product shall be considered confidential information by Animal and Plant Health Inspection Service until at least two additional product licenses are issued for the product or unless use of the test is authorized by the licensee, in which case, such potency test may be published as part of the Standard Requirement for the product.

(a) Until a potency test is published as part of the Standard Requirement for the product, reference to such a test shall be made in the filed Outline of Production and the test shall be conducted.

(b) When a potency test has been published as part of the Standard Requirement, such test shall be conducted unless the product is specifically exempted as provided in § 113.4.

[40 FR 14084, Mar. 28, 1975, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.10 Testing of bulk material for export or for further manufacture.

When a product is prepared in a licensed establishment for export in large multiple-dose containers as provided in § 112.8(d) or (e) of this subchapter or for further manufacturing purposes as provided in § 114.3(d) of this subchapter, samples of the bulk material shall be subjected to all required tests prescribed in the filed Outline of Production or Standard Requirements for the product. Samples of concentrated liquid product shall be diluted to a volume equal to the contents of the sample times the concentration factor prior to initiating potency tests.

[49 FR 45846, Nov. 21, 1984]

STANDARD PROCEDURES

§ 113.25 Culture media for detection of bacteria and fungi.

(a) Ingredients for which standards are prescribed in the United States Pharmacopeia, or elsewhere in this part, shall conform to such standards. In lieu of preparing the media from the individual ingredients, they may be made from dehydrated mixtures which, when rehydrated with purified water, have the same or equivalent composition as such media and have growth-promoting buffering, and oxygen tension-controlling properties equal to or better than such media. The formulas for the composition of the culture media prescribed in §§ 113.26 and 113.27 are set forth in the United States Pharmacopeia, 19th Edition.

(b) The licensee shall test each quantity of medium prepared at one time from individual ingredients and the first quantity prepared from each lot of commercial dehydrated medium for growth-promoting qualities. If any portion of a lot of commercial dehydrated medium is held for 90 days or longer after being so tested, it shall be retested before use. Two or more strains of micro-organisms that are exacting in their nutritive requirements shall be used. More than one dilution shall be used to demonstrate the adequacy of the medium to support the growth of a minimum number of micro-organisms.

(c) The sterility of the medium shall be confirmed by incubating an adequate number of test vessels and examining each for growth. Additional control may be used by incubation of representative uninoculated test vessels for the required incubation period during each test.

(d) A determination shall be made by the licensee for each biological product of the ratio of inoculum to medium which shall result in sufficient dilution of such product to prevent bacteriostatic and fungistatic activity. The determination may be made by tests on a representative biological product for each group of comparable products containing identical preservatives at equal or lower concentrations. Inhibitors or neutralizers of preservatives, approved by the Administrator, may be considered in determining the proper ratio.

[35 FR 16039, Oct. 13, 1970, as amended at 37 FR 2430, Feb. 1, 1972; 41 FR 27715, July 6, 1976; 56 FR 66784, Dec. 26, 1991]

§ 113.26 Detection of viable bacteria and fungi except in live vaccine.

Each serial and subserial of biological product except live vaccines shall be tested as prescribed in this section unless otherwise specified by the Administrator. When cell lines, primary cells, or ingredients of animal origin used in the preparation of a biological product are required to be free of viable bacteria and fungi, they shall also be tested as prescribed in this section.

(a) The media to be used shall be as follows:

(1) Fluid Thioglycollate Medium with 0.5 percent beef extract shall be used to test for bacteria in biological products containing clostridial toxoids, bacterins, and bacterin-toxoids.

(2) Fluid Thioglycollate Medium with or without 0.5 percent beef extract shall be used to test for bacteria in biological products other than clostridial toxoids, bacterins, and bacterin-toxoids.

(3) Soybean-Casein Digest Medium shall be used to test biological products for fungi; provided, that Fluid Thioglycollate Medium without beef extract shall be substituted when testing biological products containing mercurial preservatives.

(b) Test procedure:

(1) Ten test vessels shall be used for each of two media selected in accordance with paragraph (a)(1), (a)(2), or (a)(3) of this section. Each test vessel shall contain sufficient medium to negate the bacteriostatic or fungistatic activity in the inoculum as determined in § 113.25(d).

(2) Inoculum:

(i) When completed product is tested, 10 final container samples from each serial and each subserial shall be tested. One ml from each sample shall be inoculated into a corresponding individual test vessel of culture medium: *Provided,* That, if each final container sample contains less than 2 ml, one-half of the contents shall be used as inoculum for each test vessel.

(ii) When cell lines, primary cells, or ingredients of animal origin are tested, at least a 20 ml test sample from each lot shall be tested. One ml shall be inoculated into each test vessel of medium.

(3) Incubation shall be for an observation period of 14 days at 30 °to 35 °C. to test for bacteria and 14 days at 20 °to 25 °C. to test for fungi.

(4) If the inoculum renders the medium turbid so that the absence of growth cannot be determined by visual examination, subcultures shall be made on the seventh to eleventh day from biological products prepared from clostridial toxoids, bacterins, and bacterin-toxoids and the third to seventh day for other biological products. Portions of the turbid medium in amounts of not less than 1.0 ml. shall be transferred to 20 to 25 ml. of fresh medium, and incubated the balance of the 14-day period.

(c) Examine the contents of all test vessels for macroscopic microbial growth during the incubation period. When demonstrated by adequate controls to be invalid, the test may be repeated. For each set of test vessels representing a serial or subserial in a valid test, the following rules shall apply:

(1) If no growth is found in any test vessel, the serial or subserial meets the requirements of the test.

(2) If growth is found in any test vessel, one retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(3) If growth is found in any test vessel of the final test, the serial, subserial, or ingredients to be used in the preparation of a biological product, as the case may be, is unsatisfactory.

[35 FR 16039, Oct. 13, 1970, as amended at 37 FR 2430, Feb. 1, 1972; 39 FR 21042, June 18, 1974; 40 FR 758, Jan. 3, 1975; 40 FR 14084, Mar. 28, 1975; 56 FR 66784, Dec. 26, 1991]

§ 113.27 Detection of extraneous viable bacteria and fungi in live vaccines.

Unless otherwise specified by the Administrator or elsewhere exempted in this part, each serial and subserial of live vaccine and each lot of Master Seed Virus and Master Seed Bacteria shall be tested for extraneous viable bacteria and fungi as prescribed in this section. A Master Seed found unsatisfactory shall not be used in vaccine production and a serial found unsatisfactory shall not be released.

(a) *Live viral vaccines.* Each serial and subserial of live viral vaccine shall be

tested for purity as prescribed in this paragraph. However, products of chicken embryo origin recommended for administration other than by parenteral injection may be tested as provided in paragraph (e) of this section.

(1) Soybean Casein Digest Medium shall be used.

(2) Ten final container samples from each serial and subserial shall be tested.

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and desiccated vaccine shall be rehydrated as recommended on the label with accompanying diluent or with sterile purified water.

(4) To test for bacteria, place 0.2 ml of vaccine from each final container into a corresponding individual vessel containing at least 120 ml of Soybean Casein Digest Medium. Additional medium shall be used if the determination required in §113.25(d) indicates the need for a greater dilution of the product. Incubation shall be at 30 °to 35 °C for 14 days.

(5) To test for fungi, place 0.2 ml of vaccine from each final container sample into a corresponding individual vessel containing at least 40 ml of Soybean Casein Digest Medium. Additional medium shall be used if the determination required in §113.25(d) indicates the need for a greater dilution of the product. Incubation shall be at 20 °to 25 °C for 14 days.

(6) Examine the contents of all test vessels macroscopically for microbial growth at the end of the incubation period. If growth in a vessel cannot be reliably determined by visual examination, judgment shall be confirmed by subcultures, microscopic examination, or both.

(7) For each set of test vessels representing a serial or subserial tested according to these procedures, the following rules shall apply:

(i) If growth is found in 2 or 3 test vessels of the initial test, 1 retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(ii) If no growth is found in 9 or 10 of the test vessels in the initial test, or 19 or 20 vessels in the retest, the serial or subserial meets the requirements of the test.

(iii) If growth is found in four or more test vessels in the initial test, or two or more in a retest, the serial or subserial is unsatisfactory.

(b) *Live bacterial vaccines.* Each serial or subserial of live bacterial vaccine shall be tested for purity as prescribed in this paragraph.

(1) Soybean Casein Digest Medium and Fluid Thioglycollate Medium shall be used.

(2) Ten final container samples from each serial and subserial shall be tested.

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and desiccated vaccine shall be rehydrated as recommended on the label with accompanying diluent or with sterile purified water. Product recommended for mass vaccination shall be rehydrated at the rate of 30 ml sterile purified water per 1,000 doses.

(4) To test for extraneous bacteria, place 0.2 ml of vaccine from each final container into a corresponding individual vessel containing at least 40 ml of Fluid Thioglycollate Medium. Additional medium shall be used if the determination required in §113.25(d) indicates the need for a greater dilution of the product. Incubation shall be at 30 °to 35 °C for 14 days.

(5) To test for extraneous fungi, place 0.2 ml of vaccine from each final container into a corresponding individual vessel containing at least 40 ml of Soybean Casein Digest Medium. Additional medium shall be used if the determination required in §113.25(d) indicates the need for a greater dilution of the product. Incubation shall be at 20 °to 25 °C for 14 days.

(6) Examine the contents of all test vessels macroscopically for atypical microbial growth at the end of the incubation period. If growth of extraneous microorganisms cannot be reliably determined by visual examination, judgment shall be confirmed by subculturing, microscopic examination, or both.

(7) For each set of test vessels representing a serial or subserial tested according to these procedures, the following rules shall apply:

(i) If extraneous growth is found in 2 or 3 test vessels of the initial test, 1 retest to rule out faulty technique may

be conducted using 20 unopened final container samples.

(ii) If no extraneous growth is found in 9 or 10 test vessels in the initial test, or 19 or 20 vessels in the retest, the serial or subserial meets the requirements of the test.

(iii) If extraneous growth is found in 4 or more test vessels in the initial test, or 2 or more in a retest, the serial or subserial is unsatisfactory.

(c) *Master Seed Virus.* Not less than 4 ml of each lot of Master Seed Virus shall be tested. Frozen liquid Master Seed Virus shall be thawed, and desiccated Master Seed Virus shall be rehydrated with Soybean Casein Digest Medium immediately prior to starting the test.

(1) To test for bacteria, place 0.2 ml of the sample of Master Seed Virus into 10 individual vessels each containing at least 120 ml of Soybean Casein Digest Medium. Incubation shall be at 30 °to 35 °C for 14 days.

(2) To test for fungi, place 0.2 ml of the sample of Master Seed Virus into 10 individual vessels each containing at least 40 ml of Soybean Casein Digest Medium. Incubation shall be at 20 °to 25 °C for 14 days.

(3) Examine the contents of all test vessels macroscopically for microbial growth at the end of the incubation period. If growth in a vessel cannot be reliably determined by visual examination, judgment shall be confirmed by subcultures, microscopic examination, or both.

(4) For each set of test vessels representing a lot of Master Seed Virus tested according to these procedures, the following rules shall apply:

(i) If growth is found in any test vessel of the initial test, one retest to rule out faulty technique may be conducted using a new sample of Master Seed Virus.

(ii) If growth is found in any test vessel of the final test, the lot of Master Seed Virus is unsatisfactory.

(d) *Master Seed Bacteria.* Not less than 4 ml of each lot of Master Seed Bacteria shall be tested. Frozen liquid Master Seed Bacteria shall be thawed, and desiccated Master Seed Bacteria shall be rehydrated with sterile purified water immediately prior to starting the test.

(1) To test for extraneous bacteria, place 0.2 ml of the sample of Master Seed Bacteria into 10 individual vessels each containing at least 40 ml of Fluid Thioglycollate Medium. Incubation shall be at 30 °to 35 °C for 14 days.

(2) To test for extraneous fungi, place 0.2 ml of the sample of Master Seed Bacteria into 10 individual vessels each containing at least 40 ml of Soybean Casein Digest Medium. Incubation shall be at 20 °to 25 °C for 14 days.

(3) Examine the contents of all test vessels macroscopically for atypical microbial growth at the end of the incubation period. If growth of extraneous microorganisms cannot be reliably determined by visual examination, judgment shall be confirmed by subcultures, microscopic examination, or both.

(4) For each set of test vessels representing a lot of Master Seed Bacteria tested according to these procedures, the following rules shall apply:

(i) If extraneous growth is found in any test vessel of the initial test, one retest to rule out faulty technique may be conducted using a new sample of Master Seed Bacteria.

(ii) If extraneous growth is found in any test vessel of the final test, the lot of Master Seed Bacteria is unsatisfactory.

(e) Live viral vaccines of chicken embryo origin recommended for administration other than by parenteral injection, which were not tested or have not been found free of bacteria and fungi by the procedures prescribed in paragraph (a) of this section, may be tested according to the procedures prescribed in this paragraph.

(1) Brain Heart Infusion Agar shall be used with 500 Kinetic (Kersey) units of penicillinase per ml of medium added just prior to pouring the plates.

(2) Ten final containers from each serial and each subserial shall be tested.

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and lyophilized vaccine shall be rehydrated to the quantity recommended on the label using the accompanying sterile diluent or sterile purified water. Product recommended for mass vaccination shall be rehydrated at the rate of 30 ml sterile purified water per 1,000 doses.

(4) From each container sample, each of 2 plates shall be inoculated with vaccine equal to 10 doses if the vaccine is recommended for poultry or 1 dose if the vaccine is recommended for other animals. Twenty ml of medium shall be added to each plate. One plate shall be incubated at 30 °to 35 °for 7 days and the other plate shall be incubated at 20 °to 25 °C for 14 days.

(5) Colony counts shall be made for each plate at the end of the incubation period. An average colony count for the 10 samples representing the serial or subserial shall be made for each incubation condition.

(6) For each set of test vessels representing a serial or subserial tested according to these procedures, the following rules shall apply:

(i) If the average count at either incubation condition exceeds 1 colony per dose for vaccines recommended for poultry, or 10 colonies per dose for vaccines recommended for other animals in the initial test, 1 retest to rule out faulty technique may be conducted using 20 unopened final containers.

(ii) If the average count at either incubation condition of the final test for a serial or subserial exceeds 1 colony per dose for vaccines recommended for poultry, or 10 colonies per dose for vaccines recommended for other animals, the serial or subserial is unsatisfactory.

[48 FR 28430, June 22, 1983, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.28 Detection of mycoplasma contamination.

The heart infusion test, using heart infusion broth and heart infusion agar, provided in this section shall be conducted when a test for mycoplasma contamination is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) Media additives provided in this paragraph shall be prepared as follows:

(1) DPN-Cysteine Solution:

(i) Use Nicotinamide adenine dinucleotide (oxidized) and L-Cysteine hydrochloride.

(ii) Prepare 1 gram/100 milliliters (ml) purified water (1 percent solution) of each. Mix the solutions together; the cysteine reduces the DPN. Filter sterilize, dispense in appropriate amounts and store frozen at −20 degrees centigrade.

(2) Inactivated horse serum—horse serum which has been inactivated at 56 °C for 30 minutes.

(b) Heart infusion broth shall be prepared as provided in this paragraph.

(1) Dissolve in 970 ml of purified water, 25 grams of heart infusion broth, 10 grams of proteose peptone No. 3, and either 5 grams of yeast autolysate or 5 ml of fresh yeast extract.

(2) Add the following:

1 percent tetrazolium chloride (ml)	5.5
1 percent thallium acetate (ml)	25
Penicillin (units)	500,000
Inactivated horse serum (ml)	100

(3) Adjust pH to 7.9 with NaOH, filter sterilize, and dispense 100 ml aliquots into 125 ml flasks and store until needed.

(4) Add 2 ml of DPN-Cysteine solution to each 100 ml of broth on day of use.

(c) Heart Infusion Agar shall be prepared as provided in this paragraph.

(1) Dissolve in 900 ml of purified water by boiling the following:

Heart infusion agar (g)	25
Heart–infusion broth (g)	10
Proteose peptone No. 3 (g)	10
1 pct thallium acetate (ml)	25

(2) Cool the medium and adjust pH to 7.9 with NaOH.

(3) Autoclave the medium.

(4) Cool the medium 30 minutes in a 56 °C waterbath.

(5) Dissolve 5 grams of yeast autolysate in 100 ml of distilled water, filter sterilize, and add to the medium.

(6) Add to the medium:

126 ml of inactivated horse serum
21 ml of DPN-Cysteine solution
525,000 units of Penicillin.
Dispense 10 ml aliquots into 60×15 mm disposable culture dishes or petri dishes.

(d) The test procedure provided in this paragraph shall be followed when conducting the mycoplasma detection test.

(1) Preparation of inoculum. Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and lyophilized vaccine shall be rehydrated to the volume recommended on the label with mycoplasma medium. In the case of a lyophilized biological product, e.g., 1,000 dose vial of poultry vaccine to be administered via the drinking

water, the vaccine shall be rehydrated to 30 ml with mycoplasma medium. In the case of a cell line or a sample of primary cells, the inoculum shall consist of the resuspended cells. Control tests shall be established as provided in paragraph (d)(4) of this section.

(2) Inoculation of plate. Plate 0.1 ml of inoculum on an agar plate and make a short, continuous streak across the plate with a pipet. Tilt the plate to allow the inoculum to flow over the surface.

(3) Inoculation of flask of medium. Transfer 1 ml of the inoculum into a flask containing 100 ml mycoplasma medium and mix thoroughly. Incubate the flask at 33 to 37 °C for 14 days during which time, one of four agar plates shall be streaked with 0.1 ml of material from the incubating flask of inoculated medium on the 3d day, one on the 7th day, one on the 10th day, and one on the 14th day post-inoculation.

(4) Control tests shall be conducted simultaneously with the detection test using techniques provided in paragraphs (d)(2) and (3) of this section, except the inoculum for the positive control test shall be selected mycoplasma cultures and the negative control test shall be uninoculated medium from the same lot used in the detection test.

(5) All plates shall be incubated in a high humidity, 4–6 percent CO_2 atmosphere at 33 °to 37 °C for 10–14 days and examined with a stereoscopic microscope at 35x to 100x or with a regular microscope at 100x.

(e) Interpretation of test results.

(1) If growth appears on at least one of the plates in the positive control test and does not appear on any of the plates in the negative control test, the test is valid.

(2) If mycoplasma colonies are found on any of the plates inoculated with material being tested, the results are positive for mycoplasma contamination.

[38 FR 29887, Oct. 30, 1973, as amended at 41 FR 6752, Feb. 13, 1976; 41 FR 32882, Aug. 6, 1976]

§ 113.29 Determination of moisture content in desiccated biological products.

Methods provided in this section must be used when a determination of moisture content in desiccated biological products is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product. Firms currently using methods other than those provided in this section for determining the moisture content in desiccated biological products have until November 5, 2004 to update their Outlines of Production to be in compliance with this requirement.

(a) Final container samples of completed product shall be tested. The weight loss of the sample due to drying in a vacuum oven shall be determined. All procedures should be performed in an environment with a relative humidity less than 45 percent. The equipment necessary to perform the test is as follows:

(1) Cylindrical weighing bottles with airtight glass stoppers.

(2) Vacuum oven equipped with validated thermometer and thermostat. A suitable air-drying device should be attached to the inlet valve.

(3) Balance, accurate to 0.1 mg (rated precision ±0.01mg).

(4) Desiccator jar equipped with phosphorous pentoxide, silica gel, or equivalent.

(5) Desiccated vaccine in original sealed vial. Sample and control should be kept at room temperature in their original airtight containers until use.

(b) Test procedure:

(1) Thoroughly cleaned and labeled sample-weighing bottles with stoppers should be allowed to dry at 60 ±3 °C under vacuum at less than 2.5 kPa.

(i) Transfer hot bottles and stoppers into the desiccator and allow to cool to room temperature.

(ii) After bottles have cooled, insert stoppers and weigh and record the weights of the bottles as ''A.''

(iii) Return weighing bottles to the desiccator.

(2) Remove the sample container seal.

(i) Using a spatula, break up the sample plug and transfer the required amount of sample to the previously tared weighing bottle.

(ii) Insert the stopper and weigh and record the weights of the weighing bottles as ''B.''

(3) Place the weighing bottle with the stopper at an angle in the vacuum

oven. Set the vacuum to < 2.5 kPa and the temperature to 60 ±3 °C.

(4) After a minimum of 3 hours of drying time, turn off the vacuum pump and allow dry air to bleed into the oven until the pressure inside the oven is equalized with the prevailing atmospheric pressure.

(5) While the bottle is still warm, replace the stopper in its normal position and transfer the weighing bottle to the desiccator.

(i) Allow a minimum of 2 hours for the weighing bottle to cool to room temperature or for its weight to reach equilibrium.

(ii) Weigh, and record the weight as ''C.''

(6) Calculate the percentage of moisture in the original sample as follows:

$(B-C)/(B-A) \times (100)$ = Percentage of residual moisture, where:

A = tare weight of weighing bottle

B − A = weight of sample before drying

B − C = weight of sample after drying

(7) The results are considered satisfactory if the percentage of residual moisture is less than or equal to the manufacturer's specification.

[68 FR 57608, Oct. 6, 2003]

§ 113.30 Detection of Salmonella contamination.

The test for detection of Salmonella contamination provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) Samples shall be collected from the bulk suspension before bacteriostatic or bactericidal agents have been added. When tissue culture products are to be tested, 1 ml of tissue extract used as the source of cells or 1 ml of the minced tissue per se shall be tested.

(b) Five ml of the liquid vaccine suspension shall be used to inoculate each 100 ml of liquid broth medium (tryptose and either selenite F or tetrathionate). The inoculated media shall be incubated 18–24 hours at 35–37 °C.

(c) Transfers shall be made to either MacConkey agar or Salmonella-Shigella agar, incubated for 18–24 hours and examined.

(d) If no growth typical of Salmonella is noted, the plates shall be incubated an additional 18–24 hours and again examined.

(e) If suspicious colonies are observed, further subculture on suitable media shall be made for positive identification. If Salmonella is found, the bulk suspension is unsatisfactory.

[38 FR 29888, Oct. 30, 1973]

§ 113.31 Detection of avian lymphoid leukosis.

The complement-fixation test for detection of avian lymphoid leukosis provided in this section shall be conducted on all biological products containing virus which has been propagated in substrates of chicken origin: *Provided,* An inactivated viral product shall be exempt from this requirement if the licensee can demonstrate to Animal and Plant Health Inspection Service that the agent used to inactivate the vaccine virus would also inactivate lymphoid leukosis virus.

(a) Propagation of contaminating lymphoid leukosis viruses, if present, shall be done in chick embryo cell cultures.

(1) Each vaccine virus, cytopathic to chick embryo fibroblast cells, shall be effectively neutralized, inactivated, or separated so that minimal amounts of lymphoid leukosis virus can be propagated on cell culture during the 21-day growth period. If a vaccine virus cannot be effectively neutralized, inactivated, or separated, a sample of another vaccine prepared the same week from material harvested from each source flock (or other sampling procedure acceptable to Animal and Plant Health Inspection Service) used for the preparation of the questionable vaccine virus that cannot be neutralized, inactivated, or separated shall be tested each week during the preparation of such questionable vaccine.

(2) When cell cultures are tested, 5 ml of the final cell suspension as prepared for seeding of production cell cultures shall be used as inoculum. When vaccines are tested, the equivalent of 200 doses of Newcastle disease vaccine or 500 doses of other vaccines for use in poultry, or one dose of vaccine for use in other animals shall be used as inoculum. Control cultures shall be

prepared from the same cell suspension as the cultures for testing the vaccine.

(3) Uninoculated chick embryo fibroblast cell cultures shall act as negative controls. One set of chick fibroblast cultures inoculated with subgroup A virus and another set inoculated with subgroup B virus shall act as positive controls, A and B respectively.

(4) The cell cultures shall be propagated at 35–37 °C for at least 21 days. They shall be passed when necessary to maintain viability and samples harvested from each passage shall be tested for group specific antigen.

(b) The microtiter complement-fixation test shall be performed using either the 50 percent or the 100 percent hemolytic end point technique to determine complement unitage. Five 50 percent hemolytic units or two 100 percent hemolytic units of complement shall be used for each test.

(1) All test materials, including positive and negative controls, shall be stored at −60 °C or colder until used in the test. Before use, each sample shall be thawed and frozen three times to disrupt intact cells and release the group specific antigen.

(2) The antiserum used in the microtiter complement-fixation test shall be a standard reagent supplied or approved by the Animal and Plant Health Inspection Service. Four units of antiserum shall be used for each test.

(3) Presence of complement-fixing activity in the harvested samples (from passages) at the 1:4 or higher dilution, in the absence of anticomplementary activity, shall be considered a positive test unless the activity can definitely be established to be caused by something other than lymphoid leukosis virus, subgroups A and/or B. Activity at the 1:2 dilution shall be considered suspicious and the sample further subcultured to determine presence or absence of the group specific antigen.

(4) Biological products or primary cells which are found contaminated with lymphoid leukosis viruses are unsatisfactory. Source flocks from which contaminated material was obtained are also unsatisfactory.

[38 FR 29888, Oct. 30, 1973, as amended at 38 FR 32917, Nov. 29, 1973; 39 FR 21042, June 18, 1974; 56 FR 66784, Dec. 26, 1991]

§ 113.32 Detection of Brucella contamination.

The test for detection of Brucella contamination provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in a filed Outline of Production for the product.

(a) One ml of the minced tissue used as the source of cells or 1 ml of the extract of the tissue prior to the addition of antibiotics, diluent and stabilizer, shall be inoculated onto each of three tryptose agar plates and incubated in a 10 percent CO_2 atmosphere at a temperature of 35–37 °C for at least 7 days.

(b) If colonies are identified as Brucella, the biological product is unsatisfactory.

(c) If colonies suspicious of Brucella are observed but cannot be identified as a Brucella species, either

(1) The biological product shall be regarded as unsatisfactory and destroyed; or

(2) Further subculture or other procedures shall be carried out until a positive identification can be made.

[38 FR 29888, Oct. 30, 1973]

§ 113.33 Mouse safety tests.

One of the mouse safety tests provided in this section shall be conducted when such test is prescribed in a Standard Requirement or in the filed Outline of Production for a biological product recommended for animals other than poultry: *Provided,* That if the inherent nature of one or more ingredients makes the biological product lethal or toxic for mice but not lethal or toxic for the animals for which it is recommended, the licensee shall demonstrate the safety of such product by an acceptable test written into such Outline of Production.

(a) Final container samples of completed product from live virus vaccines shall be tested for safety using young adult mice in accordance with the test provided in this paragraph.

(1) Vaccine, prepared for use as recommended on the label, shall be tested. Eight mice shall be inoculated intracerebrally with 0.03 ml and eight mice shall be inoculated intraperitoneally with 0.5 ml. Both groups shall be observed for 7 days.

(2) If unfavorable reactions attributable to the product occur in two or more mice in either group during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur in two or more mice in either group, the test shall be declared inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

(b) Bulk or final container samples of completed product from liquid products, such as but not limited to antiserums and bacterins, shall be tested for safety in accordance with the test provided in this paragraph.

(1) Unless otherwise prescribed in the Standard Requirement or approved in a filed Outline of Production for the product, a 0.5 ml dose shall be injected intraperitoneally or subcutaneously into eight mice and the animals observed for 7 days.

(2) If unfavorable reactions attributable to the product occur in any of the mice during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

[38 FR 34727, Dec. 18, 1973, as amended at 39 FR 16857, May 10, 1974]

§ 113.34 Detection of hemagglutinating viruses.

The test for detection of hemagglutinating viruses provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) Final container samples of completed product rehydrated as recommended on the label shall be used as inoculum: *Provided,* That poultry vaccines distributed without diluent shall be rehydrated with 30 ml of sterile distilled water per 1,000 doses and used as inoculum. When one or more fractions are to be used in combination with Newcastle Disease Vaccine, test samples shall be collected from bulk suspensions of each prior to mixing with the Newcastle Disease Vaccine.

(b) Each of ten 9- to 10-day-old embryonating eggs from Newcastle disease susceptible flocks shall be inoculated into the allantoic cavity with 0.2 ml of the undiluted inoculum.

(1) Test five uninoculated embryos of the same age and from the same flock as those used for the test as negative controls.

(2) Test an allantoic fluid sample of Newcastle disease virus as a positive control.

(c) Three to five days post-inoculation, a sample of allantoic fluid from each egg shall be tested separately by a rapid plate test for hemagglutinating activity using a 0.5 percent suspension of fresh chicken red blood cells.

(d) If the results are inconclusive, one or two blind passages shall be made using fluids from each of the original test eggs. Fluids from dead and live embryos may be pooled separately for inoculum in these passages.

(e) If hemagglutinating activity attributable to the product is observed, the serial is unsatisfactory.

[38 FR 29889, Oct. 30, 1973]

§ 113.35 Detection of viricidal activity.

The test for detection of viricidal activity provided in this section shall be conducted when such a test is prescribed in an applicable standard requirement or in the filed Outline of Production for each inactivated liquid biological product used as diluent for a desiccated live virus vaccine in a combination package.

(a) Bulk or final container samples of completed product from each serial shall be tested.

(b) The product shall be tested with each virus fraction for which it is to be used as a diluent. If the vaccine to be rehydrated contains more than one virus fraction, the test shall be conducted with each fraction after neutralization of the other fraction(s), and/or dilution of the vaccine beyond the titer range of the other fraction(s), or the test shall be conducted using representative single-fraction desiccated

vaccines which are prepared by the licensee and which are licensed. *Provided*, That the Administrator may authorize licensees to prepare and use unlicensed single-fraction vaccines for this purpose.

(c) Test procedure: (1) Rehydrate at least two vials of the vaccine with the liquid product under test according to label recommendations and pool the contents.

(2) Rehydrate at least two vials of the vaccine with the same volume of sterile purified water and pool the contents.

(3) Neutralize to remove other fractions, if necessary.

(4) Hold the two pools of vaccine at room temperature (20 °to 25 °C) for 2 hours. The holding period shall begin when rehydration is completed.

(5) Titrate the virus(es) in each pool of vaccine as provided in the filed Outline of Production or an applicable standard requirement.

(6) Compare respective titers.

(d) If the titer of the vaccine virus(es) rehydrated with the product under test is more than 0.7 $\log_{10}$ below the titer of the vaccine virus(es) rehydrated with sterile purified water, the product is unsatisfactory for use as diluent.

(e) If the product is unsatisfactory in the first test, one retest to rule out faulty techniques may be conducted using four vials of the vaccine for each pool and the acceptability of the product judged by the results of the second test.

(f) Liquid products found to be unsatisfactory for use as diluent by this test are not prohibited from release as separate licensed products if labeled as prescribed in §112.7(g).

[44 FR 25412, May 1, 1979, as amended at 56 FR 66784, Dec. 26, 1991; 64 FR 43044, Aug. 9, 1999]

§ 113.36 Detection of pathogens by the chicken inoculation test.

The test for detection of extraneous pathogens provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) The biological product to be tested shall be prepared for use as recommended on the label, or in the case of desiccated vaccine to be used in poultry, rehydrated with sterile distilled water at the rate of 30 ml per 1,000 doses.

(b) At least 25 healthy susceptible young chickens, properly identified and obtained from the same source and hatch, shall be immunized at least 14 days prior to being put on test. The immunizing agent shall be the same as the product to be tested but from a serial previously tested and found satisfactory.

(c) At least 20 of the previously immunized birds shall be inoculated with 10 label doses of the vaccine being tested by each of the following routes: Subcutaneous, intratracheal, eye-drop, and comb scarification (1 cm^2). Twenty birds may be used for each route or combination of routes.

(d) At least five birds shall be isolated as control birds.

(e) All birds shall be observed for 21 days for signs of septicemic diseases, respiratory diseases, or other pathologic conditions.

(f) If the controls remain healthy and unfavorable reactions attributable to the product occur in the vaccinates, the serial or subserial tested is unsatisfactory. If the controls do not remain healthy or if unfavorable reactions not attributable to the product occur in the vaccinates, or both, the test shall be declared inconclusive and may be repeated: *Provided*, That, if the test is not repeated, the serial of subserial tested shall be considered unsatisfactory.

[38 FR 29889, Oct. 30, 1973, as amended at 39 FR 21042, June 18, 1974; 43 FR 7610, Feb. 24, 1978]

§ 113.37 Detection of pathogens by the chicken embryo inoculation test.

The test for detection of extraneous pathogens provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) The biological product to be tested shall be prepared for use as recommended on the label, or in the case of desiccated vaccine to be used in poultry, rehydrated with sterile distilled water at the rate of 30 ml per 1,000 doses.

(b) One volume of the prepared vaccine shall be mixed with up to nine volumes of sterile heat-inactivated specific antiserum to neutralize the vaccine virus in the product. Each lot of antiserum shall be demonstrated by virus neutralization tests not to inhibit other viruses known to be possible contaminants.

(c) After neutralization, 0.2 ml of the vaccine-serum mixture shall be inoculated into each of at least 20 fully susceptible chicken embryos.

(1) Twenty embryos, 9 to 11 days old, shall be inoculated on the chorioallantoic membrane (CAM) with 0.1 ml, and in the allantoic sac with 0.1 ml.

(2) Eggs shall be candled daily for 7 days. Deaths occurring during the first 24 hours shall be disregarded but at least 18 viable embryos shall survive 24 hours post-inoculation for a valid test. Examine all embryos and CAM's from embryos which die after the first day. When necessary, embryo subcultures shall be made to determine the cause of a death. The test shall be concluded on the seventh day post-inoculation and the surviving embryos (including CAM's) examined.

(d) If death and/or abnormality attributable to the inoculum occur, the serial is unsatisfactory: *Provided*, That, if there is a vaccine virus override, the test may be repeated, using a higher titered antiserum.

[38 FR 29889, Oct. 30, 1973, as amended at 39 FR 21042, June 18, 1974]

§ 113.38 Guinea pig safety test.

The guinea pig safety test provided in this section shall be conducted when prescribed in a Standard Requirement or approved Outline of Production for a biological product. When desiccated products are tested, final container samples of completed product prepared for administration in the manner recommended on the label shall be used. When liquid products are tested, either bulk or final container samples of completed product shall be used.

(a) Unless otherwise specified in the Standard Requirement or approved Outline of Production for the product, a 2 ml dose shall be injected either intramuscularly or subcutaneously into each of two guinea pigs and the animals observed for 7 days.

(b) If unfavorable reactions attributable to the product occur in either of the guinea pigs during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

[39 FR 16857, May 10, 1974; 39 FR 20368, June 10, 1974]

§ 113.39 Cat safety tests.

The safety tests provided in this section shall be conducted when prescribed in a standard requirement or in the filed Outline of Production for a biological product recommended for use in cats.

(a) The cat safety test provided in this paragraph shall be used when the Master Seed Virus is tested for safety.

(1) The test animals shall be determined to be susceptible to the virus under test as follows:

(i) Throat swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of the virus. Blood samples shall also be drawn and individual serum samples tested for antibody to the virus.

(ii) The cats shall be considered susceptible if swabs are negative for virus isolation and the serums are free of virus antibody at the 1:2 final dilution in a 50 percent plaque reduction test or other serum-neutralization test of equal sensitivity.

(iii) When determining susceptibility to a virus which does not lend itself to the methods in paragraphs (a)(1)(i) and (ii) of this section, a method acceptable to Animal and Plant Health Inspection Service shall be used.

(2) Each of at least 10 susceptible cats shall be administered a sample of the Master Seed Virus equivalent to the amount of virus to be used in one cat dose of the vaccine, by the method to be recommended on the label, and the cats observed each day for 14 days.

(3) If unfavorable reactions attributable to the virus occur in any of the cats during the observation period, the Master Seed Virus is unsatisfactory. If unfavorable reactions occur which are

not attributable to the Master Seed Virus, the test shall be declared inconclusive and repeated: *Provided,* That, if not repeated, the Master Seed Virus shall be unsatisfactory.

(b) The cat safety test provided in this paragraph shall be used when a serial of vaccine is tested for safety before release.

(1) Each of two healthy cats shall be administered 10 cat doses by the method recommended on the label and the cats observed each day for 14 days.

(2) If unfavorable reactions attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated: *Provided,* That, if not repeated, the serial shall be unsatisfactory.

[44 FR 58898, Oct. 12, 1979, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.40 Dog safety tests.

The safety tests provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a biological product recommended for use in dogs. Serials which are not found to be satisfactory when tested pursuant to the procedures in this section may not be released for shipment.

(a) The dog safety test provided in this paragraph shall be used when the Master Seed Virus is tested for safety.

(1) The test animals shall be determined to be susceptible to the virus under test by a method acceptable to the Animal and Plant Health Inspection Service.

(2) Each of at least 10 susceptible dogs shall be administered a sample of the Master Seed Virus equivalent to the amount of virus to be used in one dog dose of the vaccine, by the method recommended on the label, and the dog shall be observed each day for 14 days.

(3) If unfavorable reactions attributable to the virus occur in any of the dogs during the observation period, the Master Seed Virus is unsatisfactory. If unfavorable reactions occur which are not attributable to the Master Seed Virus, the test shall be declared inconclusive and may be repeated: *Provided:* That, if the test is not repeated, the Master Seed Virus shall be considered unsatisfactory.

(b) The dog safety test provided in this paragraph shall be used when a serial of vaccine is tested for safety before release.

(1) Each of two healthy dogs shall be administered 10 dog doses by the method recommended on the label and the dogs shall be observed each day for 14 days.

(2) If unfavorable reactions attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the biological product, the test shall be declared inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial shall be considered unsatisfactory.

[60 FR 14358, Mar. 17, 1995]

§ 113.41 Calf safety test.

The calf safety test provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a product.

(a) *Test procedure.* Each of two calves shall be injected with the equivalent of 10 doses of vaccine administered in the manner recommended on the label and observed each day for 21 days.

(b) *Interpretation.* If unfavorable reactions attributable to the product occur in either of the calves during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

[39 FR 27428, July 29, 1974]

§ 113.42 Detection of lymphocytic choriomeningitis contamination.

The test for detection of lymphocytic choriomeningitis (LCM) virus provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in a filed Outline of Production. Vaccine virus may be neutralized with specific antiserum when necessary.

(a) Each of at least 10 mice obtained from a source free of LCM shall be injected in the footpad of a hindfoot with 0.02 ml of the material being tested and observed each day for 21 days.

(b) If any of the mice show swelling in the injected footpad or if more than one becomes systemically abnormal, the material being tested is unsatisfactory.

[42 FR 6794, Feb. 4, 1977]

§ 113.43 Detection of chlamydial agents.

The test for chlamydial agents provided in this section shall be conducted when such a test is prescribed in an applicable standard requirement or in a filed Outline of Production.

(a) The yolk sac of 6-day-old chicken embryos shall be injected. Three groups of 10 embryos shall be used sequentially.

(1) The inoculum for each embryo in the first group shall consist of 0.5 ml of a mixture of equal parts of the seed virus with phosphate buffered saline that may contain Streptomycin, Vancomycin, Kanamycin, or a combination thereof. Not more than 2 mg/ml of each antibiotic shall be used.

(2) On the 10th day postinoculation, the yolk sac of viable embryos shall be harvested, pooled, homogenized as a 20 percent suspension in phosphate buffered saline antibiotic diluent, and 0.5 ml of the mixture injected into the second group of chicken embryos. This process shall be repeated for the injection of the third group of embryos using the yolk sacs of viable embryos from the second group.

(3) For each of the three passages, embryo deaths occurring within 48 hours of injection shall be disregarded, except that if more than three such deaths occur at any passage, that passage shall be repeated.

(b) If one or more embryo deaths occur at any passage after 48 hours postinjection, the yolk sacs from each of the dead embryos shall be subcultured into 10 additional embryos. If one or more embryo deaths again occur due to chlamydial agents, the Master Seed Virus is unsatisfactory for use to produce vaccine.

[44 FR 58899, Oct. 12, 1979]

§ 113.44 Swine safety test.

The swine safety test provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a product.

(a) *Test procedure.* (1) Inject each of two swine of the minimum age for which the product is recommended with the equivalent of two doses of bacterial vaccine or 10 doses of viral vaccine.

(2) Administer vaccine in the manner recommended on the label.

(3) Observe swine each day for 21 days.

(b) *Interpretation.* If unfavorable reactions attributable to the product occur in either of the swine during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated; *Provided,* That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

[48 FR 33476, July 22, 1983]

§ 113.45 Sheep safety test.

The sheep safety test provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a product.

(a) *Test procedure.* (1) Inject each of two sheep of the minimum age for which the product is recommended with the equivalent of two doses of bacterial vaccine or 10 doses of viral vaccine.

(2) Administer vaccine in the manner recommended on the label.

(3) Observe sheep each day for 21 days.

(b) *Interpretation.* If unfavorable reactions attributable to the product occur in either of the sheep during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated; *Provided,* That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

[48 FR 33476, July 22, 1983]

§ 113.46 Detection of cytopathogenic and/or hemadsorbing agents.

The tests for detection of cytopathogenic and/or hemadsorbing agents provided in this section shall be conducted when prescribed in an applicable Standard Requirement or in the filed Outline of Production for a product.

(a) *Test for cytopathogenic agents.* One or more monolayers that are at least 6 cm^2 and at least 7 days from the last subculture shall be tested as provided in this paragraph.

(1) Stain each monolayer with a suitable cytological stain.

(2) Examine the entire area of each stained monolayer for evidence of inclusion bodies, abnormal number of giant cells, or other cytopathology indicative of cell abnormalities attributable to an extraneous agent.

(b) *Test for hemadsorbing agents.* One or more monolayers that are at least 6 cm^2 and at least 7 days from the last subculture shall be tested as provided in this paragraph.

(1) Wash the monolayer with several changes of phosphate buffered saline.

(2) Add an appropriate volume of a 0.2 percent red blood cell suspension to uniformly cover the surface of the monolayer of cultured cells. Suspensions of washed guinea pig and chicken red blood cells shall be used. These suspensions may be mixed before addition to the monolayer or they may be added separately to individual monolayers.

(3) Incubate the monolayer at 4° C for 30 minutes, wash with phosphate buffered saline, and examine for hemadsorption.

(4) If no hemadsorption is apparent, repeat step (b)(2) of this section and incubate the monolayers at 20–25 °C for 30 minutes, wash with phosphate buffered saline, and examine again for hemadsorption. If desired, separate monolayers may be used for each incubation temperature.

(c) If specific cytopathology or hemadsorption attributable to an extraneous agent is found, the material under test is unsatisfactory and shall not be used to prepare biological products. If an extraneous agent is suspected because of cytopathology or hemadsorption and cannot be eliminated as a possibility by additional testing, the material under test is unsatisfactory.

[50 FR 441, Jan. 4, 1985, as amended at 58 FR 50252, Sept. 27, 1993]

§ 113.47 Detection of extraneous viruses by the fluorescent antibody technique.

The test for detection of extraneous viruses by the fluorescent antibody technique provided in this section shall be conducted when prescribed in an applicable Standard Requirement or in a filed Outline of Production for a product.

(a) Monolayer cultures of cells (monolayers), at least 7 days after the last subculturing, shall be processed and stained with the appropriate antiviral fluorochrome-conjugated antibody as specified in paragraph (b) of this section.

(1) Three groups of one or more monolayers shall be required for each specific virus prescribed in paragraph (b) of this section.

(i) At the time of the last subculturing, one group of test monolayers shall be inoculated with approximately 100–300 $FAID_{50}$ of the specific virus being tested for as positive controls.

(ii) One group of monolayers shall be the ''material under test.''

(iii) One group of monolayers, that are of the same type of cells as the test monolayers and that have been tested as prescribed in §§ 113.51 or 113.52 (whichever is applicable), shall be prepared as negative controls.

(2) Each group of monolayers shall have a total area of at least 6 cm^2.

(3) Positive control monolayers may be fixed (processed so as to arrest growth and assure attachment of the monolayer to the surface of the vessel in which they are grown) before 7 days after subculturing if fluorescence is enhanced by doing so, *Provided,* That a monolayer of the material under test is also fixed at the same time as the positive control and a monolayer of the material under test is also fixed at least seven days after subculturing. Monolayers that are fixed before 7 days after subculturing shall be stained at the same time as the test monolayers and negative controls fixed at least 7 days after subculturing.

(b) The antiviral fluorochrome-conjugated antibodies to be used shall depend on the type of cells required to be tested for extraneous viruses as specified in an applicable Standard Requirement or in a filed Outline of Production. Antiviral fluorochrome-conjugated antibodies specific for the extraneous viruses shall be applied to each respective type of cell in accordance with the following list. Under certain circumstances, additional tests may need to be conducted, as determined by the Administrator. When a specific antiviral fluorochrome-conjugated antibody is used in testing for the listed extraneous viruses specified in more than one cell type, it need only be applied to the most susceptible cell type.

(1) All cells shall be tested for:

(i) Bovine virus diarrhea virus;

(ii) Reovirus; and

(iii) Rabies virus.

(2) Bovine, caprine, and ovine cells shall, in addition, be tested for:

(i) Bluetongue virus;

(ii) Bovine adenoviruses;

(iii) Bovine parvovirus; and

(iv) Bovine respiratory syncytial virus.

(3) Canine cells shall, in addition, be tested for:

(i) Canine coronavirus;

(ii) Canine distemper virus; and

(iii) Canine parvovirus.

(4) Equine cells shall, in addition, be tested for:

(i) Equine herpesvirus; and

(ii) Equine viral arteritis virus.

(5) Feline cells shall, in addition, be tested for:

(i) Feline infectious peritonitis virus; and

(ii) Feline panleukopenia virus.

(6) Porcine cells shall, in addition, be tested for:

(i) Porcine adenovirus;

(ii) Porcine parvovirus;

(iii) transmissible gastroenteritis virus; and

(iv) Porcine hemagglutinating encephalitis virus.

(7) Firms that do not have rabies virus on premises either for research or production purposes are exempt from having to produce positive rabies virus control monolayers. Fixed positive rabies virus control monolayers will be provided by the National Veterinary Services Laboratories.

(c) After staining, each group of monolayers shall be examined for the presence of specific fluorescence attributable to the presence of extraneous viruses.

(1) If the material under test shows any evidence of specific viral fluorescence, it is unsatisfactory and may not be used; *Provided,* That, if specific fluorescence attributable to the virus being tested for is absent in the positive control monolayers, the test is inconclusive and may be repeated.

(2) If the fluorescence of the monolayers inoculated with the specific virus as positive controls is equivocal, or if the negative monolayers show equivocal fluorescence indicating possible viral contamination, or both, the test shall be declared inconclusive, and may be repeated; *Provided,* That, if the test is not repeated, the material under test shall be regarded as unsatisfactory for use in the production of biologics.

[60 FR 24548, May 9, 1995]

INGREDIENT REQUIREMENTS

§ 113.50 Ingredients of biological products.

All ingredients used in a licensed biological product shall meet accepted standards of purity and quality; shall be sufficiently nontoxic so that the amount present in the recommended dose of the product shall not be toxic to the recipient; and in the combinations used shall not denature the specific substances in the product below the minimum acceptable potency within the dating period when stored at the recommended temperature.

[38 FR 29889, Oct. 30, 1973]

§ 113.51 Requirements for primary cells used for production of biologics.

Primary cells used to prepare biological products shall be derived from normal tissue of healthy animals. When prescribed in an applicable Standard Requirement or in the filed Outline of Production, each batch of

primary cells used to prepare a biological product shall be tested as prescribed in this section. A batch of primary cells found unsatisfactory by any prescribed test shall not be used. A serial of biological product shall not be released if produced from primary cells that are found unsatisfactory by any prescribed test.

(a) Final container samples of completed product or samples of the final pool of harvested material or samples of each subculture of cells used to prepare the biological product shall be shown free of mycoplasma as prescribed in §113.28. The sample for testing shall consist of at least 75 cm^2 of actively growing cells or the equivalent in harvest fluids; *Provided,* That all sources of cells in the batch of primary cells are represented.

(b) Final container samples of completed product or samples of the final pool of harvested material or samples of each subculture of cells used to prepare the biological product shall be shown free of bacteria and fungi as prescribed in §113.26 or §113.27 (whichever is applicable).

(c) A monolayer at least 75 cm^2 from each batch of primary cells or each subculture of primary cells used to prepare a biological product shall be shown free of extraneous agents as prescribed in this paragraph.

(1) The test monolayer shall be maintained using the medium (with additives) and under conditions similar to those used to prepare biological products.

(i) Monolayers of avian origin shall be maintained for at least 14 days and shall be subcultured at least once during the maintenance period. All but the last subculture shall result in a new monolayer of at least 75 cm^2. The last subculture shall meet the minimum area requirement specified in §§113.46 and 113.47.

(ii) Monolayers not of avian origin shall be maintained for at least 28 days and shall be subcultured at least twice during the maintenance period. All but the last subculture shall result in a new monolayer of at least 75 cm^2. The last subculture shall meet the minimum area requirement specified in §§113.46 and 113.47.

(2) Monolayers shall be examined regularly throughout the required maintenance period for evidence of the presence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the batch of primary cells is unsatisfactory.

(3) At the conclusion of the required maintenance period, monolayers shall be tested for:

(i) Cytopathogenic and/or hemadsorbing agents as prescribed in §113.46;

(ii) Extraneous viruses by the fluorescent antibody technique as prescribed in §113.47.

[50 FR 442, Jan. 4, 1985, as amended at 60 FR 24549, May 9, 1995]

§113.52 Requirements for cell lines used for production of biologics.

When prescribed in an applicable Standard Requirement or in a filed Outline of Production each cell line used to prepare a biological product shall be tested as prescribed in this section. A cell line found unsatisfactory by any prescribed test shall not be used. A serial of biological product shall not be released if produced from a cell line that is found unsatisfactory by any prescribed test.

(a) *General requirements.* (1) A complete record of the cell line shall be kept, such as, but not limited to, the source, passage history, and medium used for propagation.

(2) A Master Cell Stock (MCS) shall be established at a specified passage level for each cell line. The passage level and identity of the MCS and the highest passage level (MCS + n) intended for use in the preparation of a biological product shall be specified in the Outline of Production for the product.

(3) Sufficient 1.0 ml or larger aliquots of MCS and MCS + n shall be prepared, kept in a frozen state, and made available to Animal and Plant Health Inspection Service (APHIS) upon request for performing the tests prescribed in this section.

(4) Each lot of cells shall be monitored for the characteristics determined to be normal for the cell line, such as, but not limited to, microscopic appearance, growth rate, acid

production, or other observable features.

(b) The MCS shall be shown to be of the same species of origin as that reported in paragraph (a)(1) of this section by the following method:

(1) At least four monolayers with a total area of at least 6 cm^2 shall be grown to at least 80 percent confluency.

(2) The monolayers shall be removed from their media, processed, stained, and examined.

(i) At least two monolayers shall be stained with an antispecies fluorchrome-conjugated antibody unrelated to the species of origin of the MCS.

(ii) At least two monolayers shall be stained with an antispecies fluorochrome-conjugated antibody specific to the species of origin of the MCS.

(iii) All monolayers shall be examined for evidence of specific fluorescence.

(3) If specific fluorescence is not found in the monolayers stained with the conjugate specific to the species of origin of the MCS, the cell line is unsatisfactory and shall not be used for vaccine production.

(4) If nonspecific fluorescence is found in the monolayers stained with conjugate from an unrelated species of origin or other results make the test results equivocal, the procedure shall be repeated until either specific fluorescence is found only in the monolayers stained with conjugate specific to the species of origin of the MCS and not in the control monolayers or specific fluorescence cannot be identified and the MCS is declared unsatisfactory.

(5) Alternate tests to determine the species of origin of the MCS may be used if approved by APHIS.

(c) The MCS and either each subculture of cells used to prepare a biological product or the final pool of harvested material (with or without the stabilizer) or final container samples of completed product for each serial of such product shall be shown to be free of mycoplasma as prescribed in §113.28. The sample for testing shall consist of at least 75 cm^2 of actively growing cells or the equivalent, in harvest fluids. The cells shall represent all sources of cells in the batch.

(d) The MCS and either each subculture used to prepare a biological product or the final pool of harvested material for each serial of such product or final container samples of completed product for each serial of such product shall be tested for bacteria and fungi as prescribed in §113.26 or §113.27 (whichever is applicable). If bacteria or fungi are found in the MCS, the MCS shall not be used. If bacteria or fungi are found in a subculture, the subculture shall not be used.

(e) A monolayer at least 75 cm^2 from each MCS shall be shown free of extraneous agents as prescribed in this paragraph.

(1) The test monolayer shall be maintained for at least 21 days using the medium (with additives) intended for growth and maintenance and under conditions similar to those used to prepare biological products.

(2) Cells shall be subcultured at least two times during the maintenance period. All but the last subculture shall result in at least one new monolayer of at least 75 cm^2. The last subculture shall meet the minimum area requirement specified in §§113.46 and 113.47 and paragraph (f) of this section.

(3) Monolayers shall be examined regularly throughout the 21-day maintenance period for evidence of the presence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the MCS is unsatisfactory.

(4) At the conclusion of the 21-day maintenance period, monolayers shall be tested for:

(i) Cytopathogenic and/or hemadsorbing agents as prescribed in §113.46; and

(ii) Extraneous agents by the fluorescent antibody technique as prescribed in §113.47.

(f) At the conclusion of the 21-day maintenance period provided in paragraph (e) of this section, at least one monolayer of at least 75 cm^2 shall also be shown free of extraneous agents as prescribed in this paragraph.

(1) Alternately freeze and thaw the monolayer(s) three times. Centrifuge the disrupted cells at no greater than 2,000×g for no more than 15 minutes to

remove cellular debris. Divide the supernatant into equal aliquots and dispense 1.0 ml onto each of at least one monolayer (at least 75 cm^2) of:

(i) Vero (African green monkey kidney) cell line;

(ii) Embryonic cells, neonatal cells, or a cell line of the same species of origin as the MCS if different than provided in paragraph (f)(1)(i) of this section;

(iii) Embryonic cells, neonatal cells, or a cell line of the species for which the vaccine is recommended if different than provided in paragraph (f)(1)(ii) of this section; and

(iv) Embryonic cells, neonatal cells, or a cell line of bovine origin if not specified in paragraphs (f)(1)(ii), and (iii) of this section.

(2) The monolayers of cells specified in paragraphs (f)(1)(i), (ii), (iii), and (iv) of this section shall be maintained for at least 14 days after inoculation with the aliquot of disrupted MCS. Monolayers shall be subcultured at least once during the maintenance period. All but the last subculture shall result in a new monolayer of at least 75 cm^2. The last subculture shall meet the minimum area requirement specified in §§ 113.46 and 113.47.

(3) Monolayers shall be examined regularly throughout the 14-day maintenance period for evidence of the presence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the MCS is unsatisfactory.

(4) At the conclusion of the 14-day maintenance period, monolayers shall be tested for:

(i) Cytopathogenic and/or hemadsorbing agents as prescribed in § 113.46; and

(ii) Extraneous viruses by the fluorescent antibody technique as prescribed in § 113.47.

(g) The karyology of cells lines used in the production of biologics shall be examined as follows. A minimum of 50 mitotic cells shall be examined at both the MCS and MCS+n. The modal number in the MCS+n shall not exceed plus or minus 15 percent of the modal number of the MCS. Any marker chromosomes present in the MCS shall persist at the MCS+n. If the modal number exceeds the limits and/or the marker chromosomes do not persist (through the MCS+n passage level), the cell line shall not be used for vaccine production.

(h) If direct or indirect evidence exists that a cell line which is intended for use in the preparation of a vaccine may induce malignancies in the species for which the product is intended, that cell line shall be tested for tumorigenicity/oncogenicity by a method acceptable to APHIS.

[50 FR 442, Jan. 4, 1985; 50 FR 3316, Jan. 24, 1985, as amended at 56 FR 66784, Dec. 26, 1991; 60 FR 24549, May 9, 1995]

§ 113.53 Requirements for ingredients of animal origin used for production of biologics.

Each lot of ingredient of animal origin which is not subjected to heat sterilization or other sterilization methods acceptable to Animal and Plant Health Inspection Service (APHIS), such as, but not limited to serum and albumin, used to prepare a biological product shall be tested as prescribed in this section by the licensee or a laboratory acceptable to VS. Results of all tests shall be recorded by the testing laboratory and made a part of the licensee's records. A lot of ingredient found unsatisfactory by any prescribed test shall not be used to prepare a biological product. A serial of biological product shall not be released if produced using an ingredient that is found unsatisfactory by any prescribed test.

(a) Samples of each lot of ingredient of animal origin which is not subjected to heat sterilization, used to prepare a biological product shall be shown free of mycoplasma by the method prescribed in § 113.28.

(b) Samples of each lot of ingredient or animal origin which is not subjected to heat sterilization of other sterilization methods acceptable to APHIS used to prepare a biological product shall be shown free of bacteria and fungi as prescribed in § 113.26.

(c) Samples of each lot of ingredient of animal origin, except porcine trypsin, which is not subjected to heat sterilization or other viricidal procedure acceptable to APHIS used in the preparation of biological products shall be tested as prescribed in this paragraph;

(1) Monolayers at least 75 cm^2 of Vero (African green monkey kidney) cell line and of primary cells or a cell line of the same species of origin as the ingredient shall be used in the test. Cell lines used shall have been found satisfactory when tested as prescribed in § 113.52 and primary cells used shall have been found satisfactory when tested as prescribed in § 113.51.

(2) At least 3.75 ml or 15 percent of the ingredient shall be used in the growth medium for the preparation of at least 75 cm^2 test monolayers. The ingredient shall also be used in the growth medium when monolayers are subcultured. If the ingredient being tested is cytotoxic when tested in this manner, other procedures may be used if approved by APHIS.

(3) The test monolayers shall be maintained for at least 21 days.

(4) Cells shall be subcultured at least two times during the maintenance period. All but the last subculture shall result in at least one new monolayer of at least 75 cm^2. The last subculture shall meet the minimum area requirements specified in §§ 113.46 and 113.47.

(5) Monolayers shall be examined regularly throughout the 21-day maintenance period for evidence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the ingredient is unsatisfactory.

(6) At the conclusion of the 21-day maintenance period, monolayers shall be tested for:

(i) Cytopathogenic and/or hemadsorbing agents as prescribed in § 113.46; and

(ii) Extraneous viruses by the fluorescent antibody technique as prescribed in § 113.47.

(d) Each lot of porcine trypsin which has not been treated to inactivate porcine parvovirus (PPV) in a manner acceptable to VS shall be tested for PPV as prescribed in this paragraph.

(1) Not less than 5.0 grams of trypsin shall be dissolved in a volume of suitable diluent sufficient to fill a centrifuge angle head. After centrifuging for 1 hour at 80,000×g, the pellet material shall be reconstituted in distilled water and inoculated into a flask containing 75 cm^2 of a 30 to 50 percent confluent monolayer culture of primary porcine cells or a porcine cell line of proven equal PPV susceptibility. An additional flask of cells shall be held as a negative control.

(2) The test and control monolayers shall be maintained for at least 14 days and subcultured at least once during the maintenance period.

(3) At the end of the 14-day maintenance period, and 4 to 7 days after the last subculturing, monolayers shall be tested for the presence of porcine parvovirus by the fluorescent antibody technique as prescribed in § 113.47(c).

(e) A sample of serum from each donor horse used to produce a lot of equine serum used in the preparation of biological products recommended for use in horses shall be tested at a laboratory approved by Animal and Plant Health Inspection Service using the Coggins test for equine infectious anemia antibodies. If antibodies to equine infectious anemia are found, the lot of serum is unsatisfactory.

[50 FR 442, Jan. 4, 1985; 50 FR 3316, Jan. 24, 1985, as amended at 56 FR 66784, Dec. 26, 1991; 60 FR 24549, May 9, 1995]

§ 113.54 Sterile diluent.

Sterile Diluent shall be supplied in a final container by the licensee when such diluent is required for rehydration or dilution of the vaccine.

(a) Sterile Diluent may be distilled or deionized water or it may be a special liquid solution formulated in accordance with an acceptable outline on file with Animal and Plant Health Inspection Service.

(b) Each quantity prepared at one time in a single container and bottled into final containers shall be designated as a serial. Each serial shall be given a number which shall be used in records, test reports, and on the final container label.

(c) Final container samples from each serial shall be tested for bacteria and fungi in accordance with the test provided in § 113.26. Any serial found to be unsatisfactory shall not be released.

[39 FR 27428, July 29, 1974, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.55 Detection of extraneous agents in Master Seed Virus.

Unless otherwise prescribed in a Standard Requirement or in a filed Outline of Production, each Master

Seed Virus (MSV) shall be tested as prescribed in this section. A MSV found unsatisfactory by any prescribed test shall not be used. A serial of biological product shall not be released if produced from a MSV that is found unsatisfactory by any prescribed test.

(a) At least a 1.0 ml aliquot per cell culture of MSV shall be dispensed onto monolayers (at least 75 cm^2 in area) of:

(1) Vero (African green monkey kidney) cell line;

(2) Embryonic cells, neonatal cells, or a cell line of the species for which the vaccine is recommended; and

(3) Embryonic cells, neonatal cells, or a cell line of the species of cells in which the MSV is presently being propagated if different than prescribed in paragraphs (a)(1) and (a)(2) of this section. Cell lines used shall have been found satisfactory when tested as prescribed in §113.52 and primary cells used shall have been found satisfactory when tested as prescribed in §113.51. If the MSV is cytopathic for or causes hemadsorption in the cells in which it is to be tested, the MSV shall be neutralized with monospecific antiserum supplied or approved by Animal and Plant Health Inspection Service (APHIS) or counteracted by a method approved by APHIS.

(b) At least one monolayer of each cell type used in the test shall be maintained as an uninoculated control.

(c) Each monolayer shall be maintained at least 14 days.

(d) Cells shall be subcultured at least once during the maintenance period. All but the last subculture shall result in at least one new monolayer at least 75 cm^2. The last subculture shall meet the minimum area requirement specified in §§113.46 and 113.47.

(e) Monolayers shall be examined regularly throughout the 14-day maintenance period for evidence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the MSV is unsatisfactory.

(f) At the conclusion of the 14-day maintenance period, monolayers shall be tested for:

(1) Cytopathogenic and/or hemadsorbing agents as prescribed in §113.46;

(2) Extraneous agents by the fluorescent antibody technique as prescribed in §113.47.

[50 FR 444, Jan. 4, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

LIVE BACTERIAL VACCINES

§113.64 General requirements for live bacterial vaccines.

When prescribed in an applicable Standard Requirement or in the filed Outline of Production, a live bacterial vaccine shall meet the requirements in this section.

(a) *Purity test.* Final container samples of completed product from each serial and subserial, and samples of each lot of Master Seed Bacteria shall be tested for the presence of extraneous viable bacteria and fungi in accordance with the test provided in §113.27(b).

(b) *Safety tests.* (1) Samples of completed product from each serial or first subserial and samples of each lot of Master Seed Bacteria shall be tested for safety in young adult mice in accordance with the test provided in §113.33(b) unless:

(i) The bacteria or agents in the vaccine are inherently lethal for mice.

(ii) The vaccine is recommended for poultry.

(2) Samples of completed product from each serial or first subserial of live bacterial vaccine shall be tested for safety in one of the species for which the product is recommended as follows:

(i) Live bacterial vaccine recommended for use in dogs shall be tested as provided in §113.40, except that dogs shall be injected with the equivalent of two doses of vaccine administered as recommended on the label.

(ii) Live bacterial vaccine recommended for use in cattle shall be tested as provided in §113.41, except that calves shall be injected with the equivalent of two doses of vaccine administered as recommended on the label.

(iii) Live bacterial vaccine recommended for use in sheep shall be tested as provided in §113.45.

(iv) Live bacterial vaccine recommended for use in swine shall be tested as provided in §113.44.

(c) *Identity test.* At least one of the identity tests provided in this paragraph shall be conducted for the Master Seed Bacteria and final container samples from each serial or first subserial of completed biological product. A known positive control (reference) provided or approved by Animal and Plant Health Inspection Service shall be included in such tests.

(1) *Fluorescent antibody test.* The direct fluorescent antibody staining technique shall be conducted using suitable smears of the vaccine bacteria. Fluorescence typical for the bacteria concerned shall be demonstrated. Fluorescence shall not occur in control smears treated with specific antiserum.

(2) *Tube agglutination test.* A tube agglutination test shall be conducted with a suitable suspension of the vaccine bacteria using the constant antigen decreasing serum method with specific antiserum. Agglutination typical for the bacteria shall be demonstrated. Agglutination shall not occur with negative serum used as a control in this test.

(3) *Slide agglutination test.* The rapid plate (slide) agglutination test shall be conducted with suitable suspensions of the vaccine bacteria using the hanging drop, slide or plate method, with specific antiserum. Agglutination typical for the bacteria shall be demonstrated by microscopic or macroscopic observation. Agglutination shall not occur with negative serum used as a control in this test.

(4) *Characterization tests.* Applicable biochemical and cultural characteristics shall be demonstrated as specified in the filed Outline of Production.

(d) *Ingredient requirements.* Ingredients used for the growth and preparation of Master Seed Bacteria and of live bacterial vaccine shall meet the requirements provided in § 113.50. Ingredients of animal origin shall meet the applicable requirements provided in § 113.53.

(e) *Moisture content.* The maximum percent moisture in desiccated vaccines shall be stated in the filed Outline of Production and shall be established by the licensee as follows:

(1) *Prelicensing.* Data obtained by conducting accelerated stability tests and bacterial counts shall be acceptable on a temporary basis.

(2) *Licensed products.* Data shall be obtained by determining the percent moisture and bacterial count at release and expiration on a minimum of 10 consecutive released serials.

(3) Final container samples of completed product from each serial and subserial must be tested for moisture content in accordance with the test provided in § 113.29.

[48 FR 33476, July 22, 1983, as amended at 54 FR 19352, May 5, 1989; 56 FR 66784, Dec. 26, 1991; 68 FR 57608, Oct. 6, 2003]

§ 113.65 Brucella Abortus Vaccine.

Brucella Abortus Vaccine shall be prepared as a desiccated live culture bacterial vaccine from smooth colonial forms of the *Brucella abortus* organism, identified as Strain 19. Each serial and subserial shall be tested for purity, potency, and moisture content. A serial or subserial found unsatisfactory by a prescribed test shall not be released.

(a) *Purity tests.* Each serial and subserial shall be tested for purity as provided in this paragraph.

(1) Macroscopic and microscopic examination shall be made on bulk samples from production containers. If organisms not typical of *Brucella abortus* organisms are evident, the serial or subserial is unsatisfactory.

(2) Two final container vials of completed product shall be tested by inoculating one tube of Dextrose Andrades broth with gas tube and one tube of thioglycollate broth from each vial. The inoculated media shall be incubated at 35 to 37 ° C for 96 hours. If growth not typical of *Brucella abortus* organisms is evident, the serial or subserial is unsatisfactory.

(3) Bacterial dissociation test. Final container samples of completed product from each serial and subserial shall be tested for bacterial dissociation. Smooth colonies are the desired form. Rough colonies are undesirable terminal dissociation forms. Intermediate and intermediate-to-rough are also undesirable.

(i) The sample container shall be rehydrated and streaked on one potato agar plate in such a manner as to produce confluent colonies. Artificial reflected light shall be used so that the

rays pass through the plate at a 45 °angle.

(ii) If the vaccine contains more than 5 percent rough colonies or more than 15 percent total undesirable colonies, the serial or subserial is unsatisfactory. If organisms or growth not characteristic of *Brucella abortus* are found, the serial or subserial is unsatisfactory. The test may be repeated one time using double the number of samples: *Provided,* That, if the test is not repeated, the serial or subserial is unsatisfactory.

(b) *Bacterial count requirements for reduced dose vaccine.* Each serial and each subserial shall be tested for potency.

(1) Two final container vials of completed product shall be tested for the number of viable organisms per dose of rehydrated vaccine. A bacterial count per vial shall be made on tryptose agar plates from suitable dilutions using 1 percent peptone as a diluent. The inoculated media shall be incubated at 35 to 37 ° C for 96 hours.

(2) If the average count of the two final container samples of freshly prepared vaccine contains less than 3.0 or more than 10.0 billion organisms per dose, the serial or subserial is unsatisfactory.

(3) If the average count on the initial test is less than the minimum or greater than the maximum required in paragraph (b)(2) of this section, the serial or subserial may be retested one time using four additional final container vials. The average count of the retest is determined. If the average count of the four vials retested is less than the required minimum or greater than the required maximum, the serial or subserial is unsatisfactory. If the average count of the four vials retested is within the required limits described in paragraph (b)(2) of this section, the following shall apply:

(i) If the average count obtained in the initial test is less than one-third or more than three times the average count obtained on the retest, the average count of the initial test shall be considered the result of test system error and the serial or subserial is satisfactory.

(ii) If the average count obtained in the initial test is one-third or more than the average retest count or three times or less than the average retest count, a new average count shall be determined from the counts of all six vials. If the new average is less than the minimum or greater than the maximum required in paragraph (b)(2) of this section, the serial or subserial is unsatisfactory.

(4) If tested at any time within the expiration period, each dose of rehydrated vaccine must contain at least 3.0 billion viable organisms per dose.

(c) *Bacterial count requirements for standard vaccine.* Each serial and subserial shall be tested for potency.

(1) Two final container samples shall be tested for the number of viable organisms per milliliter of rehydrated vaccine. One bacterial count per vial shall be made on tryptose agar plates from suitable dilutions using 1 percent peptone as a diluent. The inoculated media shall be incubated at 35 to 37 ° C for 96 hours.

(2) If the average count of the two final container samples of freshly prepared vaccine does not contain at least 10 billion viable organisms per milliliter, the serial or subserial is unsatisfactory.

(3) If the initial bacterial count is less than 10 billion organisms per milliliter, the serial or subserial may be retested one time using four samples. If the average count of the four vials retested is less than the required minimum, the serial or subserial is unsatisfactory.

(4) If tested at any time within the expiration period, each milliliter of rehydrated vaccine does not contain at least 5 billion viable organisms per milliliter, the serial or subserial is unsatisfactory.

[39 FR 16857, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, and amended at 40 FR 758, Jan. 3, 1975; 50 FR 23794, Jan. 6, 1985]

§ 113.66 Anthrax Spore Vaccine—Nonencapsulated.

Anthrax Spore Vaccine—Nonencapsulated shall be a live spore suspension prepared from nonencapsulated variants of *Bacillus anthracis.* Only Master Seed which has been established as pure, safe, and immunogenic shall be used for production. All serials of vaccine shall be prepared from the

first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity as follows:

(1) Forty-two susceptible guinea pigs from the same source each weighing 400 to 500 grams, shall be used as test animals (30 vaccinates and 12 controls).

(2) An arithmetic mean spore count of vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The guinea pigs used as vaccinates shall be injected as recommended on the label with a predetermined number of vaccine spores. To confirm the dosage, five replicate spore counts shall be conducted on a sample of the vaccine dilution used.

(3) Fourteen to fifteen days postvaccination the vaccinates and controls shall each be challenged with not less than 4,500 guinea pig LD_{50} of a virulent suspension of *Bacillus anthracis* furnished or approved by Animal and Plant Health Inspection Service and observed for 10 days.

(4) If at least 10 of the 12 controls do not die from *Bacillus anthracis* within the 10-day postchallenge observation period the test is invalid and may be repeated.

(5) If at least 27 of 30 of the vaccinates do not survive the 10-day postchallenge observation period, the Master Seed is unsatisfactory.

(6) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. The vaccinates and controls must meet the criteria prescribed in paragraphs (b)(4) and (b)(5) of this section.

(7) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test Requirements for Release.* Each serial and subserial shall meet the applicable general requirements prescribed in 9 CFR 113.64 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* Samples of completed product from each serial or first subserial shall be tested for safety in sheep or goats by the methods described in 9 CFR 113.45(a).

(2) *Spore Count Requirements.* Final container samples of completed product shall be tested for spore count. Samples shall be diluted in tenfold steps. Each dilution expected to yield 30 to 300 colonies per plate shall be plated in triplicate on tryptose agar, inverted, and incubated at 35 to 70 °C for 24 hours to 28 hours. Each plate having uniformly distributed colonies shall be counted and an average count determined. To be eligible for release, each serial and each subserial shall have a spore count sufficiently greater than that of the vaccine used in the immunogenicity test to assure that when tested at any time within the expiration period, each serial and subserial shall have a spore count of at least twice that used in the immunogenicity test but not less than 2,000,000 spores per dose.

[50 FR 23794, June 6, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.67 Erysipelothrix Rhusiopathiae Vaccine.

Erysipelothrix Rhusiopathiae Vaccine shall be prepared as a desiccated live culture of an avirulent or modified strain of *Erysipelothrix rhusiopathiae.* Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production.

(a) The Master Seed shall meet the applicable requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The selected bacterial count from the lot of Master Seed shall be established as follows:

(1) Thirty *Erysipelothrix rhusiopathiae* susceptible swine shall be used as test animals (20 vaccinates and 10 controls) for each route of administration recommended on the label.

(2) An arithmetic mean count of the colony forming units from vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted.

The 20 swine to be used as vaccinates shall be injected as recommended on the label with a predetermined quantity of vaccine bacteria. The 10 control swine shall be held separately from the vaccinates. To confirm the dosage calculation, an arithmetic mean count shall be established by conducting five replicate titrations on a sample of the bacterial vaccine dilution used. Only plates containing between 30 and 300 colonies shall be considered in a valid test.

(3) The vaccinates and controls shall be examined and their average body temperature determined prior to challenge. Fourteen to twenty-one days postvaccination, the vaccinates and controls shall be challenged with a virulent *Erysipelothrix rhusiopathiae* culture and observed for 7 days. The challenge culture and instructions for preparation and use shall be obtained from Animal and Plant Health Inspection Service.

(4) A satisfactory challenge shall be evidenced in the controls by a high body temperature or clinical signs including, but not limited to acute illness with hyperemia of the abdomen and ears, possibly terminating in sudden death; moribundity, with or without metastatic skin lesions; depression with anorexia, stiffness, and/or joint involvement; or any combination of these symptoms and lesions.

(5) If at least 80 percent of the controls do not show characteristic signs during the observation period including, but not limited to a body temperature of 105.6 ° F or higher on at least 2 consecutive days, the test shall be considered inconclusive: *Provided,* That control pigs which meet the criteria requirements for susceptibility except for high body temperature shall be considered susceptible if sacrificed and organisms identified as *Erysipelothrix rhusiopathiae* can be isolated from the blood, spleen, or other organs.

(6) To demonstrate immunity after challenge, the vaccinates shall remain free of clinical signs and the body temperature shall not exceed 104.6 ° F on 2 or more consecutive days. If at least 90 percent of the vaccinates do not remain free from clinical signs and high body temperature throughout the observation period, the Master Seed is unsatisfactory.

(7) The Master Seed shall be retested for immunogenicity in 3 years. Only five vaccinates and five controls need to be used in the retest: *Provided,* That at least four of five vaccinates and four of the five controls shall meet the criteria prescribed in paragraphs (b)(5) and (b)(6) of this section.

(8) An Outline of Production change shall be made before authority for use of a new Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable requirements in §113.64 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* Samples of completed product from each serial or first subserial shall be tested for safety in young adult mice as prescribed in §113.33(b) and in swine as prescribed in §113.44.

(2) *Bacterial count requirements.* Final container samples of completed product from each serial and each subserial shall be tested for bacterial count using the method used in paragraph (b)(2) of this section. Two replicate titrations shall be conducted on each sample. To be eligible for release, each serial and subserial shall have a bacterial count sufficiently greater than that of the vaccine used in the immunogenicity test to assure that, when tested at any time within the expiration period, each serial and subserial shall have a bacterial count two times greater than that used in such immunogenicity test.

[50 FR 23795, June 6, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

§113.68 Pasteurella Haemolytica Vaccine, Bovine.

Pasteurella Haemolytica Vaccine, Bovine, shall be prepared as a desiccated live culture bacterial vaccine of an avirulent or modified strain of *Pasteurella haemolytica,* identified as serotype 1. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production. All serials of vaccine shall

be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The immunogenicity of a selected bacterial count from the lot of Master Seed shall be established as follows:

(1) Fifteen *Pasteurella haemolytica* susceptible calves shall be used as test animals (10 vaccinates and 5 controls) for each route of administration recommended on the label.

(2) An arithmetic mean count of the colony forming units from vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The 10 calves to be used as vaccinates shall be injected as recommended on the label with a predetermined quantity of vaccine bacteria. The five control calves shall be held separately from the vaccinates. To confirm the dosage calculation, five replicate titrations on a sample of the bacterial vaccine used. Only plates containing between 30 and 300 colonies shall be considered a valid test.

(3) The vaccinates and controls shall be examined and their average body temperature determined prior to challenge. Fourteen to twenty-one days post vaccination, the vaccinates and controls shall each be challenged by the respiratory route with a (virulent) pneumonia producing *Pasteurella haemolytica* culture and observed for 4 to 7 days. The challenge culture and instructions for preparation for use shall be furnished or approved by the Animal and Plant Health Inspection Service.

(4) A satisfactory challenge shall be evidenced in the controls by progression of clinical signs consistent with respiratory system infection following challenge, including but not limited to lacrimation, mucoid nasal exudates, expiratory dyspnea, tachypnea, pulmonary rales, and cough possibly terminating in death; moribundity, depression with anorexia, diarrhea with substantial weight loss; or any combination of these symptoms.

(5) Lung lesion response to challenge will be assessed in all calves. Lung lesions will be assessed at necropsy in calves that succumb to challenge. Surviving calves will be euthanized on day 4 to 7 following challenge and lung lesions assessed at necropsy. Lung lesion scores will be used in the assessment of the response to challenge exposure. If a significant difference in lung lesion scores cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service, the Master Seed is unsatisfactory.

(6) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest: *Provided*, that, at least four of five vaccinates and four of five controls shall meet the criteria prescribed in paragraphs (b)(4) and (b)(5) of this section.

(7) An Outline of Production change must be made before authority for use of a new lot of Master Seed is granted by the Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §§ 113.8 and 113.64 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* Samples of completed product from each serial or first subserial shall be tested for safety in calves as provided in §§ 113.41(a) and 113.41(b) except, that the equivalent of two doses of vaccine shall be used and administered in the manner recommended on the label.

(2) *Bacterial count requirements.* Final container samples of completed product shall be tested for bacterial count using the method used in paragraph (b)(2) of this section. Two replicate titrations shall be conducted on each serial and subserial. Each sample shall be rehydrated with accompanying sterile diluent to the volume indicated on the label. To be eligible for release, each serial and subserial shall have a bacterial count sufficiently greater than that of the vaccine used in the

immunogenicity test to assure that, when tested at any time within the expiration period, each serial and subserial shall have a bacterial count at least two times greater than that used in the immunogenicity test.

[55 FR 35559, Aug. 31, 1990]

§ 113.69 Pasteurella Multocida Vaccine, Bovine.

Pasteurella Multocida Vaccine, Bovine, shall be prepared as a desiccated live culture bacterial vaccine of an avirulent or modified strain of *Pasteurella multocida,* of bovine origin. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The immunogenicity of a selected bacterial count from the lot of Master Seed shall be established as follows:

(1) Fifteen *Pasteurella multocida* susceptible calves shall be used as test animals (10 vaccinates and 5 controls) for each route of administration recommended on the label.

(2) An arithmetic mean count of the colony forming units from vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The 10 calves to be used as vaccinates shall be injected as recommended on the label with a predetermined quantity of vaccine bacteria. The five control calves shall be held separately from the vaccinates. To confirm the dosage calculation, arithmetic mean count shall be established by conducting five replicate titrations on a sample of the bacterial vaccine used. Only plates containing between 30 and 300 colonies shall be considered a valid test.

(3) The vaccinates and controls shall be examined and their average body temperature determined prior to challenge. Fourteen to twenty-one days post vaccination, the vaccinates and controls shall each be challenged by the respiratory route with a (virulent) pneumonia producing *Pasteurella multocida* culture and observed for 4 to 10 days. The challenge culture and instructions for preparation for use shall be furnished or approved by the Animal and Plant Health Inspection Service.

(4) A satisfactory challenge shall be evidenced in the controls by progression of clinical signs consistent with respiratory system infection following challenge, including but not limited to acute illness with higher body temperature and respiration rate, lacrimation, mucoid nasal exudate, expiratory dyspnea, tachypnea, pulmonary rales, and cough, possibly terminating in death; moribundity, depression with anorexia; diarrhea with substantial weight loss; or any combination of these symptoms.

(5) Lung lesion response to challenge will be assessed in all calves. Lung lesions will be assessed at necropsy in calves that succumb to challenge. Surviving calves will be euthanized on day 4 to 10 following challenge and lung lesions assessed at necropsy. Lung lesion scores will be used in the assessment of the response to challenge exposure. If a significant difference in lung lesion scores cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service, the Master Seed is unsatisfactory.

(6) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest: *Provided,* that, at least four of five vaccinates and four of five controls shall meet the criteria prescribed in paragraphs (b)(4) and (b)(5) of this section.

(7) An Outline of Production change must be made before authority for use of a new lot of Master Seed is granted by the Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §§ 113.8 and 113.64 and the requirements in this paragraph. Any serial or subserial found unsatisfactory

by a prescribed test shall not be released.

(1) *Safety Test.* Samples of completed product from each serial or first subserial shall be tested for safety in calves as provided in §§ 113.41(a) and 113.41(b), except that the equivalent of two doses of vaccine shall be used and administered in the manner recommended on the label.

(2) *Bacterial count requirements.* Final container samples of completed product shall be tested for bacterial count using the method used in paragraph (b)(2) of this section. Two replicate titrations shall be conducted on each serial and subserial. Each sample shall be rehydrated with accompanying sterile diluent to the volume indicated on the label. To be eligible for release, each serial and subserial shall have a bacterial count sufficiently greater than that of the vaccine used in the immunogenicity test count per dose established to assure that, when tested at any time within the expiration period, each serial and subserial shall have a bacterial count at least two times greater than that used in the immunogenicity test.

[55 FR 35560, Aug. 31, 1990]

§ 113.70 Pasteurella Multocida Vaccine, Avian Isolate.

Pasteurella Multocida Vaccine, Avian Isolate, shall be prepared as a desiccated live culture of an avirulent or modified strain of *Pasteurella multocida*. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity in each species and for each serotype for which the Master Seed is claimed to give protection.

(1) Thirty *Pasteurella multocida* susceptible birds shall be used as test animals (20 vaccinates and 10 controls) for each bird species, route of administration, and serotype for which protection is claimed on the label.

(2) An arithmetic mean count of colony forming units from vaccine produced from the highest passage of Master Seed shall be established before the immunogenicity test is conducted. The 20 birds to be used as vaccinates shall be inoculated, as recommended on the label with a predetermined quantity of vaccine bacteria. The 10 control birds shall be held separately from the vaccinates. To confirm the dosage calculation, an arithmetic mean count shall be established by conducting five replicate titrations on a sample of the bacterial vaccine used. Only plates containing between 30 and 300 colonies shall be considered in a valid test.

(3) Not less than 14 days after vaccination, each of 20 vaccinates and each of 10 unvaccinated controls shall be challenged intramuscularly or by other methods acceptable to the Animal and Plant Health Inspection Service with a virulent *Pasteurella multocida* strain, for which protection is claimed, and observed daily for a 14 day postchallenge period.

(4) Eight or more of the unvaccinated controls must die for the test to be valid. If at least 16 of 20 of the vaccinates do not survive the 14-day postchallenge period, the Master Seed is unsatisfactory at the selected bacterial count.

(5) The Master Seed shall be retested for immunogenicity in 3 years and shall meet the criteria prescribed in paragraph (b)(4) of this section.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable requirements in §§ 113.8 and 113.64 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* Samples of completed product from each serial or first subserial shall be tested for safety.

(i) Ten birds of a species for which the vaccine is recommended shall be given the equivalent of 10 doses each of the vaccine and observed for 10 days. If the vaccine is recommended for more than one species, only one species needs to be tested.

(ii) If unfavorable reactions attributable to the vaccine occur during the observation period in two or more of the test birds, the serial is unsatisfactory.

(iii) If unfavorable reactions occur which are not attributable to the test vaccine, the test is inconclusive and may be repeated. If the results of the next test are not satisfactory, or if the test is not repeated, the serial shall be considered unsatisfactory.

(2) *Bacterial count requirements.* Final container samples of completed product shall be tested for bacterial count using the method used in paragraph (b)(2) of this section. Two replicate titrations shall be conducted on each serial and subserial. Each sample shall be rehydrated with accompanying sterile diluent to the volume indicated on the label. To be eligible for release, each serial and subserial shall have a bacterial count sufficiently greater than that of the vaccine used in the immunogenicity test count per dose established to assure that, when tested at any time within the expiration period, each serial and subserial shall have a bacterial count at least two times greater than that used in the immunogenicity test.

[55 FR 35560, Aug. 31, 1990, as amended at 59 FR 19633, Apr. 25, 1994; 64 FR 43044, Aug. 9, 1999]

§ 113.71 Chlamydia Psittaci Vaccine (Feline Pneumonitis), Live Chlamydia.

Chlamydia Psittaci Vaccine (Feline Pneumonitis), Live Chlamydia, shall be prepared from chlamydia-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable requirements prescribed in §113.300 and the requirements in this section. Master Seed propagated in chicken embryos shall be tested for pathogens by the chicken embryo test prescribed in §113.37. If found unsatisfactory by any prescribed test, the Master Seed shall not be used.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The immunogenicity of a selected dose from the lot of Master Seed shall be established as follows:

(1) Thirty feline pneumonitis susceptible cats shall be used as test animals (20 vaccinates and 10 controls). Blood samples shall be drawn and individual serum samples tested. The cats shall be considered suitable for use if all serums are negative for pneumonitis antibody in a complement fixation test or other test of equal sensitivity.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The 20 cats used as vaccinates shall be administered a predetermined quantity of vaccine by the method to be recommended on the label and the remaining 10 cats shall be held as controls. To confirm the dosage calculations, five replicate titrations shall be conducted on a sample of the vaccine dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Fourteen or more days after the final dose of vaccine, the vaccinates and controls shall each be challenged intranasally with a minimum of 10,000 yolk sac LD50 of virulent feline pneumonitis furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 28 days postchallenge. The rectal temperature of each animal shall be taken and the presence or absence of clinical signs noted and recorded each day.

(i) If less than 8 of 10 controls show clinical signs of feline pneumonitis infection other than fever, the test is inconclusive and may be repeated.

(ii) If a significant difference in clinical signs other than fever or chlamydia shedding cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Either 10 vaccinates and 6 controls or 5 vaccinates and 3 controls shall be used in the retest.

(i) If less than five of six or three of three of the controls in the retest show clinical signs of feline pneumonitis infection other than fever, the test is inconclusive and may be repeated.

(ii) A significant difference in clinical signs shall be demonstrated between vaccinates and controls in a valid test as prescribed in paragraph (c)(3)(ii) of this section.

(5) An Outline of Production change must be made before authority for use of a new lot of Master Seed is granted by the Animal and Plant Health Inspection Service.

(c) *Test requirements for release:* Except for § 113.300(a)(3)(ii), each serial and subserial shall meet the requirements prescribed in § 113.300 and in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) The test for pathogens prescribed in § 113.37 shall be conducted on each serial or one subserial of avian origin vaccine.

(2) *Chlamydia titer requirements.* Final container samples of completed product shall be tested for chlamydia titer using the titration method used in paragraph (b)(2) of this section. To be eligible for release, each serial and each subserial shall have a titer sufficiently greater than the titer of vaccine used in the immunogenicity test prescribed in paragraph (b) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a titer 0.7 greater than that used in such immunogenicity test but not less than 2.5 ID50 per dose.

[55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

INACTIVATED BACTERIAL PRODUCTS

§ 113.100 General requirements for inactivated bacterial products.

Unless otherwise prescribed in an applicable Standard Requirement or in the filed Outline of Production, an inactivated bacterial product shall meet the applicable requirements in this section.

(a) *Purity tests.* (1) Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(2) Each lot of Master Seed Bacteria shall be tested for the presence of extraneous viable bacteria and fungi in accordance with the test provided in § 113.27(d).

(b) *Safety tests.* Bulk or final container samples of completed product from each serial shall be tested for safety in young adult mice in accordance with the test provided in § 113.33(b) unless:

(1) The product contains material which is inherently lethal for mice. In such instances, the guinea pig safety test provided in § 113.38 shall be conducted in place of the mouse safety test.

(2) The product is recommended for poultry. In such instances, the product shall be safety tested in poultry as defined in the specific Standard Requirement or Outline of Production for the product.

(3) The product is recommended for fish, other aquatic species, or reptiles. In such instances, the product shall be safety tested in fish, other aquatic species, or reptiles as required by specific Standard Requirement or Outline of Production for the product.

(c) *Identity test.* Methods of identification of Master Seed Bacteria to the genus and species level by laboratory tests shall be sufficient to distinguish the bacteria from other similar bacteria according to criteria described in the most recent edition of ''Bergey's Manual of Systematic Bacteriology'' or the American Society for Microbiology ''Manual of Clinical Microbiology''. If Master Seed Bacteria are referred to by serotype, serovar, subtype, pilus type, strain or other taxonomic subdivision below the species level, adequate testing must be used to identify the bacteria to that level. Tests which may be used to identify Master Seed Bacteria include, but are not limited to:

(1) Cultural characteristics,

(2) Staining reaction,

(3) Biochemical reactivity,

(4) Fluorescent antibody tests,

(5) Serologic tests,

(6) Toxin typing,

(7) Somatic or flagellar antigen characterization, and

(8) Restriction endonuclease analysis.

(d) *Ingredient requirements.* Ingredients used for the growth and preparation of Master Seed Bacteria and of

final product shall meet the requirements provided in § 113.50. Ingredients of animal origin shall meet the applicable requirements provided in § 113.53.

(e) Only serials tested for viricidal activity in accordance with the test provided in § 113.35 and found satisfactory by such test shall be packaged as diluent for desiccated fractions in combination packages.

(f) If formaldehyde is used as the inactivating agent, and the serial has not been found satisfactory by the viricidal activity test, bulk or final container samples of completed product from each serial must be tested for residual free formaldehyde content using the ferric chloride test.[2] Firms currently using tests for residual free formaldehyde content other than the ferric chloride test have until July 14, 2004 to update their Outline of Production to be in compliance with this requirement.

(1) The residual free formaldehyde content of biological products containing clostridial antigens must not exceed 1.85 grams per liter (g/L).

(2) The residual free formaldehyde content of bacterins, bacterin-toxoids, and toxoids, other than those containing clostridial antigens, must not exceed 0.74 grams per liter (g/L).

[39 FR 16862, May 10, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 60 FR 14355, Mar. 17, 1995; 68 FR 35283, June 13, 2003]

§ 113.101 Leptospira Pomona Bacterin.

Leptospira Pomona Bacterin shall be produced from a culture of *Leptospira pomona* which has been inactivated and is nontoxic. Each serial of biological product containing *Leptospira pomona* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product shall be diluted with physiological saline so that each 0.25 ml contains not more than 1/800th of the dose recommended on the label and shall be tested for potency, using the two-stage test provided in this paragraph.

(1) *Vaccinates.* Inject each of at least 10 but not more than 12 young adult hamsters, each weighing 50 to 90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly, in accordance with the label recommendations for use.

(2) *Controls.* Retain at least 10 but not more than 12 additional hamsters from the same group as unvaccinated controls.

(3) *Challenge.* From 14 to 18 days postvaccination, challenge each of 10 vaccinates and each of 10 controls intraperitoneally with a suspension of virulent *Leptospira pomona* organisms, using a dose of 10–10,000 hamster LD_{50} as determined by titration.

(4) *Post-challenge period.* Observe the vaccinates and controls for 14 days post-challenge and record all deaths. If eight or more controls die of leptospirosis, the test is valid and the results shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total dead hamsters for satisfactory serial	Cumulative total dead hamsters for unsatisfactory serial
1	10	10	2 or less	5 or more.
2	10	20	5 or less	6 or more.

(5) If three or four vaccinates die in the first stage, the second stage shall be conducted in a manner identical to the first stage.

(6) If the second stage is used, each serial shall be evaluated according to the second part of the table. On the basis of cumulative results, each serial shall either pass or fail.

[39 FR 16862, May 10, 1974, as amended at 40 FR 20067, May 8, 1975; 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

[2] The procedures for performing the ferric chloride test for residual free formaldehyde may be obtained from USDA, APHIS, Center for Veterinary Biologics-Laboratory, 1800 Dayton Road, P.O. Box 844, Ames, IA 50010.

§ 113.102 Leptospira Icterohaemorrhagiae Bacterin.

Leptospira Icterohaemorrhagiae Bacterin shall be produced from a culture of *Leptospira icterohaemorrhagiae* which has been inactivated and is nontoxic. Each serial of biological product containing *Leptospira icterohaemorrhagiae* fraction shall meet the applicable requirements in § 113.100 and be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product shall be diluted with physiological saline so that each 0.25 ml contains not more than 1/80th of the dose recommended on the label and shall be tested for potency, using the two-stage test provided in this paragraph.

(1) *Vaccinates.* Inject each of at least 10 but not more than 12 young adult hamsters, each weighing 50 to 90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly, in accordance with the label recommendations for use.

(2) *Controls.* Retain at least 10 but not more than 12 additional hamsters from the same group as unvaccinated controls.

(3) *Challenge.* From 14 to 18 days postvaccination, challenge each of 10 vaccinates and each of 10 controls intraperitoneally with a suspension of virulent *Leptospira icterohaemorrhagiae* organisms, using a dose of 10–10,000 hamster LD_{50} as determined by titration.

(4) *Post-challenge period.* Observe the vaccinates and controls for 14 days post-challenge and record all deaths. If eight or more controls die from leptospirosis, the test is valid and the results shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total dead hamsters for satisfactory serial	Cumulative total dead hamsters for unsatisfactory serial
1	10	10	2 or less	5 or more.
2	10	20	5 or less	6 or more.

(5) If three or four vaccinates die in the first stage, the second stage shall be used. The second stage shall be conducted in a manner identical to the first stage.

(6) If the second stage is used, each serial shall be evaluated according to the second part of the table. On the basis of cumulative results, each serial shall either pass or fail.

[39 FR 16862, May 10, 1974, as amended at 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.103 Leptospira Canicola Bacterin.

Leptospira Canicola Bacterin shall be produced from a culture of *Leptospira canicola* which has been inactivated and is nontoxic. Each serial of biological product containing *Leptospira canicola* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. Serials found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product shall be diluted with physiological saline so that each 0.25 ml contains not more than 1/80th of the dose recommended on the label and shall be tested for potency, using the two-stage test provided in this paragraph.

(1) *Vaccinates.* Inject each of at least 10 but not more than 12 young adult hamsters, each weighing 50 to 90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly, in accordance with the label recommendations for use.

(2) *Controls.* Retain at least 10 but not more than 12 additional hamsters from the same group as unvaccinated controls.

(3) *Challenge.* From 14 to 18 days postvaccination, challenge each of 10 vaccinates and each of 10 controls intraperitoneally with a suspension of virulent *Leptospira canicola* organisms, using a dose of 10–10,000 hamster LD_{50} as determined by titration.

(4) *Post-challenge period.* Observe the vaccinates and controls for 14 days post-challenge and record all deaths. If eight or more controls die from leptospirosis, test is valid and the results shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total dead hamsters for satisfactory serial	Cumulative total dead hamsters for unsatisfactory serial
1	10	10	2 or less	5 or more.
2	10	20	5 or less	6 or more.

(5) If three or four vaccinates die in the first stage, the second stage shall be used. The second stage shall be conducted in a manner identical to the first stage.

(6) If the second stage is used, each serial shall be evaluated according to the second part of the table. On the basis of cumulative results, each serial shall either pass or fail.

[39 FR 16862, May 10, 1974, as amended at 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.104 Leptospira Grippotyphosa Bacterin.

Leptospira Grippotyphosa Bacterin shall be produced from a culture of *Leptospira grippotyphosa* which has been inactivated and is nontoxic. Each serial of biological product containing *Leptospira grippotyphosa* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product shall be diluted with physiological saline so that each 0.25 ml contains not more than 1/800th of the dose recommended on the label and shall be tested for potency, using the two-stage test provided in this paragraph.

(1) *Vaccinates.* Inject each of at least 10 but not more than 12 young adult hamsters, each weighing 50 to 90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly, in accordance with the label recommendations for use.

(2) *Controls.* Retain at least 10 but not more than 12 additional hamsters from the same group as unvaccinated controls.

(3) *Challenge.* From 14 to 18 days postvaccination, challenge each of 10 vaccinates and each of 10 controls intraperitoneally with a suspension of virulent *Leptospira grippotyphosa* organisms, using a dose of 10–10,000 hamster LD_{50} as determined by titration.

(4) *Post-challenge period.* Observe the vaccinates and controls for 14 days post-challenge and record all deaths. If eight or more controls die of leptospirosis, the test is valid and the results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of vaccinates	Dead hamsters for acceptance	Dead hamsters for rejection
1	10	2 or less	5 or more.
2	20	5 or less	6 or more.

(5) If three or four vaccinates die in the first stage, the second stage shall be conducted in a manner identical to the first stage.

(6) If the second stage is used, each serial shall be evaluated according to the second part of the table. On the basis of cumulative results, each serial shall either pass or fail.

[40 FR 17003, Apr. 16, 1975, as amended at 40 FR 23989, June 4, 1975; 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.105 Leptospira Hardjo Bacterin.

Leptospira Hardjo Bacterin shall be produced from a culture of *Leptospira hardjo* which has been inactivated and is nontoxic. Each serial of biological product containing *Leptospira hardjo* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the test written into the filed Outline of Production.

[40 FR 17003, Apr. 16, 1975, as amended at 40 FR 20067, May 8, 1975. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.106 Clostridium Chauvoei Bacterin.

Clostridium Chauvoei Bacterin shall be produced from a culture of *Clostridium chauvoei* which has been inactivated and is nontoxic. Each serial of biological product containing *Clostridium chauvoei* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. Serials found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the two-stage test provided in this paragraph.

(1) Each of at least 8 but not more than 10 guinea pigs, each weighing 300 to 500 grams, shall be injected subcutaneously with a guinea pig dose. A second guinea pig dose shall be injected 21 to 23 days after the first dose. Each guinea pig dose shall be one-fifth of the dose recommended on the label for a calf.

(2) *Clostridium chauvoei* challenge material, available upon request from Animal and Plant Health Inspection Service, shall be used for challenge 14 to 15 days following the last injection of the product. Each of eight vaccinates and each of five additional nonvaccinated guinea pigs for controls shall be injected intramuscularly with approximately 100 LD_{50} of challenge material. This dose shall be determined by statistical analysis of results of titrations of the challenge material. The vaccinates and controls shall be observed for 3 days postchallenge and all deaths recorded.

(3) For a valid test, at least 80 percent of the controls shall die within the 3 day post-challenge observation period. If this requirement is met, the results of the potency test shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of deaths for a satisfactory test	Cumulative total number of deaths for an unsatisfactory test
1	8	8	1 or less	3 or more.
2	8	16	4 or less	5 or more.

The second stage shall be required only when exactly two animals die in the first stage. The second stage shall be conducted in a manner identical to the first stage.

[39 FR 16862, May 10, 1974, as amended at 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990 and amended at 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.107 Clostridium Haemolyticum Bacterin.

Clostridium Haemolyticum Bacterin shall be produced from a culture of *Clostridium haemolyticum* which has been inactivated and is nontoxic. Each serial of biological product containing *Clostridium haemolyticum* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity,

safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the two-stage test provided in this paragraph.

(1) Each of at least 8 but not more than 10 guinea pigs, each weighing 300 to 500 grams, shall be injected subcutaneously with a guinea pig dose. A second guinea pig dose shall be injected 21 to 23 days after the first dose. Each guinea pig dose shall be one-fifth of the dose recommended on the label for a calf.

(2) *Clostridium haemolyticum* challenge material, available upon request from Animal and Plant Health Inspection Service, shall be used for challenge 14 to 15 days following the last injection of the product. Each of eight vaccinates and each of five additional nonvaccinated guinea pigs for controls shall be injected intramuscularly with approximately 100 LD_{50} of challenge material. This dose shall be determined by statistical analysis of results of titrations of the challenge material. The vaccinates and controls shall be observed for 3 days postchallenge and all deaths recorded.

(3) For a valid test, at least 80 percent of the controls shall die within the 3 day post-challenge observation period. If this requirement is met, the results of the potency test shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of deaths for a satisfactory test	Cumulative total number of deaths for an unsatisfactory test
1	8	8	1 or less	3 or more.
2	8	16	4 or less	5 or more.

The second stage shall be required only when exactly two animals die in the first stage. The second stage shall be conducted in a manner identical to the first stage.

[39 FR 16862, May 10, 1974, as amended at 40 FR 20067, May 8, 1975; 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.108 Clostridium Novyi Bacterin-Toxoid.

Clostridium Novyi Bacterin-Toxoid shall be produced from a culture of *Clostridium novyi* which has been inactivated and is nontoxic. Each serial of biological product containing *Clostridium novyi* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the Alpha toxin-neutralization test provided in this paragraph.

(1) When used in this test, the following words and terms shall mean:

(i) *International antitoxin unit.* (I.U.) That quantity of Alpha Antitoxin which reacts with Lo and L+ doses of Standard Toxin according to their definitions.

(ii) *Lo dose.* The largest quantity of toxin which can be mixed with one unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) *L+ dose.* The smallest quantity of toxin which can be mixed with one unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.

(iv) *Standard antitoxin.* The Alpha Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International *Clostridium novyi* Alpha Antitoxin Standard and which is either supplied by or acceptable to the Animal and Plant

Health Inspection Service. The antitoxin unit value shall be stated on the label.

(v) *Standard toxin.* The Alpha toxin preparation which is supplied by or is acceptable to the Animal and Plant Health Inspection Service.

(vi) *Diluent.* The solution used to make proper dilutions prescribed in this test. Such solutions shall be made by dissolving 1 gram of peptone and 0.25 gram of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 121 °C for 25 minutes; and storing at 4 °C until used.

(2) Each of at least eight rabbits of a strain acceptable to the Animal and Plant Health Inspection Service, each weighing 4–8 pounds, shall be injected subcutaneously with not more than half of the recommended cattle dose. *Provided,* That, if the product is recommended only for sheep, half of the recommended sheep dose shall be used. A second dose shall be given not less than 20 days nor more than 23 days after the first dose.

(3) Fourteen to seventeen days after the second dose, all surviving rabbits shall be bled, and the serum tested for antitoxin content.

(i) At least seven rabbits are required to make an acceptable serum pool.

(ii) Equal quantities of serum from each rabbit shall be combined and tested as a single pooled serum.

(iii) If less than seven rabbits are available, the test is invalid and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(4) The antitoxin content of the rabbit serums shall be determined by the serum neutralization test as follows:

(i) Make a dilution of Standard Antitoxin to contain 0.1 International Unit of antitoxin per ml.

(ii) Make a dilution of Standard Toxin in which 0.1 Lo dose is contained in a volume of 1 ml or less. Make a second dilution of Standard Toxin in which 0.1 L+ dose is contained in a volume of 1 ml or less.

(iii) Combine 0.1 International Unit of Standard Antitoxin with 0.1 Lo dose of diluted Standard Toxin and combine 0.1 International Unit of Standard Antitoxin with 0.1 L+ dose of diluted Standard Toxin. Each mixture is adjusted to a final volume of 2.0 ml with diluent.

(iv) Combine 0.1 Lo dose of diluted Standard Toxin with a 0.2 ml volume of undiluted serum. The mixture is adjusted to a final volume of 2.0 ml with diluent.

(v) Neutralize all toxin-antitoxin mixtures at room temperature for 1 hour and hold in ice water until injections of mice can be made.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 72 hours post injection and record all deaths.

(5) Test Interpretation shall be as follows:

(i) If any mice inoculated with the mixture of 0.1 International Unit of Standard Antitoxin and 0.1 Lo doses of Standard Toxin die, the results of the serum neutralization test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) If less than 80 percent of the mice inoculated with the mixture of 0.1 International Unit of Standard Antitoxin and 0.1 L+ doses of Standard Toxin die, the results of the serum neutralization test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(iii) If any mice inoculated with the mixture of 0.2 ml undiluted serum with 0.1 Lo dose of Standard Toxin die, the serum is considered to contain less than 0.50 International Units per ml.

(iv) If the single pooled serum from seven or more rabbits contains less than 0.5 International Unit per ml, the serial is unsatisfactory.

[39 FR 16862, May 10, 1974, as amended at 45 FR 40101, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990; 56 FR 37825, Aug. 9, 1991, as amended at 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.109 Clostridium Sordellii Bacterin-Toxoid.

Clostridium Sordellii Bacterin-Toxoid shall be produced from a culture of *Clostridium sordellii* which has been inactivated and is nontoxic. Each serial

of biological product containing *Clostridium sordellii* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the toxin-neutralization test provided in this paragraph.

(1) When used in this test, the following words and terms shall mean:

(i) *International antitoxin unit.* (I.U.) That quantity of antitoxin which reacts with Lo and L+ doses of Standard Toxin according to their definitions.

(ii) *Lo dose.* The largest quantity of toxin which can be mixed with one unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) *L+ dose.* The smallest quantity of toxin which can be mixed with one unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.

(iv) *Standard antitoxin.* The antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International *Clostridium sordellii* Antitoxin Standard and which is either supplied by or acceptable to the Animal and Plant Health Inspection Service. The antitoxin unit value shall be stated on the label.

(v) *Standard toxin.* The toxin preparation which is supplied by or is acceptable to the Animal and Plant Health Inspection Service.

(vi) *Diluent.* The solution used to make proper dilutions prescribed in this test. Such solutions shall be made by dissolving 1 gram of peptone and 0.25 gram of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 121 °C for 25 minutes; and storing at 4 °C until used.

(2) Each of at least eight rabbits of a strain acceptable to the Animal and Plant Health Inspection Service, each weighing 4–8 pounds, shall be injected subcutaneously with not more than half of the recommended cattle dose: *Provided,* That, if the product is recommended only for sheep, half of the recommended sheep dose shall be used. A second dose shall be given not less than 20 days nor more than 23 days after the first dose.

(3) Fourteen to seventeen days after the second dose, all surviving rabbits shall be bled, and the serum tested for antitoxin content.

(i) At least seven rabbits are required to make an acceptable serum pool.

(ii) Equal quantities of serum from each rabbit shall be combined and tested as a single pooled serum.

(iii) If less than seven rabbits are available, the test is invalid and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(4) The antitoxin content of the rabbit serums shall be determined by the serum neutralization test as follows:

(i) Make a dilution of Standard Antitoxin to contain 1.0 international unit of antitoxin per ml.

(ii) Make a dilution of Standard Toxin in which 1.0 Lo dose is contained in a volume of 1 ml or less. Make a second dilution of Standard Toxin in which 1.0 L+ dose is contained in a volume of 1 ml or less.

(iii) Combine 1.0 International Unit Standard Antitoxin with 1.0 Lo dose of diluted Standard Toxin and combine 1.0 International Unit of Standard Antitoxin with 1.0 L+ dose of diluted Standard Toxin. Each mixture is adjusted to a final volume of 2.0 ml with diluent.

(iv) Combine 1.0 Lo dose of diluted Standard Toxin with a 1.0 ml volume of undiluted serum. This mixture is adjusted to a final volume of 2.0 ml with diluent.

(v) Neutralize all toxin-antitoxin mixtures at room temperature for 1 hour and hold in ice water until injections of mice can be made.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 72 hours post injection and record all deaths.

(5) Test Interpretation shall be as follows:

(i) If any mice inoculated with the mixture of 1.0 International Unit of Standard Antitoxin and 1.0 Lo doses of Standard Toxin die, the results of the serum neutralization test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) If less than 80 percent of the mice inoculated with the mixture of 1.0 International Unit of Standard Antitoxin and 1.0 L+ doses of Standard Toxin die, the results of the serum neutralization test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(iii) If any mice inoculated with the mixture of 1.0 ml undiluted serum with 1.0 Lo dose of Standard Toxin die, the serum is considered to contain less than 1.0 International Units per ml.

(iv) If the single pooled serum from seven or more rabbits contains less than 1.0 International Unit per ml, the serial is unsatisfactory.

[39 FR 16862, May 10, 1974, as amended at 42 FR 61247, Dec. 2, 1977; 45 FR 40101, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990; 56 FR 37826, Aug. 9, 1991; 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.110 Clostridium Botulinum Type C Bacterin-Toxoid.

Clostridium Botulinum Type C Bacterin-Toxoid shall be produced from a culture of *Clostridium botulinum* Type C which has been inactivated and is nontoxic. Each serial of biological product containing *Clostridium botulinum* Type C fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency, using susceptible mink as test animals. At least five vaccinates and three unvaccinated controls of the same source and approximately the same age shall be used.

(1) Each of the vaccinates shall be injected subcutaneously with the dose recommended on the label for mink. Twenty-one to twenty-eight days post-injection, the vaccinates and the controls shall be challenged intraperitoneally with botulinum Type C toxin which has been titrated in mice to provide for a $10^{4.0}$ mouse MLD dose. The titration technique shall include inoculation of the mice intraperitoneally.

(2) The vaccinates and controls shall be observed for 7 days post-challenge and signs of botulism and deaths noted. For a valid test, the controls shall die of botulism. If the test is valid and 80 percent of the vaccinates do not remain free of botulism, the serial is unsatisfactory.

[39 FR 16862, May 10, 1974, as amended at 40 FR 759, Jan. 3, 1975. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.111 Clostridium Perfringens Type C Toxoid and Bacterin-Toxoid.

Clostridium Perfringens Type C Toxoid and Clostridium Perfringens Type C Bacterin-Toxoid shall be produced from a culture of *Clostridium perfringens* Type C which has been inactivated and is nontoxic. Each serial shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the Beta toxin-neutralization test provided in this paragraph.

(1) When used in this test, the following words and terms shall mean:

(i) *International antitoxin unit.* (I.U.) That quantity of Beta Antitoxin which reacts with L_0 and L^+ doses of Standard Toxin according to their definitions.

(ii) L_0 dose. The largest quantity of toxin which can be mixed with one unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) L_+ *dose.* The smallest quantity of toxin which can be mixed with one unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.

(iv) *Standard antitoxin.* The Beta Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International *Clostridium perfringens* Beta Antitoxin Standard and which is either supplied by or acceptable to Animal and Plant Health Inspection Service. The antitoxin unit value shall be stated on the label.

(v) *Standard toxin.* The Beta toxin preparation which is supplied by or is acceptable to Animal and Plant Health Inspection Service.

(vi) *Diluent.* The solution used to make proper dilutions prescribed in this test. Such solutions shall be made by dissolving 1 gram of peptone and 0.25 grams of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 250 °F for 25 minutes; and storing at 4 °C until used.

(2) Each of at least eight rabbits of a strain acceptable to APHIS, each weighing 4–8 pounds, shall be injected subcutaneously with not more than half of the largest recommended dose for any species indicated on the product label. A second equivalent dose shall be given not less than 20 days nor more than 23 days after the first does.

(3) Fourteen to seventeen days after the second dose, all surviving rabbits shall be bled and the serum tested for antitoxin content.

(i) At least seven rabbits are required to make an acceptable serum pool.

(ii) Equal quantities of serum from each rabbit shall be combined and tested as a single pooled serum.

(iii) If less than seven rabbits are available, the test is invalid and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(4) The antitoxin content of the rabbit serums shall be determined as follows:

(i) Make a dilution of Standard Antitoxin to contain 10 International Units of antitoxin per ml.

(ii) Make one dilution of Standard Toxin to contain 10 L_0 doses per ml and make a second dilution of Standard Toxin to contain 10 L_+ doses per ml.

(iii) Combine 10 International Units of Standard Antitoxin with 10 L_0 doses of diluted Standard Toxin and combine 10 International Units of Standard Antitoxin with 10 L_+ doses of diluted Standard Toxin.

(iv) Combine 1 ml of undiluted serum with 10 L_0 doses of diluted Standard Toxin.

(v) Neutralize all toxin-antitoxin mixtures at room temperature for 1 hour and hold in ice water until injections of mice can be made.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 24 hours post-injection and record all deaths.

(5) Test Interpretation shall be as follows:

(i) If any mice inoculated with the mixture of 10 International Units of Standard Antitoxin and 10 L_0 doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) If less than 80 percent of the mice inoculated with mixture of 10 International Units of Standard Antitoxin and 10 L_+ doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(iii) If any mice inoculated with the mixture of serum with 10 L_0 doses of

Standard Toxin die, the serum is considered to contain less than 10 International Units per ml. and the serial is unsatisfactory

[39 FR 16862, May 10, 1974, as amended at 40 FR 759, Jan. 3, 1975; 40 FR 41088, Sept. 5, 1975. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66785, Dec. 26, 1991; 62 FR 31330, June 9, 1997]

§ 113.112 Clostridium Perfringens Type D Toxoid and Bacterin-Toxoid.

Clostridium Perfringens Type D Toxoid and Clostridium Perfringens Type D Bacterin-Toxoid shall be produced from a culture of *Clostridium perfringens* Type D which has been inactivated and is nontoxic. Each serial shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the Epsilon toxin-neutralization test provided in this paragraph.

(1) When used in this test, the following words and terms shall mean:

(i) *International antitoxin unit.* (I.U.) That quantity of Epsilon Antitoxin which reacts with L_0 and L_+ doses of Standard Toxin according to their definitions.

(ii) *L_0 dose.* The largest quantity of toxin which can be mixed with one-tenth unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) *L_+ dose.* The smallest quantity of toxin which can be mixed with one-tenth unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.

(iv) *Standard antitoxin.* The Epsilon Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International *Clostridium perfringens* Epsilon Antitoxin Standard and which is either supplied by or acceptable to Animal and Plant Health Inspection Service. The antitoxin unit value shall be stated on the label.

(v) *Standard toxin.* The Epsilon toxin preparation which is supplied by or is acceptable to Animal and Plant Health Inspection Service.

(vi) *Diluent.* The solution used to make proper dilutions prescribed in this test. Such solutions shall be made by dissolving 1 gram of peptone and 0.25 gram of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 250 °F for 25 minutes; and storing at 4 °C until used.

(2) Each of at least eight rabbits of a strain acceptable to APHIS, each weighing 4—8 pounds, shall be injected subcutaneously with not more than half of the largest recommended dose for any species indicated on the product label. A second equivalent dose shall be given not less than 20 days nor more than 23 days after the first dose.

(3) Fourteen to seventeen days after the second dose, all surviving rabbits shall be bled, and the serum tested for antitoxin content.

(i) At least seven rabbits are required to make an acceptable serum pool.

(ii) Equal quantities of serum from each rabbit shall be combined and tested as a single pooled serum.

(iii) If less than seven rabbits are available, the test is invalid and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(4) The antitoxin content of the rabbit serums shall be determined as follows:

(i) Make a dilution of Standard Antitoxin to contain 1 International Unit of antitoxin per ml.

(ii) Make one dilution of Standard Toxin to contain 10 L_o doses per ml and make a second dilution of Standard Toxin to contain 10 L_+ doses per ml.

(iii) Combine 1 International Unit of Standard Antitoxin with 10 L_o doses of diluted Standard Toxin and Combine 1 International Unit of Standard Antitoxin with 10 L_+ doses of diluted Standard Toxin.

(iv) Dilute 1 ml of serum with 1 ml of diluent (1:2) and combine 1 ml of this

solution with 10 L_o doses of diluted Standard Toxin.

(v) Neutralize all toxin-antitoxin mixtures at room temperature for 1 hour and hold in ice water until injections of mice can be made.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 24 hours post-injection and record all deaths.

(5) Test Interpretation shall be as follows:

(i) If any mice inoculated with the mixture of 1 International Unit of Standard Antitoxin and 10 L_o doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) If less than 80 percent of the mice inoculated with mixture of 1 International Unit of Standard Antitoxin and 10 L_+ doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(iii) If any mice inoculated with the mixture of serum with 10 L_o doses of Standard Toxin die, the serum is considered to contain less than 2 International Units per ml, and the serial is unsatisfactory.

[39 FR 16865, May 10, 1974; 39 FR 20783, June 14, 1974. Redesignated at 39 FR 25463, July 11, 1974, and amended at 40 FR 759, Jan. 3, 1975; 40 FR 41088, Sept. 5, 1975. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66785, Dec. 26, 1991; 62 FR 31331, June 9, 1997]

§ 113.113 Autogenous biologics.

Autogenous biologics shall be prepared from cultures of microorganisms which have been inactivated and are nontoxic. Such products shall be prepared only for use by or under the direction of a veterinarian under a veterinarian-client-patient relationship, *Provided,* That, such products may be prepared for use under the direction of a person of appropriate expertise in specialized situations such as aquaculture, if approved by the Administrator.

Each serial of an autogenous biologic shall meet the requirements in this section, and if found unsatisfactory by any prescribed test shall not be used.

(a) *Seed requirements.* The microorganisms used as seed to prepare autogenous biologics shall be microorganisms which are isolated from sick or dead animals in the herd of origin and which there is reason to believe are the causative agent(s) of the current disease affecting such animals.

(1) More than one microorganism isolated from the same herd may be used as seed.

(2) Under normal circumstances, microorganisms from one herd must not be used to prepare an autogenous biologic for another herd. The Administrator, however, may authorize preparation of an autogenous biologic for use in herds adjacent to the herd of origin, when adjacent herds are considered to be at risk. To request authorization to prepare a product for use in herds adjacent to the herd of origin, the establishment seeking authorization must submit to the Administrator (in c/o the Director, Center for Veterinary Biologics, Inspection and Compliance, 510 South 17th Street, Suite 104, Ames, IA 50010–8197) the following information. (If any of the data are unavailable, the applicant for authorization should indicate that such data are unavailable and why.)

(i) Name, address, and phone number of the owner of the herd of origin.

(ii) Attending veterinarian's name, address, and phone number.

(iii) Animal species and number in herd of origin.

(iv) Identification of microorganism(s), at least to genus.

(v) Diagnosis or clinical signs of the disease observed.

(vi) Name and address of the person who isolated the microorganism(s) and the date of isolation.

(vii) Number of doses of autogenous biologic requested and vaccination schedule.

(viii) Each adjacent herd owner's name, address, and phone number.

(ix) Number of animals and species in each adjacent herd.

(x) The attending veterinarian's or approved specialist's assessment of the

involvement of the adjacent herd(s) with the disease observed.

The applicant shall give notice to the State Veterinarian or other appropriate State Official in writing when an autogenous biologic is to be used in adjacent herds.

(3) The Administrator may authorize preparation of an autogenous biologic for use in herds which are not adjacent to the herd of origin, but which he or she considers to be at risk of infection with the same microorganism(s). Except as provided below, the same information which is required for preparation of such product for use in herds adjacent to the herd of origin must be submitted to the Administrator (in c/o the Director, Center for Veterinary Biologics, Inspection and Compliance, 510 South 17th Street, Suite 104, Ames, IA 50010–8197) for authorization to prepare a product for use in herds not adjacent to the herd of origin. Because the recipient herd involved may not be known when autogenous biologics are to be used in other geographic areas, the following data may be used in place of the data required in paragraphs (a)(2)(viii) and (a)(2)(ix) of this section.

(i) Names and addresses of practitioners in the area in place of the name, address, and phone number of the adjacent herd owner.

(ii) The geographic designations of the area involved.

(iii) A summary of the epidemiology of the disease situation that links the designated geographic areas with the herd of origin.

In addition, an applicant for authorization under this paragraph (a)(3) shall provide written approval from the State Veterinarian or other appropriate State Official in the State in which the autogenous biologic is to be used in nonadjacent herds.

(4) Under normal circumstances, microorganism(s) used for the production of autogenous biologics may not be older than 15 months from the date of isolation, or 12 months from the date of harvest of the first serial of product produced from the microorganism(s), whichever comes first. The Administrator, however, may authorize production of additional serials from microorganism(s) older than the above stated time periods, *Provided*, That, the person requesting such authorization submits the following supporting information to the address listed in paragraph (a)(3):

(i) The attending veterinarian's or approved specialist's current assessment of the continued involvement of a herd with the originally isolated microorganism(s), including a summary of the diagnostic work that has been done to support this assessment.

(ii) Evidence of satisfactory protection from the previous use of the autogenous biologic produced from the microorganisms involved.

(iii) Any other information the Administrator may require in order to determine the need to use the microorganism to make additional serials.

(b) *Restrictions.* Unless otherwise authorized by the Administrator, each serial of an autogenous biologic shall be subject to the following restrictions:

(1) Microorganisms used to prepare autogenous biologics shall not be maintained in the licensed establishment beyond the time authorized for use in production.

(2) The expiration date of the autogenous biologic shall not exceed 18 months from the date of harvest.

(c) *Testing requirements for autogenous biologics.* (1) Final container samples of completed product from the first serial or subserial of an autogenous biologic produced from an isolate shall be tested for purity as prescribed in § 113.26, and for safety as prescribed in § 113.33(b) or § 113.38 except that:

(i) When the number of final containers in a serial or subserial is 50 or less, two final container samples from each serial and subserial shall be tested as prescribed in § 113.26(b): *Provided*, That, 1 ml aliquots from each sample may be inoculated into five corresponding individual test vessels of each of the test media required.

(ii) Serials which are satisfactory after the third day of observation of purity test cultures and of safety test animals may be released for shipment to the customer and the tests continued throughout the required period; and

(iii) Serials released on the basis of satisfactory results of third day observations shall be immediately recalled if evidence of contamination occurs in

test cultures or if any of the test animals used to demonstrate product safety, sicken, or die during the observation period.

(iv) Test summaries must be submitted to the Administrator (in c/o the Director, Center for Veterinary Biologics, Inspection and Compliance, 510 South 17th Street, Suite 104, Ames, IA 50010–8197) on a quarterly basis by the 21st day of January, April, July, and October or more often as required by the Administrator.

(2) Each serial or subserial of autogenous bacterial product other than the first serial or subserial produced from an isolate shall meet the applicable general requirements prescribed in §113.100 and the special requirements prescribed in this section. Each serial or subserial of autogenous viral product other than the first serial or subserial produced from an isolate shall meet the applicable general requirements prescribed in §113.200 and the special requirements prescribed in this section. A serial or subserial found unsatisfactory by any prescribed test shall not be released.

(i) *Purity test*. Final container samples of completed product from each serial and subserial shall be tested for viable bacteria and fungi as provided in §113.26. When the number of final containers in a serial or subserial is 50 or less, two final container samples from each serial and subserial shall be tested as prescribed in §113.26(b): *Provided*, That, 1 ml aliquots from each sample may be inoculated into five corresponding individual test vessels of each of the test media required.

(ii) *Safety test*. Bulk of final container samples of completed product from each serial shall be tested for safety as provided in §113.33 (b) or §113.38.

(iii) *Identification*. All microorganisms used for the production of autogenous biologics shall be identified as follows: Bacteria, fungi, and mycoplasma shall be identified at least to genus and species. Viruses shall be identified at least to family. After 15 months from the date of isolation, or 12 months from the harvest date of the first serial of autogenous product produced from a microorganism, whichever comes first, characterization and identification shall be completed to strain and/or serotype before such microorganism may be used for production.

(iv) *Antigenicity, or immunogenicity, and potency*. Persons seeking authorization to prepare additional serials of autogenous biologics from microorganisms that are older than 24 months from the date of isolation, shall be required to conduct the following additional tests:

(A) Completed product shall be tested for antigenicity or immunogenicity in the species for which the product is recommended or in another animal species whose immunological response has been shown in the scientific literature to correlate with the response of the species for which the product is recommended. Such tests shall be conducted in accordance with a protocol developed by the licensee and approved by the Administrator and the results submitted to the Director, Center for Veterinary Biologics, Licensing and Policy Development, 510 South 17th Street, Suite 104, Ames, IA 50010–8197 for review. Microorganisms not shown to be antigenic (that is, not shown to induce a significant serological response) or immunogenic by such approved tests shall not be used for the preparation of such product.

(B) Bulk or final container samples of completed product from each serial of such autogenous biologics containing fractions for which standard requirement potency test procedures have been established shall be tested for potency in accordance with applicable standard requirement potency tests provided in 9 CFR part 113. If the culture of microorganisms used to produce such fractions is shown to be of a different strain or serotype than the reagent or challenge microorganisms used in the standard requirement potency test, reagents or challenges of the same strain or serotype as the microorganism used for production may be used.

(C) If no standard requirement potency test procedures have been established for a fraction(s) in the autogenous biologic, such fraction(s) of each serial of product shall be tested for potency using a developmental potency test described in the filed outline of production or shall at least be standardized to contain an antigenic mass

for such fraction(s) that has been shown to be antigenic or immunogenic in accordance with paragraph (c)(2)(iv)(A) of this section.

[57 FR 38756, Aug. 27, 1992, as amended at 59 FR 67616, Dec. 30, 1994; 64 FR 43044, Aug. 9, 1999; 67 FR 15714, Apr. 3, 2002]

§ 113.114 Tetanus Toxoid.

Tetanus Toxoid shall be produced from a culture of *Clostridium tetani* which has been inactivated and is nontoxic. The toxoid may be either absorbed, precipitated, or purified and concentrated. Each serial of biological product containing *tetanus toxoid* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial or subserial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency. A group of 10 guinea pigs consisting of an equal number of males and females weighing 450 to 550 grams shall each be injected subcutaneously with 0.4 of the largest dose recommended on the product labels.

(1) Six weeks after injection, all surviving guinea pigs shall be bled and equal portions of serum, but not less than 0.5 ml from each, shall be pooled. For a valid test, the pool shall contain the serum from at least eight animals.

(2) The antitoxin titer of the pooled serum shall be determined in antitoxin units (A.U.) per ml using an enzyme-linked immunosorbent assay method acceptable to the Animal and Plant Health Inspection Service.

(3) If the antitoxin titer of the serum pool is at least 2.0 A.U. per ml, the serial is satisfactory. If the antitoxin titer of the serum pool is less than 2.0 A.U. per ml, the serial may be retested by the following procedure: *Provided,* That, if the serial is not retested, it shall be declared unsatisfactory.

(4) For serials in which the serum pool contains less than 2.0 A.U. per ml, the individual serum that constituted the pool may be tested by the enzyme-linked immunosorbent assay. If at least 80 percent of the individual serums have an antitoxin titer of at least 2.0 A.U. per ml, the serial is satisfactory. If less than 80 percent of the individual serums have an antitoxin titer of at least 2.0 A.U. per ml, the serial may be retested in 10 guinea pigs using the procedure described in (c)(1) and (2) above. The antitoxin titer of the pooled serum from the guinea pigs used in the retest shall be averaged with the antitoxin level of the pooled serum from the initial test. If the average of the two pools is at least 2.0 A.U. per ml, the serial is satisfactory. If the average of the two pools is less than 2.0 A.U. per ml, the serial is unsatisfactory and shall not be retested further.

[39 FR 16862, May 10, 1974, as amended at 46 FR 23224, Apr. 24, 1981; 50 FR 24905, June 14, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 37827, Aug. 9, 1991; 56 FR 66785, Dec. 26, 1991]

§ 113.115 Staphylococcus Aureus Bacterin-Toxoid.

Staphylococcus Aureus Bacterin-Toxoid shall be prepared from toxoided broth cultures of selected toxogenic strains of *Staphylococcus aureus* which has been inactivated and is nontoxic. Each serial of biological product containing Staphylococcus Aureus Bacterin-Toxoid shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product shall be tested for safety as provided in § 113.33(b). Also, the rabbits used in the potency test provided in paragraph (c) of this section shall constitute an additional safety test. If unfavorable reactions attributable to the product occur

in any of the rabbits during the observation period, the serial is unsatisfactory.

(c) *Potency test.* Rabbits, each weighing 2000–3000 grams, shall be used as test animals. Either a five rabbit individual serum test or an eight rabbit pooled serum test shall be conducted. At the start of the test, individual serums from the five rabbits or pooled serums from the eight rabbits shall contain less than 0.2 alpha antitoxin units per ml.

(1) Each rabbit shall be given a series of not more than three intramuscular injections at 7 day intervals (1.0 ml, 2.0 ml, 3.0 ml) and observed from 7–14 days following the third injection. At the end of the observation period, a blood sample shall be taken from each rabbit.

(2) The sample of serum from each rabbit, if the five rabbit individual test is conducted or a pooled sample of equal quantities of serum from the rabbits if the eight rabbit pooled serum test is conducted, shall be tested to determine the staphylococcus alpha antitoxin units per ml as provided in paragraphs (c)(3), (4), (5), (6), (7), and (8) of this section.

(3) Inactivate rabbit serum 56 °C for 30 minutes.

(4) Make serial twofold dilutions of the serum samples and conduct the test, using 1 ml of the serial dilutions. Appropriate controls should be included for accurate interpretations.

(5) Add 1 ml of the standardized toxin containing the established ''Lh'' dose. The ''Lh'' dose is the amount of toxin which when mixed with one unit of standard antitoxin produces a 50 percent hemolysis of rabbit red blood cells.

(6) Incubate toxin-antitoxin mixture at room temperature for 30 minutes and add 1 ml of a 1.5 percent suspension of washed freshly drawn rabbit red blood cells suspended in normal saline to each tube. Mix and incubate the combined product in a 37 °C water bath for 1 hour. Refrigerate at 5 °C overnight.

(7) Read the hemolysis produced and establish the 50 percent end point. The 50 percent end point of hemolysis should be established by determining the size of the button produced by the unlysed red blood cells.

(8) Determine the units of antitoxin per 1 ml of serum.

(9) If the individual samples from four of the five rabbits in the individual serum test or the pooled samples from the eight rabbits in the pooled serum test do not contain three alpha antitoxin units per ml, the serial is unsatisfactory.

[39 FR 16862, May 10, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.116 Pasteurella Multocida Bacterin, Avian Isolate, Type 4.

Pasteurella Multocida Bacterin, Avian Isolate, Type 4 shall be prepared from cultures of *Pasteurella multocida,* avian isolate, Type 4 (Little and Lyons classification), which have been inactivated, and are nontoxic. Each serial of biological product containing Pasteurella Multocida Bacterin, Avian Isolate, Type 4, shall meet the applicable requirements in §113.100 and shall be tested for purity, safety, and potency, as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in 9 CFR 113.26.

(b) *Safety test.* Observation of the vaccinated turkeys during the prechallenge period of the potency test provided in paragraph (c) of this section shall constitute the safety test. If unfavorable reactions that are attributable to the product occur, the serial is unsatisfactory. If unfavorable reactions that are not attributable to the product occur in one turkey, test results shall be determined by observing the remaining 20 turkeys. The test is inconclusive and may be repeated if unfavorable reactions that are not attributable to the product occur in two or more turkeys, but the serial is unsatisfactory if the test is not repeated.

(c) *Potency test.* Bulk or final container samples of completed product shall be tested for potency of the Type 4 strain, using the two-stage test provided in this paragraph. Turkeys at least 6 weeks old obtained from the same source and hatch shall be properly identified and used as provided in this paragraph.

(1) *Vaccinates.* Each of not more than 21 turkeys shall be vaccinated with the dose and by the route recommended on the label. A second dose shall be given after 3 weeks and the turkeys observed for an additional 2-week prechallenge period.

(2) *Unvaccinated controls.* Each of not more than 11 turkeys shall be held as controls.

(3) *Challenge.* Not less than 14 days after the second dose, each of 20 vaccinates, and each of 10 unvaccinated controls shall be challenged intramuscularly with virulent *Pasteurella multocida,* Strain P–1662, Type 4 (Little and Lyons classification) and observed daily for a 14-day postchallenge period. Only dead birds shall be considered in evaluating the product.

(4) *Validity requirements.* Eight or more unvaccinated controls must die for the test to be valid. If this requirement is met, the potency test results are evaluated according to stage one of the following table. The test is inconclusive and may be repeated if the validity requirement is not met, but the serial is unsatisfactory if the test is not repeated.

Stage	Number of vaccinates	Cumulative number of vaccinates	Cummulative total number of dead vaccinates for_____	
			Satisfactory serial	Unsatisfactory serial
1	20	20	6 or less	9 or more.
2	20	40	15 or less ...	16 or more.

(5) The serial shall pass or fail based on the stage one results of the potency test. However, the second stage may be conducted if seven or eight vaccinates die in stage one, but the serial is unsatisfactory if the second stage is not conducted.

(6) The second stage shall be conducted in a manner identical to the first stage. The serial shall be evaluated according to stage two of the table. On the basis of accumulated results from the data of both stage tests, a serial shall either pass or fail the second stage.

[47 FR 5795, Feb. 4, 1982; 47 FR 6817, Feb. 17, 1982, as amended at 52 FR 9117, Mar. 23, 1987. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.117 Pasteurella Multocida Bacterin, Avian Isolate, Type 1.

Pasteurella Multocida Bacterin, Avian Isolate, Type 1, shall be prepared from cultures of *Pasteurella multocida,* avian isolate, Type 1 (Little and Lyons classification), which have been inactivated and are nontoxic. Each serial of biological product containing Pasteurella Multocida Bacterin, Avian Isolate, Type 1, shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Observation of the vaccinated chickens during the prechallenged period of the potency test provided in paragraph (c) of this section shall constitute the safety test. If unfavorable reactions that are attributable to the product occur, the serial is unsatisfactory. If unfavorable reactions that are not attributable to the product occur in one chicken, test results shall be determined by observing the remaining 20 chickens. The test is inconclusive and may be repeated if unfavorable reactions that are not attributable to the product occur in two or more chickens, but the serial is unsatisfactory if the test is not repeated.

(c) *Potency test.* Bulk or final container samples of completed product shall be tested for potency of the Type 1 strain, using the two-stage test provided in this paragraph. Chickens, at least 12 weeks of age, obtained from the same source and hatch, shall be properly identified and used as provided in this paragraph.

(1) *Vaccinates.* Each of not more than 21 chickens shall be injected with the dose and by the route recommended on the label. A second dose shall be injected after 3 weeks and the chickens observed for an additional 2 week prechallenge period.

(2) *Unvaccinated controls.* Each of not more than 11 chickens shall be held as controls.

(3) *Challenge.* Not less than 14 days after the second injection, each of 20 vaccinates, and each of 10 unvaccinated

controls shall be challenged intramuscularly with a minimum of 250 colony-forming units of virulent *Pasteurella multocida*, Strain X–73, Type 1 (Little and Lyons classification) and observed daily for a 14-day postchallenge period. Only dead birds shall be considered in evaluating the product.

(4) *Validity requirements.* Eight or more unvaccinated controls must die for the test to be valid. If these requirement are met, the potency test results are evaluated according to stage one of the following table. The test is inconclusive and may be repeated if the validity requirements are not met, but the serial is unsatisfactory if the test is not repeated.

Stage	Number of vaccinates	Cumulative number of vaccinates	Cummulative total number of dead vaccinates for_____	
			Satisfactory serial	Unsatisfactory serial
1	20	20	6 or less	9 or more.
2	20	40	15 or less ..	16 or more.

(5) The serial shall pass or fail based on the stage one results of the potency test. However, the second stage may be conducted if seven or eight vaccinates die in stage one, but the serial is unsatisfactory if the second stage is not conducted.

(6) The second stage shall be conducted in a manner identical to the first stage. The serial shall be evaluated according to stage two of the table. On the basis of accumulated results from the data of both stage tests, a serial shall either pass or fail the second stage.

[39 FR 16866, May 10, 1974; 39 FR 20368, June 10, 1974, as amended at 40 FR 759, Jan. 3, 1975; 40 FR 23989, June 4, 1975; 47 FR 5195, Feb. 4, 1982; 52 FR 9118, Mar. 23, 1987. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.118 Pasteurella Multocida Bacterin, Avian Isolate, Type 3.

Pasteurella Multocida Bacterin, Avian Isolate, Type 3, shall be prepared from culture of *Pasteurella multocida*, avian isolate, Type 3 (Little and Lyons classification), which have been inactivated and are nontoxic. Each serial of biological product containing Pasteurella Multocida Bacterin, Avian Isolate, Type 3, shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency, as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Observation of the vaccinated turkeys during the prechallenge period of the potency test provided in paragraph (c) of this section shall constitute the safety test. If unfavorable reactions that are attributable to the product occur, the serial is unsatisfactory. If unfavorable reactions that are not attributable to the product occur in one turkey, test results shall be determined by observing the remaining 20 turkeys. The test is inconclusive and may be repeated if unfavorable reactions that are not attributable to the product occur in two or more turkeys, but the serial is unsatisfactory if the test is not repeated.

(c) *Potency test.* Bulk or final container samples of completed product shall be tested for potency of the Type 3 strain, using the two-stage test provided in this paragraph. Turkeys, at least 6 weeks of age, obtained from the same source and hatch, shall be properly identified and used as provided in this paragraph.

(1) *Vaccinates.* Each of not more than 21 turkeys shall be injected with the dose and by the route recommended on the label. A second dose shall be injected after 3 weeks and the turkeys observed for an additional 2 week prechallenge period.

(2) *Unvaccinated controls.* Each of not more than 11 turkeys shall be held as controls.

(3) *Challenge.* Not less than 14 days after the second injection, each of 20 vaccinates, and each of 10 unvaccinated controls shall be challenged intramuscularly with a minimum of 150 colony-forming units of virulent *Pasteurella multocida*, Strain P–1059, Type 3 (Little and Lyons Classification) and observed daily for a 14-day postchallenge period. Only dead birds shall be considered in evaluating the product.

(4) *Validity requirements.* Eight or more unvaccinated controls must die for the test to be valid. If these requirements are met, the potency test results are evaluated according to stage one of the following table. The test is inconclusive and may be repeated if the validity requirements are not met, but the serial is unsatisfactory if the test is not repeated.

Stage	Number of vaccinates	Cumulative number of vaccinates	Cummulative total number of dead vaccinates for_____	
			Satisfactory serial	Unsatisfactory serial
1	20	20	6 or less	9 or more.
2	20	40	15 or less ...	16 or more.

(5) The serial shall pass or fail based on the stage one results of the potency test. However, the second stage may be conducted if seven or eight vaccinates die in stage one, but the serial is unsatisfactory if the second stage is not conducted.

(6) The second stage shall be conducted in a manner identical to the first stage. The serial shall be evaluated according to stage two of the table. On the basis of accumulated results from the data of both stage tests, a serial shall either pass or fail the second stage.

[39 FR 16862, May 10, 1974, as amended at 40 FR 759, Jan. 3, 1975; 47 FR 5196, Feb. 4, 1982; 52 FR 9118, Mar. 23, 1987. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.119 Erysipelothrix Rhusiopathiae Bacterin.

Erysipelothrix Rhusiopathiae Bacterin shall be produced from a culture of *Erysipelothrix rhusiopathiae* which has been inactivated and is nontoxic. Each serial of biological product containing *Erysipelothrix rhusiopathiae* shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the mouse protection test provided in this paragraph. A mouse dose shall be 1/10 of the least dose recommended on the label for swine. Such swine dose shall not be less than 1 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Animal and Plant Health Inspection Service.

(2) At least three threefold dilutions shall be made with the Standard and the same threefold dilutions shall be made for each Unknown. Dilutions shall be made with physiological saline solution.

(3) For each dilution of the Standard and each dilution of an Unknown, a group of at least 20 mice, each weighing 16 to 22 grams, shall be used. Each mouse in each group shall be injected subcutaneously with one mouse dose of the appropriate dilution.

(4) Each of 20 injected mice from each group shall be challenged subcutaneously 14 to 21 days after being injected. A dose containing at least 100 mouse LD_{50} of a suitable culture of *Erysipelothrix rhusiopathiae* shall be used. All survivors in each group of mice shall be recorded 10 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.6, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test: *Provided,* That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than 0.6, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than 0.6, the serial is unsatisfactory without further testing. If the average RP obtained in the retests is equal to or greater than 0.6, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory for potency.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than 0.6, the serial is unsatisfactory.

[39 FR 16862, May 10, 1974, as amended at 40 FR 759, Jan. 3, 1975; 40 FR 20067, May 8, 1975; 40 FR 51414, Nov. 5, 1975; 44 FR 71408, Dec. 11, 1979; 50 FR 23795, June 6, 1985; 51 FR 23731, July 1, 1986. Redesignated at 55 FR 35562, Aug. 31, 1990; 56 FR 66558, Dec. 24, 1991; 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.120 Salmonella Typhimurium Bacterin.

Salmonella Typhimurium Bacterin shall be prepared from a culture of *Salmonella typhimurium* which has been inactivated and is nontoxic. Each serial of biological product containing *Salmonella typhimurium* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the mouse test provided in this paragraph. A mouse dose shall be 1/20 of the least dose recommended on the label for other animals which shall not be less than 2 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Animal and Plant Health Inspection Service.

(2) At least three tenfold dilutions shall be made with the Standard and the same tenfold dilutions shall be made for each Unknown. The dilutions shall be made in Phosphate Buffered Saline.

(3) For each dilution of the Standard and each dilution of an Unknown, a group of at least 20 mice, each weighing 16-22 grams, shall be used. Each

mouse in a group shall be injected intraperitoneally with one mouse dose of the appropriate dilution. Each mouse shall be revaccinated on day 14, using the same schedule.

(4) Each of 20 vaccinated mice per group shall be challenged intraperitoneally 7–10 days after the second vaccination with a 0.25 ml dose containing 100-10,000 mouse LD_{50} as determined by titration, of a suitable culture of *Salmonella typhimurium*. All survivors in each group of mice shall be recorded 14 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the Standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.30, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test; *Provided*, That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of this section, the serial is unsatisfactory.

[40 FR 17003, Apr. 16, 1975, as amended at 42 FR 59487, Nov. 18, 1977; 48 FR 31008, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.121 Pasteurella Multocida Bacterin.

Pasteurella Multocida Bacterin shall be prepared from a culture of *Pasteurella multocida* strains other than avian which have been inactivated and are nontoxic. Each serial of biological product containing *Pasteurella multocida* fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b). The subcutaneous route is to be used.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the mouse test provided in this paragraph. A mouse dose shall be 1/20 of the least dose recommended on the label for other animals which shall not be less than 2 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Animal and Plant Health Inspection Service.

(2) At least three fivefold dilutions shall be made with the Standard and the same fivefold dilutions shall be made for each Unknown. The dilutions will be made in Phosphate Buffered Saline.

(3) For each dilution of the Standard and each dilution of each Unknown, a group of at least 20 mice, each weighing 16–22 grams, shall be used. Each mouse in a group shall be injected intraperitoneally with one mouse dose of the appropriate dilution. Each mouse shall be revaccinated on day 14, using the same schedule.

(4) Each of 20 injected mice per group shall be challenged intraperitoneally 10–12 days after the second vaccination with a 0.2 ml dose containing 100–10,000 mouse LD_{50}, as determined by titration, of a suitable culture of *Pasteurella multocida*. All survivors in each group of mice shall be recorded 10 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the Standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.50, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test: *Provided,* That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of

this section, the serial is unsatisfactory.

[40 FR 17004, Apr. 16, 1975, as amended at 42 FR 59487, Nov. 18, 1977; 48 FR 31008, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.122 Salmonella Choleraesuis Bacterin.

Salmonella Choleraesuis Bacterin shall be prepared from a culture of *Salmonella choleraesuis* which has been inactivated and is nontoxic. Each serial of biological product containing *Salmonella choleraesuis* fraction shall meet the applicable requirements in 9 CFR 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in 9 CFR 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in 9 CFR 113.33(b).

The subcutaneous route shall be used when the product is in combination with Pasteurella Multocida Bacterin.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the mouse test provided in this paragraph. A mouse dose shall be 1/20 of the least dose recommended on the label for other animals which shall not be less than 2 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Veterinary Services.

(2) At least three fivefold dilutions shall be made with the Standard and the same fivefold dilution shall be made for each Unknown. The dilutions shall be made in Phosphate-Buffered Saline.

(3) For each dilution of the Standard and each dilution of an Unknown, a group of at least 20 mice, each weighing 16 to 22 grams, shall be used. Each mouse in a group shall be injected intraperitoneally with one mouse dose of the appropriate dilution. Each mouse shall be revaccinated on day 14, using the same schedule.

(4) Each of 20 vaccinated mice per group shall be challenged intraperitoneally 7 to 10 days after the second vaccination with a 0.25 ml dose containing 10–1,000 mouse LD_{50} as determined by titration of a suitable culture of *Salmonella choleraesuis.* All survivors in each group of mice shall be recorded 14 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the Standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.50, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test; *Provided,* That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of this section, the serial is unsatisfactory.

[43 FR 25077, June 9, 1978, as amended at 48 FR 31008, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.123 Salmonella Dublin Bacterin.

Salmonella Dublin Bacterin shall be prepared from a culture of *Salmonella dublin* which has been inactivated and is nontoxic. Each serial of biological product containing *Salmonella dublin* fraction shall meet the applicable requirements in 9 CFR 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in 9 CFR 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in 9 CFR 113.33(b).

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the mouse test provided in this paragraph. A mouse dose shall be 1/20 of the least dose recommended on the label for other animals which shall not be less than 2 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Veterinary Services.

(2) At least three tenfold dilutions shall be made with the Standard and the same tenfold dilutions shall be made for each Unknown. The dilutions shall be made in Phosphate-Buffered Saline.

(3) For each dilution of the Standard and each dilution of an Unknown, a group of at least 20 mice, each weighing 16 to 22 grams, shall be used. Each mouse in a group shall be injected intraperitoneally with one mouse dose of the appropriate dilution. Each mouse shall be revaccinated on day 14, using the same schedule.

(4) Each of 20 vaccinated mice per group shall be challenged intraperitoneally 7 to 10 days after the second vaccination with a 0.25 ml dose containing 1,000–100,000 mouse LD_{50} as determined by titration of a suitable culture of *Salmonella dublin*. All survivors in each group of mice shall be recorded 14 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the Standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.30, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown cannot be calculated because

the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test; *Provided,* That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of this section, the serial is unsatisfactory.

[43 FR 25077, June 9, 1978, as amended at 48 FR 31009, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

KILLED VIRUS VACCINES

§ 113.200 General requirements for killed virus vaccines.

When prescribed in an applicable Standard Requirement or in the filed Outline of Production, a killed virus vaccine shall meet the applicable requirements in this section.

(a) *Killing agent.* The vaccine virus shall be killed (inactivated) by an appropriate agent. The procedure involved may be referred to as inactivation. Suitable tests to assure complete inactivation shall be written into the filed Outline of Production.

(b) *Cell culture requirements.* If cell cultures are used in the preparation of the vaccine, primary cells shall meet the requirements in § 113.51 and cell lines shall meet the requirements in § 113.52.

(c) *Purity tests*—(1) *Bacteria and fungi.* Final container samples of completed product from each serial shall be tested as prescribed in § 113.26.

(2) *Avian origin vaccine.* Bulk pooled material or final container samples from each serial shall also be tested for:

(i) *Salmonella* contamination as prescribed in § 113.30; and

(ii) *Lymphoid leukosis virus* contamination as prescribed in § 113.31; and

(iii) *Hemagglutinating viruses* as prescribed in § 113.34.

(3) *Mycoplasma.* If the licensee cannot demonstrate that the agent used to kill the vaccine virus would also kill mycoplasma, each serial of the vaccine shall be tested for mycoplasma as prescribed in § 113.28, prior to adding the killing agent. Material found to contain mycoplasma is unsatisfactory for use.

(4) *Extraneous viruses.* Each lot of Master Seed Virus used to prepare killed virus vaccine recommended for animals other than poultry shall meet the requirements for extraneous viruses as prescribed in § 113.55.

(d) *Safety tests.* Final container samples of completed product from each serial shall be tested for safety in guinea pigs as prescribed in § 113.38 and for safety in mice as prescribed in § 113.33: *Provided,* That, vaccines recommended for use only in poultry are exempt from this requirement.

(e) *Viricidal activity test.* Only serials tested for viricidal activity in accordance with the test provided in § 113.35 and found satisfactory by such test shall be packaged as diluent for desiccated fractions in combination packages.

(f) *Formaldehyde content.* If formaldehyde is used as the killing agent, the residual free formaldehyde content must not exceed 0.74 grams per liter (g/L) as determined using the ferric chloride test.[3] Firms currently using tests for residual free formaldehyde content other than the ferric chloride test have until July 14, 2004 to update their Outline of Production to be in compliance with this requirement.

[39 FR 27428, July 29, 1974, as amended at 40 FR 23989, June 4, 1975; 43 FR 49528, Oct. 24, 1978. Redesignated at 55 FR 35562, Aug. 31, 1990; 68 FR 35283, June 13, 2003]

§ 113.201 Canine Distemper Vaccine, Killed Virus.

Canine Distemper Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.200.

(b) The immunogenicity of vaccine prepared from the Master Seed Virus in accordance with the Outline of Production shall be established. Vaccine used for this test shall be at the highest passage from the Master Seed and prepared at the minimum preinactivation titer specified in the Outline of Production.

(1) Twenty-five canine distemper susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine distemper to determine susceptibility. A constant virus-varying serum neutralization test in cell culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(i) The 20 dogs used as vaccinates shall be injected with one dose of vaccine by the method recommended on the label. If a second dose is recommended, the second dose shall be administered at the time specified on the label.

(ii) At least 14 days after the last inoculation, the vaccinates and controls shall each be challenged intracerebrally with canine distemper virus furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 21 days.

(iii) If at least four of the five controls do not die and the survivor, if any, does not show clinical signs of canine distemper, the test is inconclusive and may be repeated.

(iv) If at least 19 of the 20 vaccinated do not survive without showing clinical signs of canine distemper during the observation period, the Master Seed Virus is unsatisfactory.

(c) *Test requirements for release.* Each serial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements for safety and potency provided in this section.

(1) *Safety test.* The vaccinates used in the potency test in paragraph (c)(2) of this section shall be observed each day during the postvaccination observation period. If unfavorable reactions occur which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial is unsatisfactory.

(2) *Potency test—serum neutralization test.* Bulk or final container samples of completed product shall be tested for potency using five susceptible dogs (four vaccinates and one control) as the test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine distemper virus to determine susceptibility.

(i) A constant virus-varying serum neutralization test in tissue culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

[3] The procedures for performing the ferric chloride test for residual free formaldehyde may be obtained from USDA, APHIS, Center for Veterinary Biologics-Laboratory, 1800 Dayton Road, P.O. Box 844, Ames, IA 50010.

(ii) *Vaccination.* Each of the four vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be administered at the time specified on the label. The dogs shall be observed each day for at least 14 days after the last inoculation.

(iii) *Serology.* At the end of the post vaccination observation period, a second blood sample shall be obtained from each of the five dogs and the serums shall be individually tested for neutralizing antibody against canine distemper virus in the same manner used to determine susceptibility.

(iv) *Interpretation of the serum neutralization test.* If the control has not remained seronegative at 1:2, the test is inconclusive and may be repeated. If at least three of the four vaccinates in a valid test have not developed titers based upon a final serum dilution of at least 1:50 and the remaining vaccinate has not developed a titer of at least 1:25, the serial is unsatisfactory except as provided in paragraphs (c)(2)(v) and (vi) of this section.

(v) *Virus challenge test.* If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the control may be challenged intracerebrally with a virulent canine distemper virus furnished or approved by the Animal and Plant Health Inspection Service and each animal observed each day for an additional 21 days.

(vi) *Interpretation of the virus challenge test.* For a serial to be satisfactory, all vaccinates must remain free from clinical signs of canine distemper while the control must die of canine distemper. If the control does not die of canine distemper, the test is inconclusive and may be repeated except, that if any of the vaccinates show signs or dies of canine distemper, the serial is unsatisfactory.

[60 FR 14359, Mar. 17, 1995]

§ 113.202 Canine Hepatitis and Canine Adenovirus Type 2 Vaccine, Killed Virus.

Canine Hepatitis and Canine Adenovirus Type 2 Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.200.

(b) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity by one or both of the following methods. Vaccine used for these tests shall be at the highest passage from the Master Seed and prepared at the minimum preinactivation titer specified in the Outline of Production.

(1) *Immunogenicity for canine hepatitis.* Twenty-five canine hepatitis susceptible dogs shall be used as test animals (20 vaccinates and 5 controls). Blood samples shall be drawn from these animals and individual serum samples tested. The dogs shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test using 50 to 300 $TCID_{50}$ of canine adenovirus.

(i) The 20 dogs to be used as vaccinates shall be injected with one dose of vaccine and the remaining five dogs held as controls. If a second dose is recommended, the second dose shall be administered at the time specified on the label.

(ii) Not less than 14 days after the last inoculation, each vaccinate and control shall be challenged intravenously with virulent infectious canine hepatitis virus furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 14 days.

(iii) If at least four of the five controls do not show severe clinical signs of infectious canine hepatitis, the test is inconclusive and may be repeated.

(iv) If at least 19 of the 20 vaccinates do not survive without showing clinical signs of infectious canine hepatitis during the observation period, the Master Seed Virus is unsatisfactory.

(2) *Immunogenicity for canine adenovirus type 2.* Thirty canine adenovirus type 2 susceptible dogs shall be used as test animals (20 vaccinates and 10 controls). Blood samples shall be drawn from these animals and

individual serum samples tested. The dogs shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test using 50 to 300 $TCID_{50}$ of canine adenovirus.

(i) The 20 dogs to be used as vaccinates shall be injected with one dose of vaccine and the remaining 10 dogs held as controls. If a second dose is recommended, the second dose shall be administered at the time specified on the label.

(ii) Not less than 14 days after the last inoculation, the vaccinates and the controls shall be challenged by exposure to a nebulized aerosol of virulent canine adenovirus type 2 furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 14 days postchallenge. The rectal temperature of each animal shall be taken and the presence of respiratory or other clinical signs of canine adenovirus type 2 noted and recorded each day.

(iii) If at least 6 of 10 controls do not show clinical signs of canine adenovirus type 2 infection other than fever, the test is inconclusive and may be repeated.

(iv) If a significant difference in clinical signs in a valid test cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service, the Master Seed Virus is unsatisfactory.

(c) *Test requirements for release.* Each serial shall meet the applicable general requirements prescribed in §113.200, the special requirements for safety provided in this section, and the applicable potency tests provided in this section.

(1) *Safety test.* The vaccinates used in the potency test in paragraph (c)(2) and/or (c)(3) of this section shall be observed each day during the postvaccination observation period. If unfavorable reactions occur which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated: *Provided,* That, if not repeated, the serial is unsatisfactory.

(2) *Potency test for canine hepatitis—serum neutralization test.* Bulk or final container samples of completed product shall be tested for potency using at least five susceptible dogs (four vaccinates and one control) as the test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine adenovirus to determine susceptibility.

(i) A constant virus-varying serum neutralization test in tissue culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(ii) *Vaccination.* Each of the vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be administered at the time specified on the label. The dogs shall be observed each day for at least 14 days after the last inoculation.

(iii) *Serology.* At the end of the postvaccination observation period, a second blood sample shall be obtained from each of the dogs and the serums shall be individually tested for neutralizing antibody against canine adenovirus in the same manner used to determine susceptibility.

(iv) *Interpretation of the serum neutralization test.* If the control(s) has not remained seronegative at 1:2, the test is inconclusive and may be repeated. If at least 75 percent of the vaccinates in a valid test have not developed titers based upon final serum dilution of at least 1:10 and the remaining vaccinate(s) has not developed a titer of at least 1:2, the serial is unsatisfactory except as provided in paragraphs (c)(2)(v) and (vi) of this section.

(v) *Virus challenge test.* If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the control(s) may be challenged intravenously with a virulent canine hepatitis virus furnished or approved by the Animal and Plant Health Inspection Service and each animal observed each day for an additional 14 days.

(vi) *Interpretation of the virus challenge test.* For a serial to be satisfactory, all vaccinates must remain free of clinical signs of canine hepatitis while the control(s) must show severe

clinical signs of canine hepatitis. If the control(s) does not show severe clinical signs of canine hepatitis, the test is inconclusive and may be repeated: *Provided,* That, if any of the vaccinates show signs or die of canine hepatitis, the serial is unsatisfactory.

(3) *Potency test for canine adenovirus type 2.* Bulk or final container samples of completed product shall be tested for potency using eight susceptible dogs (five vaccinates and three controls) as the test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine adenovirus to determine susceptibility.

(i) A constant virus-varying serum neutralization test in tissue culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(ii) *Vaccination.* Each of the five vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be administered at the time specified on the label. The dogs shall be observed each day for at least 14 days after the last inoculation.

(iii) Not less than 14 days after the last inoculation, the vaccinates and the controls shall be challenged by exposure to a nebulized aerosol of virulent canine adenovirus type 2 furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 14 days postchallenge. The rectal temperature of each animal shall be taken and the presence of respiratory or other clinical signs of canine adenovirus type 2 noted and recorded each day.

(iv) If at least two of three controls do not show clinical signs of canine adenovirus type 2 other than fever, the test is inconclusive and may be repeated.

(v) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service and prescribed in the Outline of Production, the serial is unsatisfactory.

[60 FR 14359, Mar. 17, 1995]

§ 113.203 Feline Panleukopenia Vaccine, Killed Virus.

Feline Panleukopenia Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed. The Master Seed shall meet the applicable requirements prescribed in § 113.200. Each serial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements for safety and potency provided in this section.

(a) *Safety test.* The vaccinates used in the potency test in paragraph (b) of this section shall be observed each day during the postvaccination observation period. If unfavorable reactions occur which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated: *Provided,* That, if not repeated, the serial is unsatisfactory.

(b) *Potency test—serum-neutralization test.* Bulk or final container samples of completed product shall be tested for potency using five susceptible cats (four vaccinates and one control) as the test animals. Blood samples drawn from each cat shall be individually tested for neutralizing antibody against feline panleukopenia virus to determine susceptibility.

(1) A constant virus-varying serum neutralization test in tissue culture using 100 to 300 $TCID_{50}$ of virus shall be used. Cats shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(2) *Vaccination.* Each of the four vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be given 7 to 10 days after the first dose and the cats observed each day for 14 to 21 days.

(3) *Serology.* At the end of the postvaccination observation period, a second blood sample shall be obtained from each of the five cats and the serums shall be individually tested for neutralizing antibody against feline

panleukopenia virus in the same manner used to determine susceptibility.

(4) *Interpretation of the SN test.* (i) If the control has not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(ii) If at least 3 of the 4 vaccinates in a valid test have not developed titers based upon final serum dilution of at least 1:8, and the remaining vaccinate has not developed a titer of at least 1:4, the serial is unsatisfactory except as provided in paragraphs (b)(5) and (6) of this section.

(5) *Virus-challenge test.* If the results of a valid SN test are unsatisfactory, the vaccinates and the control may be challenged with a virulent feline panleukopenia virus furnished by Veterinary Services and each animal observed each day for an additional 14 days.

(6) *Interpretation of the virus-challenge test.* If the control does not show clinical signs of feline panleukopenia, the test is inconclusive and may be repeated except, that if any of the vaccinates show such signs, the serial is unsatisfactory. Clinical signs of feline panleukopenia shall include a pronounced leukopenia wherein the white blood cell count drops to 4,000 or less per cubic mm or the white cell count drops to less than 25 percent of the normal level established by an average of three or more counts taken prior to challenge.

[39 FR 27428, July 29, 1974, as amended at 40 FR 759, Jan. 3, 1975; 43 FR 41186, Sept. 15, 1978; 43 FR 50162, Oct. 27, 1978; 50 FR 23796, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

§ 113.204 Mink Enteritis Vaccine, Killed Virus.

Mink Enteritis Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids or tissues obtained from mink that have developed mink enteritis following inoculation with virulent mink enteritis virus. Each serial shall meet the applicable requirements prescribed in §113.200 and special requirements prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety test.* Vaccinates used in the potency test in paragraph (b) of this section shall be observed each day prior to challenge. If unfavorable reactions attributable to the vaccine occur, the serial is unsatisfactory. If unfavorable reactions not attributable to the vaccine occur, the test shall be declared inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial is unsatisfactory.

(b) *Potency test.* Bulk or final container samples of completed product shall be tested for potency using 10 mink enteritis susceptible mink (five vaccinates and five controls) as follows:

(1) *Vaccination.* Each of the five vaccinates shall be injected with one dose of vaccine as recommended on the label and observed each day for 14 days.

(2) *Challenge.* At least 2 weeks after the last inoculation, the five vaccinates and the five controls shall be challenged with virulent mink enteritis virus and observed each day for 12 days. Fecal material shall be collected on one day between days 4–8 (inclusive) postchallenge from each test animal that remains free of enteric signs and tested for the presence of mink enteritis virus by cell culture with fluorescent antibody examination.

(3) *Interpretation.* A serial is satisfactory if at least 80 percent of the vaccinates remain free of enteric signs and do not shed virus in the feces, while at least 80 percent of the controls develop clinical signs of mink enteritis or shed virus in the feces. If at least 80 percent of the vaccinates remain free of enteric signs and do not shed virus in the feces, while less than 80 percent of the controls develop clinical signs of mink enteritis or shed virus in the feces, the test is considered inconclusive and may be repeated: *Provided,* That, if at least 80 percent of the vaccinates do not remain well and free of detectable virus in the feces, the serial is unsatisfactory.

[39 FR 27428, July 29, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991; 60 FR 14361, Mar. 17, 1995]

§ 113.205 Newcastle Disease Vaccine, Killed Virus.

Newcastle Disease Vaccine (Killed Virus) shall be prepared from virus-

bearing tissues or fluids obtained from embryonated chicken eggs or cell cultures. With the exception of § 113.200(c)(2)(iii), each serial shall meet the applicable general requirements prescribed in § 113.200 and special requirements prescribed in this section. A serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety test.* The prechallenge part of the potency test in paragraph (b) of this section shall constitute a safety test. If unfavorable reactions attributable to the product occur in any of the vaccinates, the serial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(b) *Potency test.* A vaccination-challenge test shall be conducted using susceptible chickens 2 to 6 weeks of age at time of vaccination, properly identified and obtained from the same source and hatch.

(1) Ten or more chickens shall be vaccinated as recommended on the label and kept isolated under observation for at least 14 days.

(2) After at least 14 days post-vaccination, the vaccinates and at least 10 unvaccinated chickens that have been kept isolated as controls shall be challenged with a virulent strain of Newcastle disease virus supplied by or approved by Veterinary Services and the vaccinates observed each day for 14 days.

(3) If at least 90 percent of the controls do not show typical signs of Newcastle disease or die, the test is inconclusive and may be repeated. If at least 90 percent of the vaccinates do not remain normal, the serial is unsatisfactory.

[39 FR 27428, July 29, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

§ 113.206 Wart Vaccine, Killed Virus.

Wart Vaccine, Killed Virus, shall be prepared from virus-bearing epidermal tumors (warts) obtained from a bovine. Each serial shall meet the requirements prescribed in this section and any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Purity.* Final container samples of completed product shall meet the requirements for purity as prescribed in § 113.200 (c)(1) and (3).

(b) *Safety.* Bulk or final container samples of completed product shall meet the requirements for safety as prescribed in §§ 113.33(b) and 113.38.

(c) *Formaldehyde content.* Bulk or final container samples of completed product shall meet the requirements for formaldehyde content as prescribed in § 113.200(f).

(d) *Potency and efficacy.* The efficacy of wart vaccine has been demonstrated to the satisfaction of Veterinary Services as being a valuable biological product. The inherent nature of the product precludes the possible development of serial to serial potency tests and none is required: *Provided,* That,

(1) The vaccine shall be a tissue extract representing at least 10 percent weight to volume suspension of wart tissue; and

(2) The vaccine shall be limited to use in the prevention of warts in cattle. Labeling recommendations shall be in accordance with § 112.7(i).

[40 FR 14084, Mar. 28, 1975, as amended at 40 FR 23989, June 4, 1975; 40 FR 30803, July 23, 1975. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

§ 113.207 Encephalomyelitis Vaccine, Eastern, Western, and Venezuelan, Killed Virus.

Encephalomyelitis Vaccine, Eastern, Western, and Venezuelan, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Each serial or subserial shall meet the requirements prescribed in this section and the general requirements prescribed in § 113.200, except those in § 113.200(d). Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety test.* Bulk samples of completed product from each serial shall be tested for encephalomyelitis virus inactivation.

(1) Each of at least ten 6 to 12 hour old chickens shall be injected subcutaneously with 0.5 ml of the product and the chickens observed each day for 10 days.

(2) If unfavorable reactions attributable to the product occur in the chickens during the observation period,

the serial is unsatisfactory. If unfavorable reactions not attributable to the product occur, the test is inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial is unsatisfactory.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency in accordance with the two-stage test provided in this paragraph. For each fraction contained in the product—Eastern type, Western type, or Venezuelan type—the serological interpretations required in this test shall be made independently. A serial or subserial found unsatisfactory for any of the fractions shall not be released.

(1) For this test, a guinea pig dose shall be one-half the amount recommended on the label for a horse and shall be administered as recommended for a horse. Each of 10 healthy guinea pigs (vaccinates) shall be injected with two guinea pig doses with an interval of 14 to 21 days between doses. Two additional guinea pigs from the same source shall be held as controls.

(2) Fourteen to 21 days after the second injection, serum samples from each vaccinate and each control shall be tested by a plaque reduction, serum neutralization test using Vero 76 cells.

(3) If the control serum samples show a titer of 1:4 or greater for any fraction, the test is inconclusive for that fraction and may be repeated: *Provided,* That, if four or more of the vaccinate serum samples show a titer of less than 1:40 for the Eastern type fraction, less than 1:40 for the Western type fraction, or less than 1:4 for the Venezuelan type fraction, the serial or subserial is unsatisfactory without further testing.

(4) If two or three of the vaccinate serum samples show a titer of less than 1:40 for the Eastern type fraction, less than 1:40 for the Western type fraction, or less than 1:4 for the Venezuelan type fraction, the second stage of the test may be used for the relevant fraction(s): *Provided,* That, if a fraction is found acceptable by the first stage of the test, the second stage need not be conducted for that fraction.

(5) If the second stage is used and four or more of the vaccinate serum samples show a titer of less than 1:40 for the Eastern type fraction or the Western type fraction, or less than 1:4 for the Venezuelan type fraction, the serial or subserial is unsatisfactory.

(6) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Vaccinates	Failures for acceptance	Failures for rejection
1	10	1 or less	4 or more.
2	20	3 or less	Do.

[39 FR 44714, Dec. 27, 1974, as amended at 40 FR 14084, Mar. 28, 1975; 42 FR 45284, Sept. 9, 1977. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991; 61 FR 67930, Dec. 26, 1996]

§ 113.208 Avian Encephalomyelitis Vaccine, Killed Virus.

Avian Encephalomyelitis Vaccine (Killed Virus) shall be prepared from virus-bearing tissues or fluids obtained from embryonated chicken eggs. Each serial shall meet the general requirements prescribed in § 113.200 and the requirements prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety tests.* (1) The prechallenge part of the potency test prescribed in paragraph (b) of this section shall constitute a safety test. If any of the vaccinates develop clinical signs of disease or die due to causes attributable to the product, the serial is unsatisfactory.

(2) An inactivation test for viable avian encephalomyelitis (AE) virus shall be conducted on each serial. The test shall be conducted using susceptible chicken embryos: *Provided,* That, if a non-embryo adapted virus is used for vaccine production, the test shall be conducted in susceptible chickens.

(i) *Chicken Embryo Test.* Each of 15 or more AE susceptible 5 or 6 day old embryos shall be injected in the yolk sac with 0.2 ml of the vaccine. For a valid test, at least 80 percent of the embryos shall survive for 48 hours post-inoculation (PI). Eleven to 13 days PI, all embryos surviving the 48 hour PI period shall be examined for gross lesions of AE; all these embryos shall be normal or the serial is unsatisfactory. Concurrently, five additional embryos from the same source shall be injected with live AE virus of the production strain to serve as positive controls. At least 4

of the 5 embryos shall show evidence of AE virus infection during the 11 to 13 day PI period or the test shall be considered inconclusive and repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) *Chicken test.* Each of 10 or more AE susceptible 7 day old chickens shall be injected intracerebrally with 0.1 ml vaccine each. The chickens shall be observed each day for 28 days. If any chickens show clinical signs of AE, the serial is unsatisfactory. Concurrently, 5 additional chickens from the same source shall be injected intracerebrally with live AE virus of the production strain to serve as positive controls. At least 4 of the 5 controls shall show evidence of AE virus infection during the observation period or the test shall be inconclusive and may be repeated: *Provided,* That, if the test is not repeated, the serial shall be unsatisfactory.

(b) *Potency test.* Bulk or final container samples of completed product from each serial or one subserial shall be tested. Ten or more AE-susceptible chickens (vaccinates), 4 weeks or older, properly identified and obtained from the same source and hatch, shall be injected as recommended on the label. At least 10 additional AE-susceptible chickens, properly identified and obtained from the same source and hatch shall be kept in isolation as controls.

(1) At least 28 days post-injection, the vaccinates and the controls shall be challenged intramuscularly with a virulent AE virus and the chickens observed each day for 21 days.

(2) If at least 80 percent of the controls do not show clinical signs of or die from AE infection, the test is inconclusive and may be repeated.

(3) If at least 80 percent of the vaccinates do not remain normal, the serial is unsatisfactory.

[39 FR 12958, Dec. 27, 1974, as amended at 40 FR 41088, Sept. 5, 1975. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

§ 113.209 Rabies Vaccine, Killed Virus.

Rabies Vaccine (Killed Virus) shall be prepared from virus-bearing cell cultures or nerve tissues obtained from animals that have developed rabies infection following injection with rabies virus. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.200 and the requirements prescribed in this section.

(1) Each lot of Master Seed Virus propagated in tissue or cells of avian origin shall also be tested for extraneous pathogens by procedures prescribed in § 113.37.

(2) Each lot of Master Seed Virus propagated in primary cell cultures of mouse or hamster origin or brain tissues of mouse origin shall be tested for lymphocytic choriomeningitis (LCM) virus by the procedure prescribed in § 113.42. If LCM virus is detected, the Master Seed Virus is unsatisfactory.

(b) The immunogenicity of vaccine prepared with virus at the highest passage from the Master Seed shall be established in each species for which the vaccine is recommended. Tests shall be conducted in accordance with a protocol filed with Animal and Plant Health Inspection Service before initiation of the tests. The vaccine shall be prepared using methods prescribed in the Outline of Production. If Rabies Vaccine is to be in combination with other fractions, the product to be tested shall include all fractions to be tested.

(1) The preinactivation virus titer must be established as soon as possible after harvest by at least five separate virus titrations. A mean relative potency value of the vaccine to be used in the host animal potency test must be established by at least five replicate potency tests conducted in accordance with the standard NIH test for potency in chapter 37 of ''Laboratory Techniques in Rabies,'' Fourth Edition (1996), edited by F.X. Meslin, M.M. Kaplan, and H. Koprowski, World Health Organization, Geneva, Switzerland (ISBN 92 4 154479 1). The provisions of chapter 37 of ''Laboratory Techniques in Rabies,'' Fourth Edition (1996), are the minimum standards for achieving compliance with this section

and are incorporated by reference. These provisions state that the challenge virus standard to be used as the challenge in the NIH test and the reference vaccine for the test are available from the national control authority. In the United States, that authority is the Animal and Plant Health Inspection Service's Center for Veterinary Biologics Laboratory, located at 1800 Dayton Avenue, P.O. Box 844, Ames, IA 50010; phone (515) 239–8331; fax (515) 239–8673. This incorporation by reference was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies may be obtained from the World Health Organization Publications Center USA, 49 Sheridan Avenue, Albany, NY 12210. Copies may be inspected at the Animal and Plant Health Inspection Service, Center for Veterinary Biologics, Licensing and Policy Development, 4700 River Road, Riverdale, MD, or at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

(2) The dose of vaccine to be used in the immunogenicity test shall be no more than the amount which, on the basis of The NIH Test For Potency, has been diluted to the proposed minimum acceptable potency value.[1]

(3) Test animals shall be uniform and have no neutralizing antibodies to rabies as determined by serum–neutralization (SN) tests.

(i) Twenty-five or more animals shall be used as vaccinates. Each shall be administered a dose of vaccine at the proposed minimum potency level and by the method specified in the Outline of Production.

(ii) Ten or more additional animals shall be held as controls.

(iii) On or about 30, 90, 180, 270, and 365 days postvaccination, all test animals shall be bled and individual serum samples tested for neutralizing antibodies to rabies virus.

(iv) All surviving test animals shall be challenged intramuscularly with virulent rabies virus furnished or approved by Animal and Plant Health Inspection Service 1 year after vaccinations, except as provided in (b)(4) of this section. The challenged animals shall be observed each day for 90 days as prescribed in §113.5(b). The brain of each test animal that dies following challenges shall be examined for rabies by the fluorescent antibody test or other method acceptable to Animal and Plant Health Inspection Service.

(v) Requirements for acceptance in challenge tests shall be death due to rabies in at least 80 percent of the controls while at least 22 of 25 or 26 of 30 or a statistically equivalent number of the vaccinates remain well for a period of 90 days.

(4) An alternative to challenging all surviving test animals in accordance with paragraph (b)(3)(iv) of this section may be used when the test animals are of species other than carnivores. Vaccinates shall be challenged at 1 year postvaccination. These shall include five vaccinates with the lowest SN titers at the 270th-day bleeding, five vaccinates with the lowest SN titers at the 365th-day bleeding, and all vaccinates with SN titers below 1:10 by the mouse SN test or below 1:16 by the rapid-fluorescent-focus-inhibition test at any bleeding. At least five SN-negative controls of each species shall be challenged at the same time as the vaccinates. All SN titers shall be titrated to an endpoint. All of the challenged vaccinates must remain well for a period of 90 days, and at least 80 percent of the controls must die of rabies for a satisfactory test without further challenge. If one or more of the vaccinates die from rabies, all the remaining vaccines, regardless of titer, along with the five controls shall be challenged. The cumulative results from the two challenges shall be evaluated for acceptance as specified in paragraph (b)(3)(v) of this section.

(5) An Outline of Production change shall be made before authority for use a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(c) If more than 1 year duration of immunity is to be claimed, a duration of immunity test for the additional time shall be conducted and interpreted as prescribed in paragraph (b) of this section for the 1 year test. The test animals shall be monitored serologically at least every 180 days. The time of challenge may be adjusted accordingly.

(d) Test requirements for release: Each serial and each subserial shall meet the general requirements prescribed in § 113.200 and special requirements in this paragraph.

(1) *Purity test.* Primary cell cultures of hamster origin or brain tissues of mouse origin used in vaccine production shall be tested for LCM virus as prescribed in § 113.42. Hamster origin cells shall be disrupted and undiluted cell fluids from each lot shall be tested. Where mouse brains are used in production, at least five mice which have not been injected with rabies virus shall be sacrificed and a 10 percent suspension of brain material shall be prepared and tested.

(2) *Safety tests.* Bulk samples from each serial shall be tested for virus inactivation and safety as follows:

(i) At the end of the inactivation period, each of 20 12 to 16 gram mice shall be injected intracerebrally with 0.03 ml and two rabbits shall be injected into each cerebral hemisphere with 0.25 ml and observed each day for 21 days. The brains of animals dying between the fourth and 21st day post-injection shall be checked for rabies virus. Material from each brain recovered shall be injected into each of five mice and the mice observed each day for 14 days. The fluorescent antibody test or serum neutralization test shall be used to confirm the presence or absence or live rabies virus. If live rabies virus is confirmed, the serial is unsatisfactory unless reprocessed in accordance with § 114.18.

(ii) A test for safety in three young seronegative animals of the most susceptible species for which the vaccine is recommended shall be conducted. Each shall in injected intramuscularly with one recommended dose of vaccine. If unfavorable reactions attributable to the product occur during a 28 day observation period, the serial is unsatisfactory.

(3) *Potency test.* Bulk or final container samples of completed product from each serial must be tested for potency by tests conducted in accordance with the standard NIH test for potency in Chapter 37 of ''Laboratory Techniques in Rabies,'' Fourth Edition (1996), which is incorporated by reference at paragraph (b)(1) of this section. The relative potency of each serial must be at least equal to that used in an approved host animal immunogenicity test.

[39 FR 44715, Dec. 27, 1974, as amended at 42 FR 6794, Feb. 4, 1977; 43 FR 49528, Oct. 24, 1978; 50 FR 20090, May 14, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990; 56 FR 66784, 66786, Dec. 26, 1991; 61 FR 31823, June 21, 1996; 64 FR 45420, Aug. 20, 1999]

§ 113.210 Feline Calicivirus Vaccine, Killed Virus.

Feline Calicivirus Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200.

(b) The Master Seed shall be tested for chlamydial agents as prescribed in § 113.43.

(c) The immunogenicity of vaccine prepared from the Master Seed in accordance with the Outline of Production shall be established by a method acceptable to Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and prepared at the minimum preinactivation titer specified in the Outline of Production.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements provided in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety.* Vaccinates used in the potency test in paragraph (d)(2) of this section shall be observed each day during the prechallenge period. If unfavorable reactions occur, including oral lesions, which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

(2) *Potency.* Bulk or final container samples of completed product shall be treated for potency as follows:

(i) Eight feline calicivirus susceptible cats (five vaccinates and three controls) shall be used as test animals. Throat and nasal swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of feline calicivirus. Blood samples shall be drawn and individual serum samples tested for neutralizing antibody. The cats shall be considered suitable for use if all swabs are negative for virus isolation and all serums are negative for calicivirus antibody at the 1:2 final dilution in a 50 percent plaque reduction test or other test of equal sensitivity.

(ii) The five cats used as vaccinates shall be administered one dose of vaccine by the method recommended on the label. If two doses are recommended, the second dose shall be given after the interval recommended on the label.

(iii) Fourteen or more days after the final dose of vaccine, the vaccinates and controls shall each be challenged intranasally with virulent feline calicivirus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days postchallenge. The rectal temperature of each animal shall be taken and the presence or absence of clinical signs, particularly lesions on the oral mucosa, noted and recorded each day.

(iv) If three of three controls do not show clinical signs of feline calicivirus infection other than fever, the test is inconclusive and may be repeated.

(v) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by Animal and Plant Health Inspection Service and prescribed in the Outline of Production, the serial is unsatisfactory.

[50 FR 433, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.211 Feline Rhinotracheitis Vaccine, Killed Virus.

Feline Rhinotracheitis Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200.

(b) The Master Seed shall be tested for chlamydial agents as prescribed in § 113.43.

(c) The immunogenicity of vaccine prepared from the Master Seed in accordance with the Outline of Production shall be established by a method acceptable to Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and prepared at the minimum preinactivation titer specified in the Outline of Production.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements provided in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* Vaccinates used in the potency test in paragraphs (d)(2) of this section shall be observed each day during the prechallenge period. If unfavorable reactions occur which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

(2) *Potency test.* Bulk or final container samples of completed product shall be tested for potency as follows:

(i) Eight feline rhinotracheitis susceptible cats (five vaccinates and three controls) shall be used as test animals. Throat and nasal swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of feline rhinotracheitis virus. Blood samples shall be drawn and individual serum samples tested for neutralizing antibody. The cats shall be considered suitable for use if all swabs are negative for virus isolation and all serums are negative for rhinotracheitis virus antibody at the 1:2 final dilution in a 50 percent plaque

reduction test or other test of equal sensitivity.

(ii) The five cats used as vaccinates shall be administered one dose of vaccine by the method recommended on the label. If two doses are recommended, the second dose shall be given after the interval recommended on the label.

(iii) Fourteen or more days after the final dose of vaccine, the vaccinates and controls shall each be challenged intranasally with virulent feline rhinotracheitis virus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days postchallenge. The rectal temperature of each animal shall be taken and the presence or absence of clinical signs noted and recorded each day.

(iv) If three of three controls do not show clinical signs of feline rhinotracheitis virus infection other than fever, the test is inconclusive and may be repeated.

(v) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by Animal and Plant Health Inspection Service and prescribed in the Outline of Production, the serial is unsatisfactory.

[50 FR 433, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.212 Bursal Disease Vaccine, Killed Virus.

Bursal Disease Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable requirements prescribed in § 113.200.

(b) Each lot of Master Seed shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly.

(c) The immunogenicity of vaccine prepared in accordance with the Outline of Production shall be established by a method acceptable to Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and prepared at the minimum preinactivation titer specified in the Outline of Production. The test shall establish that the vaccine, when used as recommended on the label, is capable of inducing an immune response in dams of sufficient magnitude to provide significant protection to offspring.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety.* Vaccinates used in the potency test in paragraph (d)(2) of this section shall be observed each day during the prechallenge period. If unfavorable reactions attributable to the vaccine occur, the serial is unsatisfactory. If unfavorable reactions which are not attributable to the vaccine occur, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

(2) *Potency.* Bulk or final container samples of completed product from each serial shall be tested for potency using the two-stage potency test provided in this paragraph.

(i) *Vaccinates.* Inject each of 21 susceptible chickens 14 to 28 days of age, properly identified and obtained from the same source and hatch, with one dose of vaccine by the route recommended on the label and observe for at least 21 days.

(ii) *Controls.* Retain at least 10 additional chickens from the same source and hatch as unvaccinated controls.

(iii) *Challenge.* Twenty-one to 28 days postvaccination, challenge 20 vaccinates and 10 controls by eyedrop with a virulent infectious bursal disease virus furnished or approved by Animal and Plant Health Inspection Service.

(iv) *Postchallenge period.* Four days postchallenge, necropsy all chickens

and examine each for gross lesions of bursal disease. For purposes of this test, gross lesions shall include peribursal edema and/or edema and/or macroscopic hemorrhage in the bursal tissue. Vaccinated chickens showing gross lesions shall be counted as failures. If at least 80 percent of the controls do not have gross lesions of bursal disease in a stage of the test, that stage is considered inconclusive and may be repeated. In a valid test, the results shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of failures for—	
			Satisfactory serial	Unsatisfactory serial
1	20	20	3 or less	6 or more.
2	20	40	8 or less	9 or more.

(v) If four or five vaccinates show lesions of bursal disease in the first stage, the second stage may be conducted in a manner identical to the first stage. If the second stage is not conducted, the serial is unsatisfactory.

(vi) If the second stage is used, each serial shall be evaluated according to the second part of the table on the basis of cumulative results.

[50 FR 434, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.213 Pseudorabies Vaccine, Killed Virus.

Pseudorabies Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200.

(b) The immunogenicity of vaccine prepared from the Master Seed in accordance with the Outline of Production shall be established by a method acceptable to Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and at the minimum preinactivation titer provided in the Outline of Production.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements provided in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety.* Vaccinates used in the potency test in paragraph (c)(2) of this section shall be observed each day during the prechallenge period. If unfavorable reactions occur, including neurological signs, which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

(2) *Potency.* Bulk or final container samples of completed product shall be tested for potency as follows:

(i) Ten pseudorabies susceptible pigs (five vaccinates and five controls) shall be used as test animals. The animals shall be at the minimal age recommended for vaccination. Blood samples shall be drawn and individual serum samples inactivated and tested for neutralizing antibody.

(ii) A constant virus-varying serum neutralization test in cell culture using 50 to 300 $TCID_{50}$ of virus shall be used. Pigs shall be considered susceptible if there is no neutralization at 1:2 final serum dilution. Other tests of equal sensitivity acceptable to Animal and Plant Health Inspection Service may be used.

(iii) The five pigs used as vaccinates shall be administered one dose of vaccine as recommended on the label. If two doses are recommended, the second dose shall be given after the interval recommended on the label.

(iv) Fourteen days or more after vaccination, blood samples shall be drawn

and individual serum samples inactivated and tested for pseudorabies virus neutralizing antibody by the method used to determine susceptibility.

(v) *Test interpretation.* If the controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated. If at least four of the five vaccinates in a valid test have not developed titers of at least 1:8, and the remaining vaccinate has not developed a titer of at least 1:4, the serial is unsatisfactory, except as provided in paragraph (c)(2)(vi) of this section.

(vi) *Virus challenge test.* If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and controls may be challenged with virulent pseudorabies virus furnished or approved by Animal and Plant Health Inspection Service. The animals shall be observed each day for 14 days postchallenge. If four of five controls do not develop central nervous system signs or die, the test is inconclusive and may be repeated. In a valid test, if two or more of the vaccinates develop clinical signs or die, the serial is unsatisfactory.

[50 FR 434, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.214 Parvovirus Vaccine, Killed Virus (Canine).

Parvovirus Vaccine, Killed Virus, recommended for use in dogs, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200.

(b) The immunogenicity of vaccine prepared in accordance with the Outline of Production shall be established as follows:

(1) Twenty-five parvovirus susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine parvovirus to determine susceptibility. A constant virus-varying serum neutralization test in cell culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution. Other tests of equal sensitivity acceptable to Animal and Plant Health Inspection Service may be used.

(2) A viral hemagglutination test or another test acceptable to Animal and Plant Health Inspection Service shall be used to measure the antigenic content of vaccine produced at the highest passage from the Master Seed before the immunogenicity test is conducted. The 20 dogs used as vaccinates shall be injected with a predetermined dose of vaccine by the method recommended on the label. To confirm the dosage calculations, five replicate tests shall be conducted on a sample of the vaccine used. If two doses are used, five replicate confirming tests shall be conducted on each dose.

(3) Fourteen days or more after the final dose of vaccine, the vaccinates and the controls shall be challenged with virulent canine parvovirus furnished or approved by Animal and Plant Health Inspection Service and the dogs observed each day for 14 days. Rectal temperature, blood lymphocyte count, and feces for viral detection shall be taken from each dog each day for at least 10 days postchallenge and the presence or absence of clinical signs noted and recorded each day.

(i) The immunogenicity of the vaccine shall be evaluated on the following criteria of infection: temperature ≥103.4 ° F; lymphopenia of ≥50 percent of prechallenge normal; clinical signs such as diarrhea, mucus in feces, or blood in feces; and viral hemagglutinins at a level of ≥1:64 in a 1:5 dilution of feces or a test of equal sensitivity. If at least 80 percent of the controls do not show at least three of the four criteria of infection during the observation period, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive the observation period without showing any more than one criterion of infection described in subparagraph (3)(i), of this section, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five susceptible dogs (four vaccinates and one control) need to be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (b)(1) of this section.

(i) Each vaccinate shall be injected with a predetermined quantity of vaccine virus as provided in paragraph (b)(2) of this section.

(ii) Fourteen to 21 days after the last vaccination, a second serum sample shall be drawn from each dog and tested for neutralizing antibody to canine parvovirus in the same manner used to determine susceptibility.

(iii) If the control has not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If three of the four vaccinates in a valid test do not develop titers based upon final serum dilution of at least 1:16, and the remaining vaccinate does not develop a titer of at least 1:8, the Master Seed is unsatisfactory, except as provided in subparagraph (4)(v) of this section.

(v) If the results of a valid SN test are unsatisfactory, the vaccinates and the control may be challenged as provided in paragraph (b)(3) of this section. If at least three of the four criteria of infection are not shown, the test is inconclusive and may be repeated, except that if any of the vaccinates show more than one criterion of infection, the Master Seed is unsatisfactory.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the requirements prescribed in §113.200 and in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Potency.* Bulk or final container samples of completed product shall be tested for antigenic content using the method used in paragraph (b)(2) of this section. To be eligible for release, each serial and each subserial shall have an antigenic content sufficiently greater than that used in the immunogenicity test to assure that, when tested at any time within the expiration period, each serial and subserial shall have an antigenic content equal to the amount used in such immunogenicity test.

(2) *Virus identity.* Bulk or final container samples shall be tested for virus identity by conducting a hemagglutination test using duplicate samples and pretreating one with specific canine parvovirus antibody. If there is not at least an eightfold reduction in hemagglutinating activity, the hemagglutination is considered to be nonspecific and the serial is unsatisfactory.

[50 FR 435, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§113.215 Bovine Virus Diarrhea Vaccine, Killed Virus.

Bovine Virus Diarrhea Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed virus which has been established as pure, safe, and immunogenic shall be used for preparing seed cultures for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in §113.200 and the requirements of this section.

(b) The immunogenicity of vaccine prepared from the Master Seed in accordance with the Outline of Production shall be established by a method acceptable to the Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and at the minimum preinactivation titer provided in the Outline of Production.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.200 and the special requirements provided in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety.* Vaccinates used in the potency test in paragraph (c)(2) of this

section shall be observed each day during the prechallenge period. If unfavorable reactions occur, including respiratory signs, which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated one time. If results of the second test are not satisfactory, or if the test is not repeated, the serial is unsatisfactory.

(2) *Potency.* Bulk or final container samples of completed product shall be tested for potency using the method described in this paragraph.

(i) Eight bovine virus diarrhea susceptible calves (five vaccinates and three controls) shall be used as test animals. Individual serum samples shall be collected, inactivated, and individually tested for neutralizing antibody.

(ii) A constant virus decreasing serum neutralization test in cell culture using 50–300 TCID50 of virus shall be used. Calves shall be considered susceptible if there is no neutralization at 1:2 final serum dilution. Other tests of equal sensitivity approved by the Animal and Plant Health Inspection Service may be used.

(iii) The five calves used as vaccinates shall be administered one dose of vaccine as recommended on the label. If two doses are recommended, the second dose shall be given according to the interval recommended on the label.

(iv) Fourteen days or more after the last vaccination, blood samples shall be drawn and the individual serum samples inactivated and tested for bovine virus diarrhea virus neutralizing antibody by the same method used to determine susceptibility.

(v) *Test interpretation.* If the controls have not remained seronegative at 1:2, the test is a No Test (NT) and may be repeated. If at least four of the five vaccinates in a valid test have not developed 50 percent endpoint titers of 1:8 or greater, the serial is unsatisfactory, except as provided in paragraph (c)(2)(vi) of this section.

(vi) *Virus Challenge Test.* If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and controls may be challenged with virulent bovine virus diarrhea virus furnished or approved by the Animal and Plant Health Inspection Service. The animals shall be observed for 14 days post-challenge. If two of the three control calves do not show a temperature rise to 104.5 °F and develop respiratory or clinical signs of bovine virus diarrhea, the test is inconclusive and may be repeated one time. If two or more vaccinates show a temperature of 104.0 °F for 2 or more days and develop respiratory or clinical or other signs, the serial is unsatisfactory.

(vii) The prevaccination and postvaccination sera from a satisfactory potency test shall be submitted to the National Veterinary Services Laboratories for confirmatory testing.

[55 FR 35562, Aug. 31, 1990]

§ 113.216 Bovine Rhinotracheitis Vaccine, Killed Virus.

Infectious Bovine Rhinotracheitis Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed virus which has been established as pure, safe, and immunogenic shall be used for preparing seed cultures for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200 and the requirements of this section.

(b) The immunogenicity of vaccine prepared in accordance with the Outline of Production shall be established by a method acceptable to the Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and at the minimum preinactivation titer provided in the Outline of Production.

(c) *Test requirements for release.* Each serial and subserial shall meet the requirements prescribed in § 113.200 and the special requirements provided in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety.* Vaccinates used in the potency test in paragraph (c)(2) of this

section shall be observed each day during the prechallenge period. If unfavorable reactions occur, which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated one time. If the results of the second test are not satisfactory, or if the test is not repeated, the serial is unsatisfactory.

(2) *Potency.* Bulk or final container samples of completed product shall be tested for potency using the method described in this paragraph.

(i) Eight infectious bovine rhinotracheitis susceptible calves (five vaccinates, three controls) shall be used as test animals. Individual serum samples shall be collected, inactivated, and individually tested for neutralizing antibody.

(ii) A constant virus decreasing serum neutralization test in cell culture using 50–300 TCID50 of virus shall be used. Calves shall be considered susceptible if there is no neutralization at 1:2 final serum dilution. Other tests of equal sensitivity acceptable to the Animal and Plant Health Inspection Service may be used.

(iii) The five calves used as vaccinates shall be administered one dose of vaccine as recommended on the label. If two doses are recommended, the second dose shall be given according to the interval recommended on the label.

(iv) Fourteen or more days after the last vaccination, blood samples shall be drawn and the individual serum samples inactivated and tested for infectious bovine rhinotracheitis virus neutralizing antibody by the same method used to determine susceptibility.

(v) *Test interpretation.* If the three controls have not remained seronegative at 1:2, the test is a No Test (NT) and may be repeated. If at least four of the five vaccinates in a valid test have not developed 50 percent endpoint titers of 1:8, the serial is unsatisfactory, except as provided in paragraph (c)(2)(vi) of this section.

(vi) *Virus Challenge Test.* If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and controls may be challenged with virulent infectious bovine rhinotracheitis virus furnished or approved by the Animal and Plant Health Inspection Service. The animals shall be observed each day for 14 days post-challenge. If two of the three control calves do not show a temperature rise to 104.5 °F and develop respiratory or other clinical signs of infectious bovine rhinotracheitis, the test is a No Test (NT) and may be repeated one time. If more than one of the vaccinates shows a temperature of 104.0°F for 2 or more days or if more than one of the vaccinates develops respiratory or clinical or other signs, the serial is unsatisfactory.

(vii) The prevaccination and postvaccination sera from a satisfactory potency test shall be submitted to the National Veterinary Services Laboratories for testing by the Animal and Plant Health Inspection Service.

[55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

LIVE VIRUS VACCINES

§ 113.300 General requirements for live virus vaccines.

When prescribed in an applicable Standard Requirement or in the filed Outline of Production, a live virus vaccine shall meet the applicable requirements in this section.

(a) *Purity tests.* (1) *Bacteria and fungi.* Final container samples of completed product and comparable samples of each lot of Master Seed Virus shall be tested for bacteria and fungi in accordance with the test provided in § 113.27.

(2) *Mycoplasma.* Final container samples of completed product and comparable samples of each lot of Master Seed Virus shall be tested for mycoplasma in accordance with the test provided in § 113.28.

(3) *Avian Origin Vaccine.* Samples of each lot of Master Seed Virus and bulk pooled material or final container samples from each serial shall also be tested for:

(i) Salmonella contamination as prescribed in § 113.30; and

(ii) Lymphoid leukosis virus contamination as prescribed in § 113.31; and

(iii) Hemagglutinating viruses as prescribed in § 113.34.

(4) *Extraneous viruses.* Each lot of Master Seed Virus used to prepare live

virus vaccine recommended for animals other than poultry shall meet the requirements for extraneous viruses as prescribed in § 113.55

(b) *Safety tests.* Samples of each lot of Master Seed Virus and final container samples of completed product from each serial or first subserial of live virus vaccine recommended for animals other than poultry shall be tested for safety in at least one species for which the vaccine is intended using methods prescribed in §§ 113.39, 113.40, 113.41, 113.44, and 113.45 or in a filed Outline of Production. The mouse safety test prescribed in § 113.33(a) shall also be conducted unless the virus or agent in the vaccine is inherently lethal for mice.

(c) *Virus identity test.* At least one of the virus identity tests provided in this paragraph or a suitable identity test prescribed in the filed Outline of Production shall be conducted on the Master Seed Virus and final container samples from each serial or first subserial of biological product.

(1) *Fluorescent antibody test.* The fluorescent antibody test shall be conducted using virus inoculated cells and uninoculated control cells. Cells shall be stained with fluorochrome conjugated specific antiserum. Fluorescence typical of the virus concerned shall be demonstrated in the inoculated cells. The control cells shall remain free of such fluorescence.

(2) *Serum neutralization test.* The serum neutralization test shall be conducted using the constant serum-decreasing virus method with specific antiserum. For positive identification, at least 100 ID_{50} of vaccine virus shall be neutralized by the antiserum.

(d) *Cell Culture Requirements.* If cell cultures are used in the preparation of Master Seed Virus or of the vaccine, primary cells shall meet the requirements prescribed in § 113.51, cell lines shall meet the requirements prescribed in § 113.52, and ingredients of animal origin shall meet the applicable requirements in § 113.53.

(e) *Moisture content.* (1) The maximum moisture content in desiccated vaccines must be stated in the filed Outline of Production.

(2) Final container samples of completed product from each serial or subserial must be tested for moisture content in accordance with the test prescribed in § 113.29.

[39 FR 27430, July 29, 1974, as amended at 43 FR 49528, Oct. 24, 1978; 50 FR 1042, Jan. 9, 1985; 54 FR 19352, May 5, 1989. Redesignated at 55 FR 35562, Aug. 31, 1990; 60 FR 24549, May 9, 1995; 68 FR 57608, Oct. 6, 2003]

§ 113.301 Ovine Ecthyma Vaccine.

Ovine Ecthyma Vaccine shall be prepared from tissue culture fluids or virus-bearing tissues obtained from sheep that have developed ovine ecthyma following inoculation with virulent ovine ecthyma virus. Ovine Ecthyma Vaccine is exempt from the requirements prescribed in §§ 113.27 and 113.300(a), (b), and (c). Each serial shall meet the moisture requirements in § 113.300(e) and the special requirements prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety tests.* (1) Bulk or final container samples of completed product from each serial shall be tested for safety as prescribed in § 113.38.

(2) The prechallenge period of the potency test shall constitute a safety test. If unfavorable reactions attributable to the vaccine occur in either of the vaccinates during the observation period, the serial is unsatisfactory.

(b) *Potency test.* Final container samples of completed product from each serial and each subserial shall be tested for potency using susceptible lambs. The vaccine shall be prepared as recommended for use on the label.

(1) Each of two lambs (vaccinates) shall be vaccinated by application of the vaccine to a scarified area on the medial surface of the thigh and observed each day for 14 days.

(2) The immunity of the two vaccinates and one or more unvaccinated lambs (controls) shall be challenged in the same manner as for vaccination, using the opposite thigh.

(3) If typical signs of ovine ecthyma, such as hyperemia, vesicles, and pustules do not develop on the controls during the first 2 weeks following challenge and persist for approximately 30 days, the test is inconclusive and may be repeated.

(4) If the vaccinates do not show a typical immune reaction, the serial is

unsatisfactory: *Provided,* That, an initial active reaction with hyperemia which resolves progressively and disappears within 2 weeks, may be characterized as a typical immune reaction.

[39 FR 27430, July 29, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

§ 113.302 Distemper Vaccine—Mink.

Distemper Vaccine—Mink shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300 and the requirements prescribed in this section.

(b) The lot of Master Seed Virus shall be tested for extraneous viruses as follows:

(1) To detect virulent canine distemper virus, each of two distemper susceptible mink or ferrets shall be inoculated with 1 ml of the Master Seed Virus and observed each day for 21 days. If undesirable reactions occur in either test animal, the lot of Master Seed Virus is unsatisfactory.

(2) Master Seed Virus propagated in chicken embryos shall be tested for pathogens by the chicken embryo test prescribed in § 113.37 except lesions typical of distemper virus may be disregarded. If found unsatisfactory, the Master Seed Virus shall not be used.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) At least 25 distemper susceptible mink shall be used as test animals. Blood samples shall be drawn from these animals and individual serum samples tested. The mink shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test with less than 500 ID_{50} of canine distemper virus. Other means of insuring susceptibility may be used if prior approval from Animal and Plant Health Inspection Service is received.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. At least 20 mink shall be vaccinated with a predetermined quantity of vaccine virus and at least 5 additional mink shall be held as unvaccinated controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) At least twenty-one days post-injection, the immunity of each of the vaccinates and the controls shall be challenged with the same size dose of virulent distemper virus and observed each day for 21 days.

(i) If at least 80 percent of the controls do not die or show severe signs of distemper, the test is inconclusive and may be repeated.

(ii) If at least 19 of 20, 27 of 30, or 36 of 40 of the vaccinates do not survive without showing clinical signs of distemper during the observation period, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided,* That five of the five vaccinates and at least four of the controls shall meet the criteria prescribed in paragraph (c)(3) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be authorized by Animal and Plant Health Inspection Service.

(d) Test requirements for release: Each serial and subserial shall meet the general requirements prescribed in § 113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Mink safety test.* Each of 2 mink shall be vaccinated with the equivalent of 10 doses of vaccine rehydrated with sterile diluent and administered in the

manner recommended on the label. The mink shall be observed each day for 21 days. If unfavorable reactions attributable to the product occur in either of the mink during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated: *Provided*, That if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

(2) *Potency Test.* An in vitro potency test shall be conducted. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer $10^{0.7}$ greater than that used in such immunogenicity test when tested by the method used in paragraph (c)(2) of this section.

[40 FR 53000, Nov. 14, 1975, as amended at 48 FR 33471, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.303 Bluetongue Vaccine.

Bluetongue Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing the seeds for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for transmissibility and reversion to virulence in sheep using a method acceptable to Animal and Plant Health Inspection Service. If reversion to virulence is demonstrated, the Master Seed is unsatisfactory.

(c) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed shall be established as follows:

(1) Twenty-five lambs, susceptible to the bluetongue virus serotype contained in the vaccine, shall be used as test animals (20 vaccinates and 5 controls). Blood samples shall be drawn from these animals and individual serums tested. A lamb shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus varying serum neutralization test with 60 to 300 $TCID_{50}$ of bluetongue virus or another method acceptable to Animal and Plant Health Inspection Service.

(2) A geometric mean titer of the vaccine produced from the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 lambs to be used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the virus dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine used.

(3) At least once during the period of 14 to 18 days postvaccination, individual serum samples shall be collected from each of the vaccinates and tested for virus neutralizing antibody using the 60 to 300 $TCID_{50}$ of bluetongue virus.

(4) Twenty-one to twenty-eight days postvaccination the vaccinates and the controls shall each be challenged with virulent bluetongue virus and observed for 14 days. The rectal temperature of each animal shall be taken and recorded for 17 consecutive days beginning 3 days prechallenge. The presence or absence of lesions or other clinical signs of bluetongue noted and recorded on each of 14 consecutive days postchallenge.

(i) If at least four of the five controls do not show clinical signs of bluetongue and a temperature rise of 3 ° F or higher over the prechallenge mean temperature, the test shall be considered inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates tested as prescribed in paragraph (c)(3) of this section do not have bluetongue neutralizing antibody titers of 1:4 final serum dilution or higher, or if more than one of the vaccinates shows a temperature rise of 3 ° F or higher than

its prechallenge mean temperature for 2 or more days, or if more than one of the vaccinates exhibits clinical signs of bluetongue, the Master Seed is unsatisfactory.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(6) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need be used in the retest: *Provided,* That five of five vaccinates and at least four of the five controls shall meet the criteria prescribed in paragraphs (c)(4) of this section.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* The mouse safety test prescribed in §113.33(a) and the lamb safety test prescribed in §113.45 shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in such immunogenicity test.

[50 FR 23796, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§113.304 Feline Panleukopenia Vaccine.

Feline Panleukopenia Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300 and the requirements prescribed in this section.

(b) The lot of Master Seed Virus shall be tested for other agents as follows:

(1) To detect virulent feline panleukopenia virus or virulent mink enteritis virus, each of two feline panleukopenia susceptible cats, as determined by the criteria prescribed in paragraph (c)(1) of this section, shall be injected subcutaneously with the equivalent of one cat dose each and the cats observed each day for 21 days. If either or both cats show signs of disease or reduced white blood cell counts below 50 percent of the normal level established by an average of three or more counts taken prior to injection, the Master Seed Virus is unsatisfactory.

(2) To detect chlamydial agents, the Master Seed Virus shall be tested as prescribed in §113.43.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five feline panleukopenia susceptible cats shall be used as test animals (20 vaccinates and 5 controls). Blood samples drawn from each cat shall be individually tested for neutralizing antibody against feline panleukopenia virus to determine susceptibility.

(i) A constant virus-carrying serum neutralization test in tissue culture using 100 to 300 $TCID_{50}$ of virus shall be used.

(ii) Cats shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 cats used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five

cats held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) Fourteen days post-injection, the vaccinates and the controls shall be challenged with virulent feline panleukopenia virus furnished by Animal and Plant Health Inspection Service and the cats observed each day for 14 days.

(i) If at least 80 percent of the controls do not show clinical signs of feline panleukopenia during the observation period, the test is inconclusive and may be repeated. Clinical signs of feline panleukopenia shall include a pronounced leukopenia wherein the white cell count drops to 4,000 or less per cubic mm, or the white cell count drops to less than 25 percent of the normal level established by an average of three or more counts taken prior to challenge.

(ii) If at least 19 of the 20 vaccinates do not survive the observation period without showing clinical signs of feline panleukopenia as described in paragraph (c)(3)(i) of this section, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Ten susceptible cats (8 vaccinates and 2 controls) shall be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (c)(1) of this section.

(i) Each vaccinate shall be injected with a predetermined quantity of vaccine virus as provided in paragraph (c)(2) of this section.

(ii) Fourteen to twenty-one days postvaccination, a second serum sample shall be drawn from each cat and tested for neutralizing antibody to feline panleukopenia virus in the same manner used to determine susceptibility.

(iii) If the two controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If at least 6 of the 8 vaccinates in a valid test do not develop titers based upon final serum dilution of at least 1:8, and the remaining vaccinates do not develop titers of at least 1:4, the Master Seed Virus is unsatisfactory except as provided in paragraph (c)(4)(v) of this section.

(v) If the results of a valid SN test are unsatisfactory, the vaccinates and the controls may be challenged as provided in paragraph (c)(3) of this section. If 100 percent of the controls do not show clinical signs of feline panleukopenia, the test is inconclusive and may be repeated except, that, if any of the vaccinates show such signs, the Master Seed Virus is unsatisfactory.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the requirements prescribed in § 113.300 and in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety Test.* The mouse safety test prescribed in § 113.33(a) and the cat safety test prescribed in § 113.39 shall be conducted.

(i) Each of two healthy cats shall be injected with 10 cat doses by the method recommended on the label and the cats observed each day for 14 days.

(ii) If unfavorable reactions attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated: *Provided,* That, if not repeated, the serial shall be unsatisfactory.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in

such immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ per dose.

[39 FR 44716, Dec. 27, 1974, as amended at 40 FR 53378, Nov. 18, 1975; 43 FR 25078, June 9, 1978; 43 FR 41186, Sept. 15, 1978; 44 FR 58900, Oct. 12, 1979; 48 FR 33471, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.305 Canine Hepatitis and Canine Adenovirus Type 2 Vaccine.

Canine Hepatitis Vaccine and Canine Adenovirus Type 2 Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used in preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 except that the dog safety test prescribed in §113.40(a) shall be conducted by the intravenous route.

(b) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity by one or both of the following methods:

(1) *Immunogenicity for canine hepatitis.* Twenty-five canine hepatitis susceptible dogs shall be used as test animals (20 vaccinates and 5 controls). Blood samples shall be drawn from these animals and individual serum samples tested. The dogs shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test using 50 to 300 $TCID_{50}$ of canine adenovirus.

(i) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 dogs to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five dogs held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(ii) Not less than 14 days postinjection, the vaccinates and the controls shall each be challenged intravenously with virulent infectious canine hepatitis virus furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 14 days.

(A) If at least four of the five controls do not show severe clinical signs of canine hepatitis, the test is inconclusive and may be repeated.

(B) If at least 19 of the 20 vaccinates do not survive without showing clinical signs of infectious canine hepatitis during the observation period, the Master Seed Virus is unsatisfactory.

(iii) The Master Seed Virus shall be retested for immunogenicity for canine hepatitis in 3 years unless use of the lot previously tested is discontinued. Ten susceptible dogs (8 vaccinates and 2 controls) shall be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (b)(1) of this section.

(A) Each vaccinate shall be injected with a predetermined quantity of vaccine virus as provided in paragraph (b)(1)(i) of this section.

(B) At least 14 days postvaccination, a second serum sample shall be drawn from each dog and tested for neutralizing antibody to canine adenovirus in the same manner used to determine susceptibility.

(C) If the two controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(D) If at least six of the eight vaccinates in a valid test do not develop titers of at least 1:10 based upon final serum dilution, the Master Seed Virus is unsatisfactory except as provided in paragraph (b)(1)(iii)(E) of this section.

(E) If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the controls may be challenged as provided in paragraph (b)(1)(ii) of this section. A Master Seed is satisfactory if all vaccinates remain free of clinical signs of canine hepatitis, while both controls develop severe clinical signs of canine hepatitis. If both controls do not show severe clinical signs of canine hepatitis, the test is inconclusive and may be repeated: *Provided,* That, if any of the vaccinates show such signs, the Master Seed Virus is unsatisfactory.

(2) *Immunogenicity for canine adenovirus Type 2.* Thirty canine

adenovirus type 2 susceptible dogs shall be used as test animals (20 vaccinates and 10 controls). Blood samples shall be drawn from these animals and individual serum samples tested. The dogs shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test using 50 to 300 $TCID_{50}$ of canine adenovirus.

(i) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 dogs to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining 10 dogs held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(ii) Not less than 14 days postinjection, the vaccinates and the controls shall be challenged by exposure to a nebulized aerosol of virulent canine adenovirus type 2 furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 14 days postchallenge. The rectal temperature of each animal shall be taken and the presence of respiratory or other clinical signs of canine adenovirus type 2 noted and recorded each day.

(A) If at least 6 of 10 controls do not show clinical signs of canine adenovirus type 2 infection other than fever, the test is inconclusive and may be repeated.

(B) If a significant difference in clinical signs in a valid test cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service, the Master Seed Virus is unsatisfactory.

(iii) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Either 10 vaccinates and 6 controls or 5 vaccinates and 3 controls shall be used in the retest.

(A) If less than 4 of 6 or 2 of 3 of the controls show clinical signs of canine adenovirus type 2 other than fever, the test is inconclusive and may be repeated.

(B) A significant difference in clinical signs shall be demonstrated between vaccinates and controls in a valid test as prescribed in paragraph (b)(2)(ii)(B) of this section.

(iv) An Outline of Production change shall be made before authorization for use of a new lot of Master Seed Virus shall be granted by the Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the requirements prescribed in § 113.300 and in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(1)(i) and/or (b)(2)(i) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test(s) prescribed in paragraph (b) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in such immunogenicity test(s) but not less than $10^{2.5}$ $TCID_{50}$ dose. If both immunogenicity tests in paragraph (b) of this section are conducted and a different amount of virus is used in each test, the virus titer requirements shall be based on the higher of the two amounts.

(2) [Reserved]

[60 FR 14361, Mar. 17, 1995]

§ 113.306 Canine Distemper Vaccine.

Canine Distemper Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) *Master Seed Virus.* The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300 and

the requirements prescribed in this section.

(1) To detect ferret virulent canine distemper virus, each of five canine distemper susceptible ferrets shall be injected with a sample of the Master Seed Virus equivalent to the amount of virus to be used in one dog dose and observed each day for 21 days. If undesirable reactions are observed during the observation period, the lot of Master Seed is unsatisfactory.

(2) Master Seed Virus propagated in tissues or cells of avian origin shall be tested for pathogens by the chicken embryo test prescribed in §113.37. If found unsatisfactory, the Master Seed Virus shall not be used.

(b) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five canine distemper susceptible dogs shall be used as test animals (20 vaccinates and 5 controls). Blood samples shall be drawn from these animals and individual serum samples tested. The dogs shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test using 50 to 300 $TCID_{50}$ of canine distemper virus.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 dogs used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five dogs held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) At least 14 days post-injection, the vaccinates and the controls shall each be challenged intracerebrally with virulent canine distemper virus furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 21 days.

(i) If at least four of the five controls do not die and the survivor, if any, does not show clinical signs of canine distemper the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive without showing clinical signs of canine distemper during the observation period, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Ten susceptible dogs (8 vaccinates and 2 controls) shall be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (b)(1) of this section.

(i) Each vaccinate shall be injected with a predetermined quantity of vaccine virus as provided in paragraph (b)(2) of this section.

(ii) At least 14 days postvaccination, a second serum sample shall be drawn from each dog and tested for neutralizing antibody to canine distemper virus in the same manner used to determine susceptibility.

(iii) If the two controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If at least 6 of the 8 vaccinates in a valid test do not develop titers of at least 1:50 based upon final serum dilution, the Master Seed Virus is unsatisfactory, except as provided in paragraph (b)(4)(v) of this section.

(v) If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the controls may be challenged as provided in paragraph (b)(3) of this section. A Master Seed is satisfactory if all vaccinates remain free of clinical signs of canine distemper, while the two controls die with clinical signs of canine distemper. If the two controls do not die with clinical signs of canine distemper, the test is inconclusive and may be repeated: *Provided,* That, if any of the vaccinates show such signs, the Master Seed Virus is unsatisfactory.

(5) An Outline of Production change shall be made before authorization for use of a new lot of Master Seed Virus shall be granted by the Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Except for §113.300(a)(3)(ii), each serial and subserial shall meet the requirements prescribed in §113.300 and in this paragraph. Final container samples of

completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) The test for pathogens prescribed in § 113.37 shall be conducted on each serial or one subserial of avian origin vaccine.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (b) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in such immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ per dose.

[60 FR 14362, Mar. 17, 1995]

§ 113.308 Encephalomyelitis Vaccine, Venezuelan.

Encephalomyelitis Vaccine, Venezuelan, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300 except (b), and the requirements prescribed in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed shall be established as follows:

(1) Tests conducted by the Department have established that horses having Venezuelan equine encephalomyelitis antibody titers of 1:20 by the hemagglutination-inhibition (HI) method or 1:40 by the serum neutralization (SN) method were immune to challenge with virulent virus. The immunogenicity test is based on the demonstration of a serological response of at least that magnitude following vaccination of serologically negative horses.

(2) At least 22 horses (20 vaccinates and 2 controls), susceptible to Venezuelan equine encephalomyelitis, shall be used as test animals. Blood samples shall be taken from each horse and the serums individually tested for neutralizing antibody. Horses shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test using 60 to 300 $TCID_{50}$ of Venezuelan equine encephalomyelitis virus.

(3) A geometric mean titer of the vaccine produced from the highest passage of the Master Seed shall be established using a method acceptable to Veterinary Services before the immunogenicity test is conducted. The 20 horses used as vaccinates shall be injected with a predetermined quantity of vaccine virus by the method to be recommended on the label. To confirm the dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(4) Twenty-one to twenty-eight days postvaccination, blood samples shall be drawn from all test animals. For a valid test, the controls shall remain seronegative at 1:2 final serum dilution. In a valid test, if at least 19 of 20 vaccinates do not have antibody titers of at least 1:20 in a hemagglutination-inhibition test or at least 1:40 in a serum neutralization test, the Master Seed is unsatisfactory.

(5) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot is discontinued. Only five vaccinates and two controls need to be used in the retest: *Provided,* That five of five vaccinates and the two controls shall meet the criteria in paragraph (b)(4) of this section.

(6) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.300 and special requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* The mouse safety test prescribed in § 113.33(b) shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the method in paragraph (b)(3) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test, but not less than $10^{2.5}$ $TCID_{50}$ per dose.

[50 FR 23797, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.309 Bovine Parainfluenza$_3$ Vaccine.

Bovine Parainfluenza$_3$ Vaccine shall be produced from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five bovine parainfluenza, susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serums tested. Also, nasal specimens shall be collected for virus isolation attempts. The calves shall be considered susceptible if:

(i) The results are negative at a 1:2 final serum dilution in a varying serum constant virus neutralization test with less than 500 $TCID_{50}$ of bovine parainfluenza$_3$ virus; and

(ii) Shall be negative to bovine parainfluenza$_3$ virus isolation attempts from the nasal specimens on the day of injection.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 calves to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five calves held as uninjected controls. To confirm the dosage calculation, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) The vaccinates and controls shall be examined for clinical signs of respiratory disease and the body temperature taken and recorded on each of the first 14 consecutive days post-injection. The vaccinates shall be bled on day 6 ±2 days post-injection.

(4) Three to four weeks post-vaccination, all calves shall be bled for serum antibodies and nasal specimens shall be collected for PI_3 virus isolation. On the same day, all vaccinates and controls shall be given acceptable challenge PI_3 virus titrating at least $10^{7.0}$ $TCID_{50}$ per ml and the animals observed for 14 days. Two ml of the challenge virus shall be instilled in each nostril or shall be inhaled as an aerosol suspension. Upon request, challenge virus and instructions shall be furnished by Animal and Plant Health Inspection Service.

(5) Each animal shall be examined for clinical signs of respiratory disease and the body temperature recorded on each of the 14 consecutive days of the post-challenge observation period. Each day for at least the first 10 days post-challenge, nasal specimens for virus isolation attempts shall be taken. All animals shall be bled on day 6 ±2 days post-challenge, and all animals shall be bled at least once 14 to 28 days post-challenge for serum antibody studies.

(6) Satisfactory Test Criteria:

(i) All virus isolations attempts shall be by culture and at least one subculture in PI_3 susceptible cells for a total of at least 14 days.

(ii) Two to four weeks post-vaccination, at least 19 of the 20 vaccinates shall have PI_3 neutralizing antibody titers of at least 1:4 and all five controls shall be negative at 1:2 dilution. None of the post-vaccination serums collected from the vaccinates on day 6 ±2 days shall reveal serum neutralization antibody titers of 1:32 or greater based upon final dilution.

(iii) Satisfactory resistance to challenge by vaccinates shall be determined by a significant difference between virus isolation rates from vaccinates and controls. The virus neutralization titers of post-challenge serums and respiratory symptoms and temperatures from all animals shall be considered in the evaluation of the test validity.

(7) Designated animal alternates for test animals showing anamnestic antibody responses (titers 1:32 or greater) on day 6 serums may be included in the study under the following provisions:

(i) No more than five alternates shall be allowed for the vaccinates and no more than two for the controls.

(ii) Alternates shall be subject to all requirements outlined for the animals for which they are alternates.

(iii) Antibody values from alternate animals may be used only to replace values from up to and including five vaccinates which develop antibody of 1:32 or greater by day 6 ±2 days post-vaccination or up to and including two controls which develop antibody titers of 1:32 or greater by day 6 ±2 days post-challenge.

(8) A sequential test procedure may be used in lieu of the 20 calf requirement. A beta value of .05 and a tolerance level of .78 shall be required.

(9) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided,* That five of five vaccinates and at least four of the controls shall meet the criteria prescribed in paragraph (c)(6) of this section.

(10) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) Test requirements for release: Each serial and subserial shall meet the applicable general requirements prescribed in § 113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested except as prescribed in paragraph (d)(1) of this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Purity test.* The test for Brucella contamination prescribed in § 113.32 shall be conducted on each batch of primary cells intended for production use.

(2) *Safety test.* The mouse safety test prescribed in § 113.33(a) and the calf safety test prescribed in § 113.41 shall be conducted.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer per dose sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ per dose.

[39 FR 44719, Dec. 27, 1974, as amended at 40 FR 41089, Sept. 5, 1975; 43 FR 49529, Oct. 24, 1978; 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 60 FR 14357, Mar. 17, 1995]

§ 113.310 Bovine Rhinotracheitis Vaccine.

Bovine Rhinotracheitis Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five infectious bovine rhinotracheitis susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serums tested. The calves shall be considered susceptible if the results are negative at a 1:2 final serum dilution by the virus plaque reduction method.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 calves to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five calves held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) At least once during a period of 14 to 28 days post-vaccination, individual serum samples shall be collected for virus-neutralization tests from each of the vaccinates. The test virus shall be 100 to 500 $TCID_{50}$ bovine rhinotracheitis virus. Results shall be used in making a determination as prescribed in paragraph (c)(6) of this section.

(4) The vaccinates and the controls shall each be challenged with virulent infectious bovine rhinotracheitis virus and observed for 14 days. The rectal temperature of each animal shall be taken and the presence or absence of respiratory or other clinical signs of bovine rhinotracheitis noted and recorded on each of the 14 consecutive days.

(5) If at least four of the five controls do not show clinical signs of infectious bovine rhinotracheitis and a marked temperature rise to 104.5 °F. or higher post-challenge, the test shall be considered inconclusive and may be repeated.

(6) If less than 19 of the post-injection serum samples tested as prescribed in paragraph (c)(3) of this section show neutralization in all tubes of the 1:2 final serum dilution, or if more than one of the vaccinates show a temperature of 103.5 °F. or higher for 2 or more days, or if more than one of the vaccinates exhibit respiratory or other clinical signs of infectious bovine rhinotracheitis, or both, the Master Seed Virus is unsatisfactory.

(7) A sequential test procedure may be used in lieu of the 20 calf requirement. A beta value of .05 and a tolerance level of .78 shall be required.

(8) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided,* That five of five vaccinates and at least four of the five controls shall meet the criteria prescribed in paragraphs (c)(5) and (6) of this section.

(9) An outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) Test requirements for release: Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested except as prescribed in paragraph (d)(1) of this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Purity test.* The test for Brucella contamination prescribed in §113.32 shall be conducted on each batch of primary cells intended for production use.

(2) *Safety test.* The mouse safety test prescribed in §113.33(a) and the calf safety test prescribed in §113.41 shall be conducted.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer per dose sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at

any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ per dose.

[39 FR 44720, Dec. 27, 1974, as amended at 40 FR 20067, May 8, 1975; 40 FR 23989, June 4, 1975; 40 FR 41089, Sept. 5, 1975; 43 FR 49529, Oct. 24, 1978; 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.311 Bovine Virus Diarrhea Vaccine.

Bovine Virus Diarrhea Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five bovine virus diarrhea susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individuals serum samples tested. The calves shall be considered susceptible to bovine virus diarrhea virus infection if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test with less than 500 $TCID_{50}$ of bovine virus diarrhea virus.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 calves to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five calves held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) At least once during a period 14 to 28 days post-vaccination, individual serum samples shall be collected for virus-neutralization tests from each of the vaccinates. The test virus shall be less than 500 $TCID_{50}$ of bovine virus diarrhea virus. The white cell count for all vaccinates and controls shall be established at least 3 days just before challenge. Results shall be used in making a determination as prescribed in paragraph (c)(5) of this section.

(4) The vaccinates and the controls shall each be challenged with virulent bovine virus diarrhea virus and observed for 14 consecutive days. The white cell count shall be determined daily on each animal from the second through the eighth day post-challenge. If leukopenia does not develop in at least four of the five controls as compared with the vaccinates, the test shall be considered inconclusive and may be repeated.

(5) If less than 19 of the post-injection serum samples, tested as prescribed in paragraph (c)(3) of this section, show neutralization in all tubes of the 1:8 dilution; or if more than one of the vaccinates exhibits respiratory or other clinical signs of bovine virus diarrhea post-challenge; or both, the Master Seed Virus is unsatisfactory.

(6) A sequential test procedure may be used in lieu of the 20 calf requirement. A beta value of .05 and a tolerance level of .78 shall be required.

(7) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided,* That five of five vaccinates and at least four of the five controls shall meet the criteria prescribed in paragraphs (c)(4) and (c)(5) of this section.

(8) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) Test requirements for release: Each serial and subserial shall meet the applicable general requirements

prescribed in §113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested except as prescribed in paragraph (d)(1) of this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Purity test.* The test for Brucella contamination prescribed in §113.32 shall be conducted on each batch of primary cells intended for production use.

(2) *Safety test.* The mouse safety test prescribed in §113.33(a) and the calf safety test prescribed in §113.41 shall be conducted.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer per dose sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ per dose.

[39 FR 44721, Dec. 27, 1974, as amended at 40 FR 20067, May 8, 1975; 40 FR 41089, Sept. 5, 1975; 43 FR 49529, Oct. 24, 1978; 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§113.312 Rabies Vaccine, Live Virus.

Rabies Vaccine shall be prepared from virus-bearing cell cultures or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300.

(1) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(2) Each lot of Master Seed Virus propagated in tissues or cells of avian origin shall be tested for pathogens by procedures prescribed in §113.37.

(3) Each lot of Master Seed Virus propagated in primary cell cultures of mouse or hamster origin or brain tissues of mouse origin shall be tested for lymphocytic choriomeningitis (LCM) virus by the procedure prescribed in §113.42. If LCM virus is detected, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be studied in each species of carnivore or domesticated wild animal for which the vaccine is specifically recommended to attempt to determine the fate of the vaccine virus. Results shall be considered in evaluating safety of vaccine virus.

(i) Obtain at least 10 unvaccinated animals, negative at 1:2 final serum dilution, of each species in which tests will be conducted. Divide each species into two groups of five animals.

(ii) For each species of animal, inject one group of five animals intramuscularly. Infiltrate a major nerve and the surrounding tissue in each of the five animals in the other group. Use 1.0 ml of high titer virus for each method of administration.

(iii) Observe all animals for signs of rabies until scheduled time to sacrifice. If animals show definite symptoms, sacrifice and check regional lymph nodes, brain, salivary glands, and kidney for rabies virus by injection of suckling mice (not more than 7 days of age). Tissues may be held frozen at −70 °C. until suckling mice are available. Inject each mouse in one litter intracerebrally with 0.02 ml of a ground tissue suspension from each organ. Observe mice each day for 21 days. If any mice die, determine if the deaths were due to rabies virus in the brain by a fluorescent antibody test.

(iv) Sacrifice animals that do not show signs of rabies according to the following schedule and check regional lymph nodes, brain, salivary glands, and kidney in suckling mice.

Route of injection	Days after injection	Number of animals
Intramuscularly	15, 20, 25, 30, 35	1 each day.
Intraneurally	3, 6, 9, 15, 30	1 each day.

(5) Each lot of Master Seed Virus shall be tested for safety in at least 10 unvaccinated serologically negative

animals of each domestic species for which the vaccine is recommended.

(i) Each group of 10 animals shall be divided into 2 groups of 5 animals. For each species, inject one group intramuscularly with 10 doses of high titer virus.

(ii) Infiltrate a major nerve of each of the animals in the other group of 5 with 10 doses of the same high titer virus. For all species except dogs and cats, multiple injections along the cervical spine in the proximity to the nerve trunks emerging from the spinal cord may be used: *Provided,* That a 1-dose volume shall be injected into each of four or more sites bilaterally.

(iii) Observe all animals each day for 90 days.

(iv) If any animals show clinical signs of rabies, sacrifice the animal and check appropriate brain tissue for rabies virus by the fluorescent antibody test and by mouse injection.

(v) If rabies is confirmed, the lot of Master Seed Virus is unsatisfactory.

(b) The immunogenicity of vaccine prepared with virus at the highest passage of the Master Seed shall be established in each species for which the vaccine is recommended. Tests shall be conducted in accordance with a protocol filed with Animal and Plant Health Inspection Service before initiation of the tests. The vaccine shall be prepared using methods prescribed in the Outline of Production. If Rabies Vaccine is to be in combination with other fractions, the product tested shall include all fractions to be recommended.

(1) A geometric mean virus titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(2) The dose of vaccine to be used in the immunogenicity test shall be no more than the amount of rehydrated vaccine which, on the basis of previous titrations, has been diluted to the proposed minimum acceptable virus titer.

(3) Test animals shall be uniform and have no neutralizing antibodies to rabies as determined by serum-neutralization (SN) tests.

(i) Twenty-five or more animals shall be used as vaccinates. Each shall be injected intramuscularly at one site in the thigh with a dose of vaccine at the proposed minimum virus titer as specified in the filed Outline of Production.

(ii) Ten or more additional animals shall be held as controls.

(iii) On or about days 30, 90, 180, 270, and 365 postvaccination, all animals shall be bled and individual serums tested for neutralizing antibodies to rabies virus.

(iv) All surviving test animals of each species shall be challenged intramuscularly with virulent rabies virus furnished or approved by Animal and Plant Health Inspection Service 1 year after vaccination, except as provided in paragraphs (b)(4), (b)(5), and (b)(6) of this section. The challenged animals shall be observed each day for 90 days as prescribed in § 113.5(b). The brain of each test animal that dies following challenge shall be examined for rabies by the fluorescent antibody test or other method acceptable to Animal and Plant Health Inspection Service.

(v) Requirements for acceptance in challenge tests shall be death due to rabies in at least 80 percent of controls while at least 22 of 25 or 26 of 30 or a statistically equivalent number of the vaccinates remain well for a period of 90 days.

(4) An alternative to challenging all surviving test animals in accordance with paragraph (b)(3)(iv) of this section may be used when the test animals are of species other than carnivores. Vaccinates shall be challenged at 1 year postvaccination. These shall include five vaccinates with the lowest SN titers at the 270th-day bleeding, five vaccinates with the lowest SN titers at the 365th-day bleeding, and all vaccinates with SN titers below 1:10 by the mouse SN test or below 1:16 by the rapid-fluorescent-focus-inhibition test at any bleeding. At least five SN-negative controls of each species shall be challenged at the same time as the vaccinates. All SN titers shall be iterated to an endpoint. All of the challenged vaccinates must remain well for a period of 90 days, and at least 80 percent of the controls must die of rabies

for a satisfactory test without further challenge. If one or more of the vaccinates die from rabies, all the remaining vaccinates, regardless of titer, along with the five controls shall be challenged. The cumulative results from the two challenges shall be evaluated for acceptance as specified in paragraph (b)(3)(v) of this section.

(5) The Master Seed Virus shall be retested for immunogenicity in 3 years and each 5 years thereafter unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest and the retest may be limited to serological response at 1 year after vaccination of the vaccinates if such response is equal to or greater than that in the original immunogenicity test and all controls remain negative. If the SN response is not satisfactory, the vaccinates and controls may be challenged. To be satisfactory, at least 4 of the 5 controls shall die of rabies and 5 of the 5 vaccinates remain well for a period of 90 days.

(6) The repeat immunogenicity tests may be terminated after 90 day SN tests if at least 10 vaccinates and at least 5 controls of each species are used and the test dose of vaccine contains the minimum acceptable virus titer throughout dating.

(i) If the 10 vaccinates have SN titers equal to or greater than the 90 day SN titers of the vaccinates in the initial immunogenicity test, the Master Seed Virus is satisfactory.

(ii) If the 10 vaccinates do not have acceptable SN titers, each vaccinate and each control shall be challenged at 1 year with virulent rabies street virus and observed for 90 days.

(iii) If at least 80 percent of the controls do not show signs of rabies during the observation period, the test is invalid and shall be repeated.

(iv) If more than 10 percent of the vaccinates show signs of rabies, the Master Seed Virus is unsatisfactory.

(7) An outline of Production change shall be made before authority for use of a new lot of Master Virus shall be granted by Animal and Plant Health Inspection Service.

(c) If more than 1 year duration of immunity is to be claimed, a duration of immunity test for the additional time shall be conducted and interpreted as prescribed in paragraph (b) of this section for the 1 year test. The test animals shall be monitored serologically at least every 180 days. The time of challenge may be adjusted accordingly.

(d) Test requirements for release: Each serial and each subserial shall meet the general requirements prescribed in §113.300 and special requirements in this paragraph.

(1) *Purity and safety tests.* Final container samples of completed product from each serial or one subserial shall be tested.

(i) The test for pathogens, prescribed in §113.37 shall be conducted on each serial or one subserial of avian origin. If necessary, neutralize the rabies virus with specific rabies antiserum.

(ii) A test for safety in three young seronegative animals of the most susceptible species for which the vaccine is recommended shall be conducted. Each shall be injected intramuscularly with 10 recommended doses of vaccine. If unfavorable reactions attributable to the product occur during a 28 day observation period, the serial is unsatisfactory.

(iii) If primary cell cultures of hamster origin or of mouse origin are used vaccine production, they shall be tested for LCM virus as prescribed in §113.42. The cells shall be disrupted and undiluted cell fluids from each lot shall be tested.

(2) *Virus titrations.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(1) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently higher than the titer of the vaccine virus used in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer equal to or greater than that used in the immunogenicity test.

(3) Young adult mice, each weighing 14 to 16 grams, shall be used as test animals when the virus in vaccine prepared with a low egg passage Flury

Strain or high cell passage Street Alabama Dufferin Strain (HCP SAD) of rabies virus is titrated. At least 10 mice for each dilution shall be used.

(i) At least 10 mice shall be used for each dilution. Each shall be injected intracerebrally with 0.03 ml.

(ii) The injected young adult mice shall be observed each day for 14 days except when testing vaccines made with HCP SAD strain of rabies virus, in which case, the mice shall be observed each day for 21 days. Deaths and paralysis occurring subsequent to the fourth day post-injection shall be noted and the LD_{50} titer calculated by the Reed and Muench Method.

(iii) Virus titer requirements for release and at expiration date shall be determined for each vaccine on the basis of data available: *Provided*, That, the lowest titer permitted at expiration date when determined by this test shall be $10^{3.0}$ LD_{50} per 0.03 ml.

(4) Suckling mice, 6 days of age or younger, shall be used as test animals when virus in vaccine prepared with a high egg passage Flury Strain of rabies virus is titrated.

(i) Six to twelve mice shall be used for each dilution. Each shall be injected intracerebrally with 0.02 ml.

(ii) The injected suckling mice shall be observed each day for 21 days. Deaths and paralysis occurring subsequent to the fourth day post-injection shall be noted and the LD_{50} titer calculated by the Reed and Muench Method; and

(iii) Virus titer requirements for release and at expiration date shall be determined for each vaccine on the basis of data available: *Provided*, That, the lowest titer permitted at expiration date when determined by this test shall be $10^{3.0}$ LD_{50} per 0.02 ml.

[39 FR 44721, Dec. 27, 1974, as amended at 40 FR 20067, May 8, 1975; 42 FR 6795, Feb. 4, 1977; 43 FR 49529, Oct. 24, 1978; 50 FR 20090, May 14, 1985; 50 FR 23797, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 61 FR 31823, June 21, 1996]

§ 113.313 Measles Vaccine.

Measles Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300. Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(b) To detect virulent canine distemper virus, each of two canine distemper susceptible ferrets shall be injected with a sample of the Master Seed Virus equivalent to the amount of virus to be used in one dog dose and observed each day for 21 days. If undesirable reactions occur in either ferret, the lot of Master Seed Virus is unsatisfactory.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five dogs, less than 12 weeks of age and free of measles antibody, shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serum samples tested. The dogs shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test with less than 500 ID_{50} of measles virus.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Twenty dogs shall be vaccinated with a predetermined quantity of vaccine virus and the remaining five dogs held as unvaccinated controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) On the day of challenge, serum samples shall be obtained from each vaccinate and individually tested for antibody against canine distemper virus. For a valid test, each vaccinate shall be negative at a 1:4 final serum

dilution in varying serum-constant virus neutralization test using less than 500 ID_{50} of canine distemper virus.

(4) At least 21 days postinoculation, the immunity of the vaccinates and controls shall be challenged by exposure to a uniform dose of aerosolized virulent canine distemper virus. All test dogs shall be observed daily for 21 days postchallenge.

(i) If at least 4 of the 5 controls do not die or show signs of distemper, including a temperature of 104.0 °F. or higher and at least 15 percent weight loss, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive without showing a temperature of 104.0 °F. or higher and a weight loss exceeding 15 percent after day 8 postchallenge, the Master Seed Virus is unsatisfactory.

(5) When approved in advance by Animal and Plant Health Inspection Service, a sequential test procedure may be used in lieu of the 20 dog requirement. A beta value of 0.05 and a tolerance level of 0.78 shall be required.

(6) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided,* That five of five vaccinates and at least four of the controls shall meet the criteria prescribed in this section.

(7) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) Test requirements for release: Each serial and subserial shall meet the general requirements prescribed in §113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety tests.* The dog safety test prescribed in §113.40 and the mouse safety test prescribed in §113.33(a) shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of the vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ ID_{50} per dose.

[40 FR 53001, Nov. 14, 1975, as amended at 43 FR 49529, Oct. 24, 1978; 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§113.314 Feline Calicivirus Vaccine.

Feline Calicivirus Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300.

(b) The Master Seed Virus shall be tested for chlamydial agents as prescribed in §113.43.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Thirty feline calicivirus susceptible cats shall be used as test animals (20 vaccinates and 10 controls). Throat swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of feline calicivirus. Blood samples shall be drawn and individual serum samples tested. The cats shall be considered suitable for use if all swabs are negative for virus isolation and if all serums are negative for calicivirus antibody at the 1:2 final dilution in a 50 percent plaque reduction test or other SN test of equal sensitivity.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus

shall be established before the immunogenicity test is conducted. The 20 cats used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method to be recommended on the label and the remaining 10 cats shall be held as controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Twenty-one or more days after the final dose of vaccine, the vaccinates and controls shall each be challenged intranasally with a minimum of 100,000 $TCID_{50}$ or plaque forming units of virulent feline calicivirus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days postchallenge. The rectal temperature of each animal shall be taken and the presence or absence of clinical signs, particularly lesions on the oral mucosa, noted and recorded each day.

(i) If less than 8 of 10 controls show clinical signs of feline calicivirus infection other than fever, the test is inconclusive and may be repeated.

(ii) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by Animal and Plant Health Inspection Service and prescribed in the Outline of Production, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Either 10 vaccinates and 6 controls or 5 vaccinates and 3 controls shall be used in the retest.

(i) If less than 5 of 6 or 3 of 3 of the controls in the retest show clinical signs of feline calicivirus infection other than fever, the test is inconclusive and may be repeated.

(ii) A significant difference in clinical signs shall be demonstrated between vaccinates and controls in a valid test as prescribed in paragraph (c)(3)(ii) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the requirements prescribed in § 113.300 and in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* The mouse safety test prescribed in § 113.33(a) and the cat safety test prescribed in § 113.39(b) shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ or plaque forming units per dose.

[44 FR 58899, Oct. 12, 1979; 44 FR 63083, Nov. 2, 1979, as amended at 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.315 Feline Rhinotracheitis Vaccine.

Feline Rhinotracheitis Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300.

(b) The Master Seed Virus shall be tested for chlamydial agents as prescribed in § 113.43.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master

Seed Virus shall be established as follows:

(1) Thirty feline rhinotracheitis susceptible cats shall be used as test animals (20 vaccinates and 10 controls). Throat swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of feline rhinotracheitis virus. Blood samples shall be drawn and individual serum samples tested. The cats shall be considered suitable for use if all swabs are negative for virus isolation and if all serums are negative for feline rhinotracheitis virus antibody at the 1:2 final dilution in a 50 percent plaque reduction test or other SN test of equal sensitivity.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 cats used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method to be recommended on the label and the remaining 10 cats shall be held as controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Twenty-one or more days after the final dose of vaccine, the vaccinates and controls shall each be challenged intranasally with a minimum of 100,000 $TCID_{50}$ or plaque forming units of virulent feline rhinotracheitis virus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days post-challenge. The rectal temperature of each animal shall be taken and the presence of respiratory or other clinical signs of feline rhinotracheitis noted and recorded each day.

(i) If less than 8 of 10 controls show clinical signs of feline rhinotracheitis infection other than fever, the test is inconclusive and may be repeated.

(ii) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by Veterinary Services and prescribed in the Outline of Production, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Either 10 vaccinates and 6 controls or 5 vaccinates and 3 controls shall be used in the retest.

(i) If less than 5 of 6 or 3 of 3 of the controls in the retest show clinical signs of feline rhinotracheitis infection other than fever, the test is inconclusive and may be repeated.

(ii) A significant difference in clinical signs shall be demonstrated between vaccinates and controls in a valid test as prescribed in paragraph (c)(3)(ii) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the requirements prescribed in §113.300 and in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* The mouse safety test prescribed in §113.33(a) and the cat safety test prescribed in §113.39(b) shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ or plaque forming units per dose.

[44 FR 58899, Oct. 12, 1979, as amended at 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.316 Canine Parainfluenza Vaccine.

Canine Parainfluenza Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five canine parainfluenza susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Nasal swabs shall be collected from each dog on the day the first dose of vaccine is administered and individually tested on susceptible cell cultures for the presence of canine parainfluenza virus. Blood samples shall also be drawn and individual serum samples tested for neutralizing antibody. Dogs shall be considered susceptible if all swabs are negative for virus isolation and if all serums are negative for canine parainfluenza antibody at a 1:2 final dilution in a constant virus-varying serum neutralization test using 50 to 300 $TCID_{50}$ of canine parainfluenza virus.

(2) A geometric mean titer of vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 dogs used as vaccinates shall be administered a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used to confirm the dosage administered. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Three to 4 weeks after the final dose of vaccine, all dogs shall be bled for serum antibodies and nasal swabs shall be collected for canine parainfluenza virus isolation. On the same day, all vaccinates and controls shall be challenged with canine parainfluenza virus furnished or approved by Animal and Plant Health Inspection Service.

(4) The rectal temperature of each dog shall be taken and the presence of respiratory or other clinical signs of canine parainfluenza virus infection noted and recorded each day for 14 consecutive days postchallenge. Nasal swabs shall be collected from each dog each day for at least 10 consecutive days postchallenge. Individual swabs shall be tested for virus isolation by culture in canine parainfluenza virus susceptible cells for at least 7 days. Results shall be evaluated according to the following criteria:

(i) If five of five controls have not remained seronegative at a final serum dilution of 1:2 during the prechallenge period, the test is inconclusive and may be repeated.

(ii) If more than one vaccinate shows febrile response, respiratory or other clinical signs of canine parainfluenza virus infection; or, if less than 19 of 20 vaccinates show serum neutralization titers of 1:4 or greater; or, if there is not a significant reduction in virus isolation rate in vaccinates when compared with controls, the Master Seed is unsatisfactory.

(5) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest: *Provided,* That five of five vaccinates and five of five controls shall meet the criteria prescribed in paragraph (b)(4) of this section.

(6) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.300 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently

greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer at least $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ per dose.

(2) [Reserved]

[50 FR 436, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.317 Parvovirus Vaccine (Canine).

Parvovirus Vaccine recommended for use in dogs shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in §113.300 and the requirements in this section.

(b) The Master Seed shall be tested for reversion to virulence in dogs using a method acceptable to Animal and Plant Health Inspection Service. If a significant increase in virulence is seen within five backpassages, the Master Seed is unsatisfactory.

(c) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five canine parvovirus susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine parvovirus to determine susceptibility. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test in cell culture using 50 to 300 $TCID_{50}$ of canine parvovirus.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 dogs used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Fourteen days or more after the final dose of vaccine the vaccinates and the controls shall be challenged with virulent canine parvovirus furnished or approved by Animal and Plant Health Inspection Service and the dogs observed each day for 14 days. Rectal temperature, blood lymphocyte count, and feces for viral detection shall be taken from each dog each day for at least 10 days postchallenge and the presence or absence of clinical signs noted and recorded each day.

(i) The immunogenicity of the Master Seed shall be evaluated on the following criteria of infection: temperature ≥103.4 ° F; lymphopenia of ≥50 percent of prechallenge normal; clinical signs such as diarrhea, mucus in feces, or blood in feces; and viral hemagglutinins at a level of ≥1:64 in a 1:5 dilution of feces or a test of equal sensitivity. If at least 80 percent of the controls do not show at least three of the four criteria of infection during the observation period, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive the observation period without showing more than one criterion of infection described in paragraph (c)(3)(i), of this section, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Five susceptible dogs (four vaccinates and one control) may be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (c)(1) of this section.

(i) Each vaccinate shall be administered a predetermined quantity of vaccine virus as provided in paragraph (c)(2) of this section.

(ii) Fourteen to 21 days after the last vaccination, a second serum sample shall be drawn from each dog and tested for neutralizing antibody to canine

parvovirus in the same manner used to determine susceptibility.

(iii) If the control has not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If three of the four vaccinates in a valid test do not develop titers of at least 1:16 final serum dilution, and the remaining vaccinate does not develop a titer of at least 1:8, the Master Seed is unsatisfactory, except as provided in paragraph (c)(4)(v) of this section.

(v) If the results of a valid SN test are unsatisfactory, the vaccinates and the control may be challenged as provided in paragraph (c)(3) of this section. If at least three of the four criteria of infection are not shown in the control dog, the test is inconclusive and may be repeated, except that if any of the vaccinates show more than one criterion of infection, the Master Seed is unsatisfactory.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine used in the immunogenicity test in paragraph (c) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test, but not less than $10^{2.5}$ ID_{50} per dose.

[50 FR 436, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.318 Pseudorabies Vaccine.

Pseudorabies Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in §113.300 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five pseudorabies susceptible pigs (20 vaccinates and 5 controls) of the youngest age for which the vaccine is recommended, shall be used as test animals. Blood samples shall be taken from each pig and the serums inactivated and individually tested for neutralizing antibody against pseudorabies virus. Pigs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test using 50 to 300 $TCID_{50}$ pseudorabies virus.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 pigs used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) Fourteen to 28 days postvaccination, the vaccinates and controls shall be challenged with virulent pseudorabies virus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days.

(i) If at least four of the five controls do not develop severe central nervous system signs or die, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates in a valid test do not remain free of signs of pseudorabies, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot is discontinued. Only five vaccinates and five controls need to be

used in the retest. Susceptibility and age requirements shall be as provided in paragraph (b)(1) of this section.

(ii) Fourteen to 28 days postvaccination, a blood sample shall be taken from each pig and the serum inactivated and tested for neutralizing antibody to pseudorabies virus by the same method used to determine susceptibility.

(iii) If the five controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If at least four of the five vaccinates in a valid test have not developed titers of 1:8 final serum dilution or greater and the remaining vaccinate a titer of 1:4 or greater, the Master Seed is unsatisfactory, except as provided in paragraph (b)(4)(v).

(v) If the results of a valid neutralization test are unsatisfactory, the vaccinates and controls may be challenged as provided in paragraph (b)(3) of this section. If at least four of five controls do not develop severe central nervous system signs or die, the test is inconclusive and may be repeated. If all five of the vaccinates in a valid test do not remain free of signs of pseudorabies, the Master Seed is unsatisfactory.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and the requirements in this paragraph.

(2) *Virus titer requirements.* Final container samples of completed product shall be titrated by the method used in paragraph (b)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer at least $10.^{0.7}$ greater than that used in the immunogenicity test, but not less than $10^{2.5}$ $TCID_{50}$ per dose.

[50 FR 437, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§§ 113.319–113.324 [Reserved]

§ 113.325 Avian Encephalomyelitis Vaccine.

Avian Encephalomyelitis Vaccine shall be prepared from virus-bearing tissues or fluids from embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly.

(c) Each lot of Master Seed Virus shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Avian encephalomyelitis susceptible chickens, all of the same age (eight weeks or older) and from the same source, shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(2) A geometric mean titer of the vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least

three appropriate (not to exceed tenfold) dilutions shall be used and the test conducted as follows:

(i) For each dilution, inoculate at least 10 embryos, 5 or 6 days old, in the yolk sac with 0.2 ml each. Twenty similar embryos obtained from the same source shall be kept as uninoculated negative controls. Disregard all deaths during the first 48 hours post-inoculation.

(ii) Eggs for each dilution shall be kept in separate containers and allowed to hatch. Sufficient precaution shall be taken to assure that chickens from each dilution remain separated. To be a valid test, at least 75 percent of the uninoculated eggs shall hatch.

(iii) On the third day after normal hatching time, count all unhatched eggs and all dead, paralyzed and ataxic chickens as positive evidence of viral infection.

(iv) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(v) Calculate the EID_{50} by the Spearman-Karber or Reed-Muench method.

(3) At least 21 days post-vaccination, the vaccinates and the controls shall be challenged intracerebrally with a virulent avian encephalomyelitis virus and observed each day for 21 days.

(4) If at least 80 percent of the controls do not show signs of avian encephalomyelitis or die, the test is inconclusive and may be repeated. If at least 19 of 20, or 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free from clinical signs of avian encephalomyelitis during the observation period, the Master Seed Virus is unsatisfactory.

(5) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(4) of this section.

(6) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in § 113.300 and the requirements prescribed in this paragraph.

(1) Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the vaccine judged accordingly.

(2) *Safety test.* Final container samples of completed product shall be tested for safety as follows:

(i) At least 25 AE susceptible birds (6 to 10 weeks of age) shall be vaccinated with the equivalent of 10 doses by each of all routes recommended on the label and be observed each day for 21 days.

(ii) If unfavorable reactions attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated, except that, if the test is not repeated, the serial shall be unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in such immunogenicity test but not less than $10^{.5}$ EID_{50} per dose.

[39 FR 44723, Dec. 27, 1974, as amended at 40 FR 18405, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 42 FR 43617, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.326 Avian Pox Vaccine.

Fowl Pox Vaccine and Pigeon Pox Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300 except paragraph (c) of this section and shall meet the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken inoculation test prescribed in § 113.36.

(c) Each lot of Master Seed Virus shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Fowl pox susceptible birds all of the same age and from the same source, shall be used as test birds. Twenty or more birds shall be used as vaccinates for each method of administration recommended on the label. Ten additional birds of the same age and from the same source as the vaccinates shall be held as unvaccinated controls.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each bird used in the test. At least three appropriate (not to exceed tenfold) dilutions shall be used and the test conducted as follows:

(i) For each dilution, inoculate at least five embryos, 9 to 11 days old, on the chorioallantoic membrane with at least 0.2 ml each. Disregard all deaths during the first 24 hours post-inoculation. To be a valid test, at least four embryos in each dilution shall remain viable beyond 24 hours.

(ii) Examine the surviving embryos for evidence of infection 5 to 7 days post-inoculation.

(iii) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(iv) Calculate the EID_{50} by the Spearman-Karber or Reed-Muench method.

(3) Fourteen to twenty-one days post-vaccination, all vaccinates and controls shall be challenged by the wing web method and observed each day for 10 days. If the wing web method was used for vaccination, the opposite wing shall be used for challenge. Challenge virus shall be provided or approved by Animal and Plant Health Inspection Service.

(4) If at least 90 percent of the controls do not develop fowl pox during the observation period, the test is inconclusive and may be repeated. If at least 19 of 20, or 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free from clinical signs of fowl pox during the observation period, the Master Seed Virus is unsatisfactory.

(5) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(4) of this section.

(6) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the requirements in § 113.36, in § 113.300 except paragraph (c), and in this paragraph.

(1) *Safety test.* Final container samples of completed product from each serial shall be tested. Vaccines recommended for use in birds 10 days of age or younger shall be tested in accordance with paragraphs (d)(1)(i), (ii), and (iii) of this section.

(i) Each of 25 susceptible birds 5 days of age or younger, properly identified and obtained from the same source and hatch, shall be vaccinated with the equivalent of 10 doses of vaccine by each of all routes recommended on the label and observed each day for 14 days. Severe clinical signs or death shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has three failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of birds	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	2 or less	4 or more.
2	50	5 or less	6 or more.

(iii) If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and may be repeated or, in lieu thereof, the serial declared unsatisfactory.

(iv) Vaccines not recommended for use in birds 10 days of age or younger shall be tested for safety as follows: Each of twenty-five 3- to 5-week-old, fowl-pox susceptible birds shall be vaccinated with the equivalent of 10 doses of vaccine by each of all routes recommended on the label and observed each day for 14 days. If any of the birds show severe clinical signs of disease or death during the observation period due to causes attributable to the product, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and may be repeated or, in lieu thereof, the serial declared unsatisfactory.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in such immunogenicity test but not less than $10^{2.0}$ EID_{50} per dose.

[39 FR 44724, Dec. 27, 1974, as amended at 40 FR 18406, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 44 FR 33051, June 8, 1979; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.327 Bronchitis Vaccine.

Bronchitis Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300 and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Bronchitis susceptible chickens, all of the same age and from the same source, shall be used in the virus-recovery test. For each method of administration recommended on the label for each serotype against which protection is claimed, twenty or more chickens shall be used as vaccinates. Ten additional chickens for each serotype against which protection is claimed shall be held as unvaccinated controls.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the

immunogenicity tests are conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in such tests. At least three approved (not to exceed tenfold) dilutions shall be used and the test conducted as follows;

(i) For each dilution, inject at least five embryos, 9 to 11 days old, in the allantoic cavity with 0.1 ml each. Deaths occurring during the first 24 hours shall be disregarded, but at least four viable embyros in each dilution shall survive beyond 24 hours of a valid test. After 5 to 8 days incubation, examine the surviving embryos for evidence of infection.

(ii) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(iii) Calculate the EID_{50} by the Spearman-Karber or Reed-Muench method.

(3) Twenty-one to twenty-eight days post-vaccination, all vaccinates and controls shall be challenged by eyedrop with virulent bronchitis virus. A separate set of vaccinates and controls shall be used for each serotype against which protection is claimed. Each challenge virus shall be approved or provided by Animal and Plant Health Inspection Service and shall titer at least $10^{4.0}$ EID_{50} per ml.

(i) Tracheal swabs shall be taken once, 5 days post-challenge, from each control and vaccinate. Each swab shall be placed in a test tube containing 3 ml of tryptose phosphate broth and antibiotics. The tube and swab shall be swirled thoroughly and if they are to be stored, be immediately frozen and be stored at below −40 °C. pending egg evaluation. For each chicken swab, at least five chicken embryos 9 to 11 days old shall be inoculated in the allantoic cavity with 0.2 ml each of broth from each tube.

(ii) All embryos surviving the third day post-inoculation shall be used in the evaluation, except that, if a swab is not represented by at least four embryos, the test of that swab is invalid and the results inconclusive. A tracheal swab shall be positive for virus recovery when any of the embryos in a valid test show typical infectious bronchitis virus lesions, such as but not limited to, stunting, curling, kidney urates, clubbed down, or death during the 4 to 7 day post-inoculation period. If less than 20 percent of the embryos which survive the third day post-inoculation die during the 4 to 7 day post-inoculation period and show no gross lesions typical of infectious bronchitis, they may be disregarded.

(iii) If less than 90 percent of the controls are positive for virus recovery, the test is inconclusive and may be repeated.

(iv) If less than 90 percent of the vaccinates are negative for virus recovery, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(3) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in §113.300 and the requirements prescribed in this paragraph, except that, if the vaccine contains more than one virus type, bulk samples taken from each type prior to mixing shall be used in the virus identity tests prescribed in §113.300(c). The additional requirements in this paragraph shall also be met.

(1) Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the vaccine judged accordingly.

(2) *Safety test.* Final container samples of completed product shall be tested to determine safety for use in bronchitis susceptible young chickens.

(i) Twenty-five susceptible chickens, 5 days of age or younger, properly identified and obtained from the same source and hatch, shall be vaccinated by the eye-drop method with the equivalent of 10 doses of vaccine and observed each day for 21 days post-vaccination. Severe respiratory signs or death shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has three failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of chickens	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	2 or less	4 or more.
2	50	5 or less	6 or more.

If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated or, in lieu thereof, the serial declared unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the procedure prescribed in paragraph (c)(2) of this section and in this paragraph.

(i) The Newcastle disease virus fraction of combined Newcastle-Bronchitis Vaccines shall be neutralized prior to titration of the bronchitis virus fraction. Equal parts of heat-inactivated Newcastle disease antiserum shall be mixed with each appropriate serial tenfold dilution of the vaccine. After inactivation, embryos shall be injected with 0.2 ml each and results calculated as a 0.1 ml dose to allow for serum dilution of the vaccine. The allantoic fluids, tested as prescribed in § 113.34 shall not show hemagglutinating activity in the lowest dilution used in the titration.

(ii) Each bronchitis virus type shall be harvested separately and a sample of bulk harvested material shall be collected prior to mixing with the other virus type(s). Each sample shall contain not less than the minimum virus titer stated in the filed Outline of Production.

(iii) To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in such immunogenicity test but not less than $10^{2.0}$ EID_{50} per dose.

[39 FR 44724, Dec. 27, 1974, as amended at 40 FR 18406, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 42 FR 43617, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 64 FR 43045, Aug. 9, 1999]

§ 113.328 Fowl Laryngotracheitis Vaccine.

Fowl Laryngotracheitis Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300 and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly. Each lot shall also be tested for safety as follows:

(1) Each of at least ten 3 to 4 week old susceptible chickens obtained from the same source and hatch as those used in the immunogenicity test prescribed in paragraph (c) of this section shall be injected intratracheally with

0.2 ml of the virus as used in the vaccine and the chickens observed each day for 14 days.

(2) If more than 20 percent of the chickens die during the observation period, the virus is unsatisfactory.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Fowl laryngotracheitis susceptible chickens all of the same age and from the same source shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed tenfold) dilutions shall be used for vaccine of chicken embryo origin and the test conducted as follows:

(i) For each dilution, inject at least five embryos, 9 to 11 days old, on the chorioallantoic membrane with 0.2 ml each. Disregard all deaths during the first 24 hours post-injection. To be a valid test, at least four embryos in each dilution shall remain viable beyond 24 hours.

(ii) Examine the surviving embryos for evidence of infection 5 to 8 days post-injection.

(iii) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(iv) Calculate the EID_{50} by the Spearman-Karber or Reed-Muench method.

(3) Tissue culture origin vaccine may be titrated by a tissue culture method approved by Animal and Plant Health Inspection Service and written into the filed Outline of Productions.

(4) Ten to fourteen days post-vaccination, all vaccinates and controls shall be challenged intratracheally or in the orbital sinus with infectious fowl laryngotracheitis virus and observed each day for 10 days. Challenge virus shall be provided or approved by Animal and Plant Health Inspection Service.

(5) If at least 80 percent of the controls do not die or show clinical signs of fowl laryngotracheitis during the observation period, the test is inconclusive and may be repeated. If at least 19 of 20, 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free of clinical signs of fowl laryngotracheitis during the observation period, the Master Seed Virus is unsatisfactory.

(6) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraphs (c)(4) and (5) of this section.

(7) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in §113.300 and the requirements prescribed in this paragraph.

(1) Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the vaccine judged accordingly.

(2) *Safety test.* Final container samples of completed product from each serial of modified live virus vaccine shall be tested for safety as provided in this paragraph. Live virus vaccine not prepared with modified live virus shall be

tested for safety as provided in the filed Outline of Production.

(i) Twenty-five 3 to 4 week old laryngotracheitis susceptible chickens shall be injected intratracheally with 0.2 ml of vaccine rehydrated at the rate of 30 ml for 1,000 doses. Chickens shall be observed each day for 14 days. Deaths shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has five, six, or seven failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of chickens	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	4 or less	8 or more.
2	50	10 or less	11 or more.

(iii) If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated or in lieu thereof, the serial declared unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method provided in paragraphs (c)(2) or (3) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in such immunogenicity test but not less than $10^{2.5}$ EID_{50} per dose for chicken embryo origin vaccine and $10^{2.0}$ EID_{50} or $10^{2.5}$ $TCID_{50}$ per dose for tissue culture origin vaccine.

[39 FR 44726, Dec. 27, 1974, as amended at 40 FR 18407, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 41 FR 44359, Oct. 8, 1976; 42 FR 43617, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.329 Newcastle Disease Vaccine.

Newcastle Disease Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300, except § 113.34, and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Newcastle Disease susceptible chickens, all of the same age and from the same source, shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed tenfold) dilutions shall be used and the test conducted as follows:

(i) For each dilution, inject at least five embryos, 9 to 11 days old, in the allantoic cavity with at least 0.1 ml each. Disregard all deaths during the first 24 hours post-injection. To be a

valid test, at least four embryos in each dilution shall remain viable beyond 24 hours.

(ii) Examine the surviving embryos for evidence of infection 5 to 7 days post-injection.

(iii) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(iv) Calculate the EID_{50} by the Spearman-Karber or Reed-Muench method.

(3) Twenty to twenty-eight days postvaccination, all vaccinates and controls shall be challenged intramuscularly with at least $10^{4.0}$ EID_{50} of virus per chicken and observed each day for 14 days. Challenge virus shall be provided or approved by Animal and Plant Health Inspection Service.

(4) If at least 90 percent of the controls do not develop clinical signs of Newcastle disease during the observation period, the test is inconclusive and may be repeated. If at least 19 of 20, or 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free from clinical signs of Newcastle disease during the observation period, the Master Seed Virus is unsatisfactory.

(5) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(4) of this section.

(6) A strain identity test acceptable to Animal and Plant Health Inspection Service shall be conducted.

(7) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in §113.300, except §113.34, and the requirements prescribed in this paragraph.

(1) Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the vaccine judged accordingly.

(2) Safety test: Final container samples of completed product from each serial shall be tested to determine whether the vaccine is safe for use in susceptible young chickens. Vaccines recommended for use in chickens 10 days of age or younger shall be tested in accordance with paragraphs (d)(2)(i), (ii), and (iii) of this section.

(i) Twenty-five susceptible chickens, 5 days of age or younger, properly identified and obtained from the same source and hatch, shall be vaccinated by the eye drop method with the equivalent of 10 doses of vaccine and the chickens observed each day for 21 days. Severe respiratory signs or death shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has 3 failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of chickens	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	2 or less	4 or more.
2	50	5 or less	6 or more.

(iii) If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and may be repeated.

(iv) Vaccines not recommended for use in chickens 10 days of age or younger shall be tested for safety as follows:

Each of twenty-five 3 to 5 week old Newcastle disease susceptible chickens shall be vaccinated as recommended on the label with the equivalent of ten doses and observed each day for 21 days. If any of the birds show severe clinical signs of disease or death during the observation period due to causes attributable to the product, the serial is unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the

titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer per dose sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{5.5}$ EID_{50} per dose.

[39 FR 44727, Dec. 27, 1974, as amended at 40 FR 18407, Apr. 28, 1975; 40 FR 23721, June 2, 1975; 40 FR 41090, Sept. 5, 1975; 42 FR 43618, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.330 Marek's Disease Vaccines.

Marek's disease vaccine shall be prepared from virus-bearing tissue culture cells. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300, and the requirements prescribed in this section. The identity test required in § 113.300(c) shall be conducted in a serotype-specific manner by a method acceptable to APHIS. Each lot of Master Seed Virus shall also be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly.

(b) *Safety test.* The Master Seed Virus shall be nonpathogenic for chickens as determined by the following procedure:

(1) Specific pathogen free chickens or embryos, negative for Marek's disease virus antibodies, and from the same source, shall be isolated into the following groups:

(i) *Group 1.* At least 50 test subjects shall be inoculated with 10 times as much viable virus as will be contained in one dose of vaccine, by the route recommended for vaccination.

(ii) *Group 2.* At least 50 test subjects shall be injected with a very virulent Marek's disease virus provided or approved by APHIS, at a dosage level that will cause gross lesions of Marek's disease in at least 80 per cent of the chickens within 50 days.

(iii) *Group 3.* Fifty uninoculated controls. For *in ovo* studies, this group should receive a sham inoculation of diluent.

(iv) *Group 4.* For studies evaluating Serotype 1 Master Seed Viruses, a group of 50 uninoculated control chickens shall be housed in contact with the group 1 vaccinated chickens.

(2) At least 40 chickens in each group shall survive to 5 days of age. All chickens that die shall be necropsied and examined for lesions of Marek's disease and cause of death. The test shall be judged according to the following criteria:

(i) At 50 days of age, the remaining chickens in group 2 shall be killed and examined for gross lesions of Marek's disease. If at least 80 percent of this group do not develop Marek's disease, the test is inconclusive and may be repeated.

(ii) At 120 days of age, the remaining chickens in groups 1, 3, and 4 shall be weighed, killed, and necropsied. If less than 30 of the chickens in group 3 survive the 120 day period, or if any of the chickens in group 3 have gross lesions of Marek's disease at necropsy, the test is declared inconclusive. If less than 30 chickens in groups 1 and 4 survive the 120 day period; or if any of the chickens in groups 1 and 4 have gross lesions of Marek's disease at necropsy; or if the average body weight of the chickens in groups 1 or 4 is significantly (statistically) different from the average in group 3 at the end of the 120 days, the lot of Master Seed Virus is unsatisfactory.

(3) For tests involving *in ovo* inoculation, hatchability results shall also be reported for each group.

(c) *Immunogenicity.* Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity at the highest passage level allowed for the product, and the virus dose to be used shall be established as follows:

(1) Specific pathogen free chickens or embryos, negative for Marek's disease antibodies, and from the same source,

shall be isolated into the following groups:

(i) *Group 1.* A minimum of 35 test subjects shall be inoculated with the vaccine, using the recommended route, at 1 day of age for chicks or 18 days of embryonation for embryos. The dose used shall be established by 5 replicate virus titrations conducted by a cell culture system or other titration method acceptable to APHIS.

(ii) *Group 2.* A minimum of 35 nonvaccinated test subjects shall be held as challenge controls.

(iii) *Group 3.* A minimum of 25 nonvaccinated test subjects shall be held as nonchallenge controls.

(iv) *Group 4.* Except for studies evaluating vaccines which contain only a Serotype 3 virus as the Marek's disease fraction, a minimum of 35 chicks shall be vaccinated at 1 day of age with a licensed Serotype 3 vaccine, in order to document the severity of the very virulent challenge.

(2) At least 30 chickens in groups 1, 2, and 4, and at least 20 chickens in group 3, shall survive to 5 days of age. All chickens in groups 1, 2, and 4 shall be challenged at 5 days of age in the following manner:

(i) For studies evaluating vaccines which contain only a Serotype 3 virus as the Marek's disease fraction, groups 1 and 2 shall be inoculated with a standard virulent challenge virus provided or approved by APHIS.

(ii) For all other Marek's disease vaccines, groups 1, 2, and 4 shall be inoculated with a very virulent challenge virus provided or approved by APHIS.

(3) All chickens shall be observed until 7 weeks of age, necropsied, and examined for grossly observable lesions consistent with Marek's disease. All chickens dying before the end of the 7 week observation period shall be necropsied and evaluated for gross lesions of Marek's disease. Any chickens not so examined shall be scored as positive for Marek's disease.

(4) For a valid test, at least 80 percent of the chickens in group 2 must develop grossly observable lesions, none of the chickens in group 3 shall develop grossly observable lesions, and (when included) greater than 20 percent of the chickens in group 4 must develop grossly observable lesions.

(5) For a valid test to be considered satisfactory, at least 80 percent of the chickens in group 1 must remain free of grossly observable lesions. The appropriate product claim resulting from a satisfactory test would be to aid in the prevention of Marek's disease, for vaccines containing only a Serotype 3 virus as the Marek's disease fraction, or to aid in the prevention of very virulent Marek's disease, for all other vaccines.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable requirements prescribed in §113.300. The identity test required in §113.300(c) shall be conducted in a serotype-specific manner by a method acceptable to APHIS. Final container samples of completed product shall also meet the requirements in paragraphs (d) (1), (2), and (3) of this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Purity test.* The chicken embryo inoculation test prescribed in §113.37 shall be conducted, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly.

(2) *Safety test.* At least 25 one-day-old, specific pathogen free chickens shall be injected, by the subcutaneous route, with the equivalent of 10 chicken doses of virus (vaccine concentrated 10X). The chickens shall be observed each day for 21 days. Chickens dying during the period shall be examined, cause of death determined, and the results recorded.

(i) If at least 20 chickens do not survive the observation period, the test is inconclusive.

(ii) If lesions of any disease or cause of death are directly attributable to the vaccine, the serial is unsatisfactory.

(iii) If less than 20 chicks survive the observation period and there are no deaths or lesions attributable to the vaccine, the test may be repeated one time, *Provided,* that if the test is not repeated, the serial shall be declared unsatisfactory.

(3) *Potency test.* The samples shall be titrated using a cell culture system or

other titration method acceptable to APHIS. For vaccines composed of more than one Marek's disease virus serotype, each fraction shall be titrated in a serotype-specific manner.

(i) Samples of desiccated vaccine shall be incubated at 37°C for 3 days before preparation for use in the potency test. Samples of desiccated or frozen vaccine shall be reconstituted in diluent according to the label recommendations, and held in an ice bath at 0°C to 4°C for 2 hours prior to use in the potency test.

(ii) For a serial or subserial to be eligible for release, each serotype contained in the vaccine shall have a virus titer per dose which is at least 3 times greater than the number of plaque forming units (pfu) used in the immunogenicity test prescribed in paragraph (c) of this section, but not less than 1000 pfu per dose.

(iii) When tested (without the pretest incubation of desiccated products) at any time within the expiration period, each serotype contained in the vaccine shall have a virus titer per dose which is at least 2 times the number of pfu used in the immunogenicity test, but not less than 750 pfu per dose.

[61 FR 33841, July 1, 1996]

§ 113.331 Bursal Disease Vaccine.

Bursal Disease Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300 and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly. Each lot of Master Seed Virus used in the preparation of modified live virus vaccines shall also be nonpathogenic to chickens as determined by the following procedures:

(1) Each of twenty-five 1-day-old bursal disease susceptible chickens (vaccinates) shall be injected subcutaneously with 10 times the recommended dose of vaccine virus and observed for 21 days. Fifteen chickens of the same source and hatch shall be kept isolated as controls.

(i) Seventeen days postvaccination, each of five controls shall be administered at least $10^{2.0}$ EID_{50} of a virulent bursal disease virus by eye-drop, isolated, and used as positive controls. The remaining controls shall be used as negative controls.

(ii) If the vaccinates do not remain free of clinical signs of bursal disease, the Master Seed Virus is unsatisfactory. If unfavorable reactions which are not attributable to the Master Seed Virus occur in more than two of the vaccinates, the test shall be declared inconclusive and may be repeated.

(iii) Twenty-one days postvaccination, the vaccinates and the controls shall be necropsied and examined for gross lesions of bursal disease. If more than two of the vaccinates have such lesions, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls or less than four of the positive controls have such lesions, the test is inconclusive and may be repeated. For purposes of this test, gross lesions shall include obvious pathological processes and/or obvious reduction in size of the bursa from normal.

(2) Each of thirty-five 3- to 4-week-old bursal disease susceptible chickens (vaccinates) shall be vaccinated with approximately one minimum protective dose of vaccine virus as determined in paragraph (c) of this section. Each of 10 chickens of the same source and hatch shall be administered at least $10^{2.0}$ EID_{50} of a virulent bursal disease virus by eye-drop, isolated, and used as positive controls. Also, each of 20 additional chickens of the same source and hatch shall be isolated and held as negative controls.

(i) Three or four days postvaccination, 10 of the vaccinates,

the 10 positive controls, and 10 of the negative controls shall be necropsied and examined for gross lesions of bursal disease. If any of the vaccinates have such lesions, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls or less than 8 of the positive controls have such lesions, the test is inconclusive and may be repeated. For purposes of this test, gross lesions shall include peri-bursal edema and/or edema and/or macroscopic hemorrhage in the bursal tissue.

(ii) Fourteen days post–vaccination, the remaining vaccinates and negative controls shall be necropsied and examined for obvious bursal atrophy. If any of the vaccinates have such atrophy, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls have such atrophy, the test is inconclusive and may be repeated.

(c) Each lot of Master Seed Virus shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Bursal Disease susceptible chickens, all of the same age (3 weeks or younger) and from the same source, shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(2) A geometric mean titer of the vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed tenfold) dilutions shall be used to conduct the titrations by a method acceptable to Animal and Plant Health Inspection Service.

(3) When the test chickens are 28 to 35 days of age but not less than 14 days postvaccination, each vaccinate and each control shall be challenged by eye-drop with a virulent bursal disease virus provided or approved by Animal and Plant Health Inspection Service.

(i) Three to five days postchallenge, all vaccinates and controls shall be necropsied and examined for gross lesions of bursal disease as described in paragraph (b)(2)(i) of this section.

(ii) If at least 19 of 20, or 27 of 30, or 36 of 40 vaccinates in each group are not free from such lesions, the Master Seed Virus is unsatisfactory, except that, if less than 90 percent of the controls have such lesions, the test is inconclusive and may be repeated.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years from the original testing unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(3) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in §113.300 and the requirements prescribed in this paragraph.

(1) *Tests for pathogens.* Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the serial judged accordingly.

(2) *Safety tests.* (i) Final container samples of completed product from each serial shall be tested to determine whether the vaccine is safe as follows:

(A) For vaccines intended for parenteral administration, each of twenty-five 1-day-old bursal disease susceptible chickens shall be vaccinated with the equivalent of 10 doses by subcutaneous injection.

(B) For vaccines intended for drinking water administration, each of

twenty-five 4- to 5-week-old bursal disease susceptible chickens shall be vaccinated orally with the equivalent of 10 doses.

(C) Ten chickens of the same source and hatch shall be maintained in isolation as negative controls. The vaccinates and controls shall be observed each day for 21 days.

(ii) If unfavorable reactions which are attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur in more than one of the controls or if unfavorable reactions which are not attributable to the biological product occur in more than two of the vaccinates, the test shall be declared inconclusive and repeated, except that, if the test is not repeated, the serial shall be unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ times greater than that used in such immunogenicity test, but not less than $10^{2.0}$ titration units (PFU or ID_{50}'s) per dose.

[44 FR 60263, Oct. 19, 1979, as amended at 44 FR 67087, Nov. 23, 1979; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 64 FR 43045, Aug. 9, 1999]

§ 113.332 Tenosynovitis Vaccine.

Tenosynovitis Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs.

Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300, except (a)(3)(ii) and (c), and the special requirements in this section.

(b) Each lot of Master Seed shall be tested for:

(1) Pathogens by the chicken inoculation test prescribed in § 113.36.

(2) Lymphoid leukosis virus contamination as follows:

(i) Each of at least 10 3-week-old or older lymphoid leukosis free chickens from the same source and hatch shall be injected intra-muscularly with an amount of Master Seed equal to 100 label doses of vaccine. At least 15 chickens of the same source and hatch shall be used as controls; 5 or more shall be unvaccinated and serve as negative controls; 5 or more shall be injected with subgroup A lymphoid leukosis virus; and 5 or more with subgroup B lymphoid leukosis virus. Each group of control chickens shall be held isolated from each other and from the vaccinates.

(ii) Twenty-one to 28 days postinoculation, blood samples shall be taken from each chicken and the serum separated using a technique conducive to virus preservation. These serums shall be used as inocula in the complement fixation for avian lymphoid leukosis (COFAL) test prescribed in § 113.31.

(iii) Serums from the vaccinates shall be tested separately, but serums within each control group may be pooled. A valid test shall have positive COFAL reactions from each virus inoculated group and negative reactions from the uninoculated controls. If any of the chickens injected with the Master Seed have positive COFAL test reactions in a valid test, the Master Seed is unsatisfactory.

(3) Identity using the following agar gel immunodiffusion test. The undiluted Master Seed may be used as test antigen or the Master Seed may be inoculated onto the chorioallantoic membrane (CAM) of fully susceptible chicken embryos and the infected CAMs ground and used as antigen. A known tenosynovitis antiserum and a known tenosynovitis antigen shall be used in the test. A precipitin line shall form between the test antigen and the known antiserum in the center well which shows identity with the line formed between the antiserum and the

known antigen, or the Master Seed is unsatisfactory.

(4) Safety using the following chicken test:

(i) For vaccines intended for use in chickens less than 14 days of age, Master Seed equal to 10 label doses shall be administered subcutaneously to each of 25 1-day-old tenosynovitis susceptible chickens.

(ii) For vaccines intended for use only in chickens 14 days of age or older, Master Seed equal to 10 label doses shall be administered subcutaneously to each of 25 4-week-old or older tenosynovitis susceptible chickens.

(iii) The vaccinates shall be observed each day for 21 days. If unfavorable reactions occur which are attributable to the vaccine, the Master Seed is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated.

(c) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Tenosynovitis susceptible chickens, of the same age and from the same source shall be used as test birds. Vaccines intended for use in very young chickens shall be administered to chickens of the youngest age for which the vaccine is recommended. Vaccines intended for use in older chickens shall be administered to 4-week-old or older chickens. Twenty or more vaccinates shall be used for each method of administration recommended on the label. Ten or more chickens shall be held as unvaccinated controls.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established using a method acceptable to Animal and Plant Health Inspection Service before the immunogenicity test is conducted. A predetermined quantity of vaccine virus shall be administered to each vaccinate. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the dose.

(3) Twenty-one to 28 days postvaccination, each vaccinate and control shall be challenged by injecting virulent virus furnished or approved by Animal and Plant Health Inspection Service into one foot pad. The vaccinates and controls shall be observed each day for 14 days. If at least 90 percent of the controls do not develop swelling and discoloration in the phalangeal joint area of the injected foot pad typical of infection with tenosynovitis virus, the test is inconclusive and may be repeated. If at least 19 of 20, 27 of 30, or 36 of 40 vaccinates do not remain free from these signs, disregarding transient swelling which subsides within 5 days postchallenge, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and controls shall meet the criteria prescribed in paragraph (c)(3) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300, except (c), and the requirements in this paragraph.

(1) *Purity.* Final container samples of completed product from each serial shall be tested for pathogens by the chicken inoculation test prescribed in §113.36.

(2) *Safety.* (i) Final container samples of completed product from each serial shall be safety tested as follows:

(A) For vaccines intended for use in very young chickens, each of 25 1-day-old tenosynovitis susceptible chickens shall be vaccinated with the equivalent of 10 doses by one method recommended on the label.

(B) For vaccines intended for use in older chickens, each of 25 4-week-old or older tenosynovitis susceptible chickens shall be vaccinated with the equivalent of 10 doses by one method recommended on the label.

(ii) The vaccinates shall be observed each day for 21 days. If unfavorable reactions occur which are attributable to the product, the serial is unsatisfactory. If unfavorable reactions occur in more than two vaccinates which are

not attributable to the product, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be titrated by the method used in paragraph (c)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer $10^{0.7}$ times greater than that used in the immunogenicity test, but not less than $10^{2.0}$ titration units (PFU or ID_{50}) per dose.

(4) *Identity.* Bulk or final container samples of completed product from each serial shall be tested for identity as prescribed in paragraph (b)(3) of this section and shall meet the criteria stated therein.

[50 FR 438, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 64 FR 43045, Aug. 9, 1999]

DIAGNOSTICS AND REAGENTS

§§ 113.400–113.405 [Reserved]

§ 113.406 Tuberculin, Intradermic.

Tuberculin, Intradermic, is a filtrate produced from cultures of Pn, C, and Dt strains of *Mycobacterium tuberculosis* (supplied by Animal and Plant Health Inspection Service) which has been inactivated and is non-toxic. Each serial shall be tested for purity, safety, potency, and special chemical tests in accordance with the conditions prescribed for each test. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Each serial shall be tested for purity as provided in this paragraph.

(1) Final container samples of completed product shall be tested for viable bacteria and fungi as prescribed in § 113.26.

(2) A 20 ml sample shall be centrifuged and the sediment examined microscopically for the presence of acidfast (Ziehl-Nielsen stain) or other microorganisms (Gram stain). A serial which contains microorganisms is unsatisfactory for release.

(b) *Safety test.* Final container samples of completed product from each serial shall be tested for safety. Two mature guinea pigs shall be injected subcutaneously with 1 ml and observed for 10 days. If unfavorable reactions attributable to the product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated: *Provided,* That if the test is not repeated, the serial shall be declared unsatisfactory.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be subjected to a comparison test using a Reference Tuberculin supplied by Animal and Plant Health Inspection Service. Test animals shall be 10 sensitized white female guinea pigs from one source which weigh 500–700 grams at the beginning of the test and which have not been used in a previous test. The comparison test shall be conducted in accordance with the procedures prescribed in paragraphs (c)(1), (2), (3), (4), (5), (6), (7), and (8) of this section.

(1) The guinea pigs shall be sensitized with a sterile heat-killed suspension of equal amounts of strains Pn, C, and Dt of *Mycobacterium tuberculosis.* The heat-killed sensitizing agent shall be injected in a volume of 0.5 ml per guinea pig. The guinea pigs shall be considered sensitized for testing not less than 30 days nor more than 120 days post-injection.

(2) The guinea pigs shall be prepared for sensitivity testing at least 4 hours prior to the injection of tuberculin. The entire abdominal and flank areas shall be clipped, a depilatory agent applied for 5–10 minutes, the area rinsed with warm water, and dried.

(3) Dilutions of 1:100, 1:200, and 1:400 shall be prepared with the Reference Tuberculin and the unknown tuberculin. Three test sites on each side of and equidistant from the abdominal midline shall be chosen on each guinea pig. Using a tuberculin syringe and needle, 0.05 ml of each dilution shall be injected intradermally at one of the

test sites which has been randomly selected for the dilution.

(4) The sensitivity of the tuberculins shall be determined 24 hours after injected by measuring the area of erythema. Measurements in millimeters shall be made anterior of the greatest diameter and perpendicular to the first measurement. The square millimeter shall be calculated by multiplying the two measurements.

(5) The total area of response for each tuberculin tested shall be determined by adding the areas of erythema for each dilution of each of the test animals in a group. The sums of the areas of erythema for all three dilutions of each tuberculin shall be added to give the total area of tuberculin response.

(6) The total tuberculin response area of the serial being tested shall be expressed as a percentage of the total tuberculin response area of the Reference Tuberculin. (The total response area of the serial divided by the total response area of the Reference Tuberculin times 100.)

(7) If the total tuberculin response area of the serial being tested does not fall between 75 percent and 125 percent of the total tuberculin response area of the Reference Tuberculin, the serial is unsatisfactory.

(8) Two unsensitized guinea pigs are given 0.05 ml intradermal injections of 1:4 and 1:10 dilutions of both the serial being tested and the Reference Tuberculin as a control for nonspecific positive reactions. If positive reactions are observed with the Reference Tuberculin, the test is considered a ''No Test'' and repeated. If positive reactions are observed with the serial being tested only, the serial is unsatisfactory.

(d) *Special chemical tests and requirements.* Final container samples of completed product from each serial shall be tested as follows:

(1) *Hydrogen ion concentration.* The hydrogen ion concentration shall be determined with a pH meter which has been standardized with a pH 7.0 buffer just prior to use. The pH of the product shall be 7.0 ±0.3.

(2) *Total nitrogen determination.* The nitrogen content shall be determined by the Kjeldahl method on duplicate 15 ml samples consisting of 5 ml from each of three vials. The total nitrogen content of the product shall be 0.18 percent ±0.06 percent.

(3) *Trichloroacetic acid precipitable nitrogen.* The determination of precipitable nitrogen by a final concentration of 4 percent trichloroacetic acid shall be made by the Kjeldahl method on duplicate 15 ml samples, consisting of 5 ml from each of three vials. The trichloroacetic acid precipitable nitrogen content shall be 0.047 percent ±0.01 percent.

(4) *Phenol determination.* The phenol content shall be determined by direct titration with a standardized bromide-bromate solution. (A correction factor of 0.04 should be subtracted from the final value in the determination of phenol in tuberculin.) The phenol content shall be 0.54 percent ±0.04 percent.

(5) *Clarity.* The product shall be optically clear and free from any extraneous particles.

[39 FR 16857, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991

§ 113.407 Pullorum antigen.

Pullorum Antigen shall be produced from a culture of representative strains of *Salmonella pullorum* which are of known antigenic composition, high agglutinability, but are not sensitive to negative and nonspecific serum. Each serial shall be tested for purity, density, preservative content, sensitivity, homogeneity, and hydrogen ion concentration. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as prescribed in § 113.26. In addition, each serial shall be free from extraneous organisms as determined by Gram staining and microscopic examination.

(b) *Nephelometric determination of bacterial density.* The bacterial density shall be 80 ±15 times McFarland No. 1 standard for stained antigen K's and 50 ±10 times McFarland No. 1 standard for tube antigen.

(c) *Preservative requirements.* (1) The formalin content of Pullorum Stained Antigen K shall be 1.0 ±0.2 percent as determined by a colorimetric method.

(2) The phenol content for Pullorum Tube Antigen shall be 0.55 ±0.05 percent as determined by direct titration with a standardized bromide-bromate solution.

(d) *Sensitivity requirements.* (1) Each serial of antigen shall be compared with a reference antigen of known sensitivity using positive and negative chicken serum. The manufacturers' recommendations for use on the accompanying label or package insert shall be followed. The recommended time limit specified for each antigen shall be carefully observed in the test.

(2) A total of at least 12 serums shall be used. This shall include at least three definitely positive, at least three weakly positive, and at least six negative serums. At least three positive chicken serums diluted with negative chicken serum shall be used to further assay comparative sensitivity between test and reference plate antigens. All test antigens shall agree closely with the reference antigen. Tests in which variation of readings between the reference and test antigen would result in a different National Poultry Improvement Plan classification shall be regarded as unsatisfactory. No unsatisfactory tests among the six or more negative serums and not more than one unsatisfactory test among the six or more positive serums shall be permitted. All tests performed shall be included for evaluation of the sensitivity assay. In the event of an unsatisfactory test using positive serums, at least three additional definitely positive and three additional weakly positive serums shall be tested. If not more than one unsatisfactory test is obtained with the additional serums, the antigen shall be acceptable.

(e) *Homogeneity requirement.* Antigens shall show no evidence of autoagglutination or unusual appearance such as the presence of flakes, specks, or a preponderance of filament forms. Microscopic examination shall be made in this determination.

(f) *Hydrogen ion concentration.* The hydrogen ion concentration shall be determined with a pH meter which has been standardized with a pH 4.0 buffer just prior to use. The pH of Pullorum Stained Antigen K shall be 4.6 ±0.4. No pH level is specified for Pullorum Tube Antigen but after dilution as recommended for use, it shall have a pH of 8.2 to 8.5.

[39 FR 16857, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, and amended at 40 FR 760, Jan. 3, 1975. Redesignated at 55 FR 35561, Aug. 31, 1990]

§ 113.408 Avian mycoplasma antigen.

Mycoplasma antigens shall be prepared from organisms, grown in broth cultures, that are inactivated and standardized. Plate antigens shall be stained with a dye acceptable to Animal and Plant Health Inspection Service (APHIS). Final container samples of completed product from each serial shall be tested for density, preservative content, homogeneity, hydrogen ion concentration, purity, sensitivity, and specificity in accordance with the conditions prescribed for each test. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Density requirements.* A 2.5 ml sample of completed antigen shall be diluted with 2.5 ml of buffer solution formulated in the same manner as the vehicle of the antigen being tested in a modified Hopkins tube and then sedimented at 1,000×g in a refrigerated centrifuge at 20 °C for 90 minutes. If the packed cell volume of the completed antigen is not 1.2 percent (±0.4 percent), the serial is unsatisfactory.

(b) *Preservative requirements.* Preservatives shall be as specified in the Outline of Production filed with APHIS in accordance with 9 CFR 114.8. If phenol is used, a direct titration with a standardized bromide-bromate solution shall be made. If the final concentration of phenol is not 0.25 percent (±0.05 percent), the serial is unsatisfactory.

(c) *Homogeneity requirements.* (1) Plate antigen shall be checked on a plate for homogeneity and autoagglutination. If plate antigen is not homogeneous and free of large visible particles (strands or clumps) or if it autoagglutinates, the serial is unsatisfactory.

(2) Stereo-microscopic examination shall be used when necessary to evaluate a granular appearing antigen.

(d) *Hydrogen ion concentration.* The hydrogen ion concentration shall be determined with a pH meter which has been standardized with a pH buffer just prior to use. The pH of Mycoplasma

Gallisepticum Antigen shall be 6.0±0.2. The pH of Mycoplasma Synoviae Antigen and Mycoplasma Meleagridis Antigen shall be 7.0±0.2.

(e) *Purity requirements.* The antigen shall be tested for viable bacteria and fungi as prescribed in § 113.26.

(f) *Sensitivity requirements.* The reactivity of each antigen shall be tested by comparing the agglutination reactions of each serial of antigen with the agglutination reactions of a standard reference antigen which is supplied by or acceptable to APHIS. A set consisting of five known positive and five known negative serums shall be used. The negative serums shall be tested against the antigens undiluted and the positive serums shall be tested against the antigens diluted 1:4 in buffer solution formulated in the same manner as the vehicle of the antigen being tested. If negative serums do not have negative reactions in this test, the serial is unsatisfactory. If the test antigen and the reference antigen do not have the same agglutination reactions with at least four of the five positive serums used, the serial is unsatisfactory.

(1) The sensitivity of Mycoplasma Gallisepticum Antigen shall be tested using a set of chicken and a set of turkey serums (the positive serums shall have varying degrees of reactivity from weakly positive to strongly positive).

(2) The sensitivity of Mycoplasma Synoviae Antigen shall be tested using chicken serums.

(3) The sensitivity of Mycoplasma Meleagridis Antigen shall be tested using turkey serums.

(g) *Specificity requirements.* Mycoplasma Synoviae Antigen shall be examined for cross-agglutination with five *Mycoplasma gallisepticum* antiserums (chicken origin); Mycoplasma Meleagridis Antigen shall be examined for cross-agglutination with five *Mycoplasma gallisepticum* antiserums (turkey origin) and five *Mycoplasma synoviae* antiserums (turkey origin). Tests shall be conducted with undiluted antigen. If cross-agglutination occurs, the serial is unsatisfactory.

[48 FR 33474, July 22, 1983. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.409 Tuberculin—PPD Bovis, Intradermic.

Tuberculin—PPD Bovis, Intradermic is a purified protein derivative produced from cultures of *Mycobacterium bovis* Strain AN–5 (supplied by Animal and Plant Health Inspection Service), which has been inactivated and is nontoxic. Each serial shall be tested for purity, safety, potency, and special chemical characteristics in accordance with the conditions prescribed for each test. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Each serial shall be tested for viable bacteria and fungi as prescribed in § 113.26.

(b) *Safety test.* Final container samples of completed product from each serial shall be tested for safety as prescribed in § 113.38.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be subjected to a comparison specificity test using a Reference PPD Tuberculin supplied by Animal and Plant Health Inspection Service.

(1) *Test animals.* White female guinea pigs from one source, which weigh 500 to 700 grams at the beginning of the test, and which have not been used in a previous test, shall be used in the specificity test. Twenty-three guinea pigs (10 sensitized with *M. bovis,* 10 sensitized with *M. avium* and three unsensitized) shall be required for each serial being tested, and 20 guinea pigs (10 sensitized with *M. bovis* and 10 sensitized with *M. avium*) shall be required for the Reference PPD Tuberculin. Allowance should be made for deaths during the sensitization period.

(2) *Sensitization of guinea pigs.*

(i) Sensitize one group of guinea pigs to *M. bovis.* Inject each animal intramuscularly with 0.5 ml of a sterile heat-killed suspension of *M. bovis* Strain AN–5 supplied by Animal and Plant Health Inspection Service.

(ii) Sensitize one group of guinea pigs to *M. avium.* Inject each animal intramuscularly with 0.5 ml of a sterile heat-killed suspension of *M. avium* Strain D–4 supplied by Animal and Plant Health Inspection Service.

(iii) Maintain an unsensitized group as control animals.

(3) Thirty-five days post-injection, the guinea pigs shall be used for tuberculin testing.

(4) The sensitized animals and controls shall be prepared at least 4 hours prior to injection of PPD tuberculin by clipping the hair from the entire abdominal and flank areas, applying a depilatory agent for 5 to 10 minutes, then rinsing with warm water and drying.

(i) Select four sites on each guinea pig for injection of PPD tuberculin. Two sites shall be on each side of the midline and spaced a sufficient distance from each other to avoid overlapping of skin reactions.

(ii) Prepare four dilutions of the Reference PPD Tuberculin and each serial of PPD tuberculin being tested so as to contain 0.6, 1.2, 2.4, and 4.8 micrograms of protein per 0.1 ml dose. Each of the four dilutions of the same tuberculin shall be randomly assigned a site on a guinea pig.

(iii) Inject one dose of each dilution at the assigned site using a tuberculin syringe.

(5) *Measurement of skin reactions.* Measure the area of erythema produced at each site on each guinea pig 24 hours following injection of PPD tuberculin. Measurements in millimeters shall be made anterior to posterior across the greatest diameter and perpendicular to the first measurement. Calculate the area of erythema in square millimeters at each site by multiplying the two measurements.

(6) *Calculation of average response per guinea pig.* Obtain the total area of erythema for each guinea pig by adding the areas of the four test sites. Add these composite areas of erythema from all guinea pigs with the same sensitization and the same PPD tuberculin injection, then divide by the number of animals in the group. The number obtained is the average response per guinea pig to the PPD tuberculin for the given type of sensitization.

(7) *Determination of specificity index.* The specificity index of a PPD tuberculin is determined by subtracting the average response obtained on *M. avium* sensitized guinea pigs from the average response obtained on *M. bovis* sensitized guinea pigs.

(8) *Validity of bioassay.* The bioassay test results obtained on serials tested concurrently in a single test series are valid if the specificity index of the reference PPD tuberculin is at least 400 square millimeters. If the results are not valid, the bioassay test series must be repeated with a different set of sensitized guinea pigs.

(9) *Reactions in unsensitized guinea pigs.* If a positive reaction (erythema) is observed in one or more of the 3 unsensitized guinea pigs, the serial is unsatisfactory.

(10) *Interpretation of specificity index.* When a bioassay is valid and reactions are not observed in unsensitized guinea pigs, the following interpretation of the specificity index will be used for classifying each serial of PPD tuberculin:

Specificity index	Classification
440 mm^2 or greater	Satisfactory.
Between 360 mm^2 and 440 mm^2	Inconclusive.
Less than 360 mm^2	Unsatisfactory.

(11) *Second stage test.* If a serial is classified as inconclusive, it can be declared unsatisfactory or undergo a second stage test. The second stage shall be conducted in a manner identical to the first stage, except that unsensitized guinea pig controls are not necessary. The results are evaluated by combining the results obtained on all guinea pigs tested in stages one and two. Calculate the average response on the 20 *M. bovis* sensitized animals and on the 20 *M. avium* sensitized animals and determine the specificity index. An inconclusive serial is satisfactory after the second stage test, if its specificity index is 400 square millimeters or more, and unsatisfactory if its specificity index is less than 400 square millimeters.

(d) *Special chemical tests and requirements.* Final container samples of completed product from each serial shall be tested as follows:

(1) *Protein concentration.* The final product shall contain a protein concentration of 1.0 ±0.1 mg/ml. The Microkjeldahl Test for Nitrogen shall be used.

(2) *Phenol content.* Phenol content of the final product shall be 0.50 percent

plus or minus 0.04 percent. A direct titration with a standardized bromide-bromate solution shall be conducted.

[41 FR 8471, Feb. 27, 1976, as amended at 41 FR 21760, May 28, 1976; 41 FR 32883, Aug. 6, 1976. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991]

ANTIBODY PRODUCTS

§ 113.450 General requirements for antibody products.

Unless otherwise prescribed in a Standard Requirement or in a filed Outline of Production, all antibody products shall meet the applicable requirements of this section.

(a) *Terminology.* The following terms in the regulations and standards concerning antibody products shall mean:

Antibody. An immunoglobulin molecule, having a precise glycoprotein structure, produced by certain cells of the B lymphocyte lineage in response to antigenic stimulation, and functioning to specifically bind and influence the antigens that induced its synthesis.

IgG (Immunoglobulin G). One of the several recognized classes of structurally related glycoproteins whose representatives include all known antibodies.

Monoclonal. Produced by, or derived from, the offspring of a single common progenitor cell.

Failure of passive transfer. A condition of neonates characterized by an abnormally low concentration of circulating maternal IgG.

(b) *Nomenclature.* Antibody products shall be named as follows:

(1) *Virus-specific products.* The true name of a virus-specific product shall: include the term ''antibody,'' specify the disease for which the product is intended, and indicate the type of animal that supplied the component antibodies. If the antibodies are monoclonal, the term ''monoclonal'' shall be used. Example: ''Duck Virus Hepatitis Antibody, Duck Origin.''

(2) *Bacterium-specific products.* The true name of a bacterium-specific product shall: include the term ''antibody'' if the component antibodies are directed against a nontoxin antigen or the term ''antitoxin'' if the component antibodies are directed against toxin, specify the organism against which the product is intended, and indicate the type of animal that supplied the component antibodies. If the antibodies are monoclonal, the term ''monoclonal'' shall be used. Example: ''Escherichia Coli Monoclonal Antibody, Murine Origin.''

(3) *Failure of passive transfer products.* The true name of a product for treatment of failure of passive transfer shall include the term ''IgG'' and indicate the type of animal that supplied the component IgG. Example: ''Bovine IgG.''

(4) *Combination products.* The true name of a product for treatment of failure of passive transfer as well as for the prevention and/or alleviation of a specific viral or bacterial disease shall be named according to the nomenclature prescribed above for virus-specific or bacterium-specific products.

(c) *Animals.* All animals used in the production of antibody products shall be healthy. Their health status shall be determined by physical examination by, or under the direct supervision of, a licensed veterinarian and by tests for infectious diseases. Such animals shall be maintained at licensed establishments: *Provided,* That cows maintained at Grade A dairies (or the equivalent) that are not injected with antigens for the purpose of stimulating the production of specific antibodies and that are used only for the purpose of supplying lacteal secretions are exempt from being maintained at a licensed establishment.

(1) No animal shall be used while showing clinical signs of disease. The presence of minor localized injuries or lesions (contusions, lacerations, burns, etc.) without body temperature elevation and without significant pain and distress shall not be construed as clinical evidence of disease.

(2) Before first use and on a regular basis, all animals used in the manufacture of antibody products shall be individually subjected to applicable tests for infectious diseases. Records of all test results shall be maintained. An animal which tests positive for an infectious disease shall not be used in the manufacture of antibody products. Retests shall be conducted as deemed necessary by the Administrator.

(i) Before first use, horses shall be tested as follows for:

(A) Equine infectious anemia (EIA) at a laboratory approved by APHIS.

(B) Piroplasmosis, dourine, and glanders at the National Veterinary Services Laboratories.

(C) Brucellosis at a laboratory approved by APHIS. Horses with standard agglutination titers of 1:50 or less can be used for production. Horses with standard agglutination titers equal to or greater than 1:100 may be tested by the Rivanol or card tests. Reactors to these supplemental tests shall not be used for production. Nonreactors to the supplemental tests shall be retested after 30 days. If the supplemental tests are negative and the agglutination titer has not increased, the animal may be used for production. Otherwise, the animal is unsatisfactory for this purpose.

(ii) Horses shall be retested annually for EIA and, if housed or pastured with any other species, shall be retested annually for brucellosis.

(iii) Before first use, cattle shall be tested as follows for:

(A) Tuberculosis by an accredited veterinarian: *Provided,* That cattle at Grade A dairies supplying only lacteal secretions need only be tested for tuberculosis in accordance with applicable Milk Ordinances or similar laws or regulations.

(B) Brucellosis at a laboratory approved by APHIS. Cattle with standard agglutination titers of 1:50 or less can be used for production. Cattle with standard agglutination titers equal to or greater than 1:100 may be tested by the Rivanol or card tests. Reactors to these supplemental tests shall not be used for production. Nonreactors to the supplemental tests shall be retested after 30 days. If the supplemental tests are negative and the agglutination titer has not increased, the animal may be used for production; otherwise, the animal is unsatisfactory for this purpose. Cattle at Grade A dairies supplying only lacteal secretions need not be tested individually for brucellosis if a portion of their secretions contribute to the herd milk pool tested as required by the brucellosis ring test. An animal of a herd testing positive by this test shall not be used in production.

(iv) Cattle shall be retested annually for both tuberculosis and brucellosis. Cattle at Grade A dairies supplying only lacteal secretions need only be tested for tuberculosis in accordance with applicable Milk Ordinances or similar laws or regulations. Cattle at Grade A dairies supplying only lacteal secretions need not be tested individually for brucellosis if a portion of their secretions contribute to the herd milk pool tested as required by the brucellosis ring test. An animal of a herd testing positive by this test shall not be used in production.

(v) For other species, appropriate tests and the frequency with which they are applied shall be specified in the filed Outline of Production for the product.

(vi) If a positive result is obtained on any prescribed test, the positive animal(s) shall be removed from the herd and the remaining animals retested. Production shall not be renewed until a negative herd test is obtained not less than 28 days following removal of the positive animal(s).

(vii) Negative animals shall be maintained separate and apart from untested or positive animals of any species. Production animals shall not be used for any other purpose, such as testing, work, or recreation.

(d) *Collection procedures.* Blood, lacteal secretions, and egg material shall be collected as described in the filed Outline of Production for the product.

(e) *Ingredient handling and processing.* Blood derivatives (serum, plasma, etc.), lacteal secretions, and egg material used in the production of antibody products shall be subjected to an appropriate procedure for the inactivation of potential contaminating microorganisms. The procedure shall be one of those described below and specified in the filed Outline of Production for the product: *Provided,* That another procedure may be substituted if demonstrated to be at least as effective by data acceptable to APHIS and specified in the filed Outline of Production for the product. These data are expected to

come from a study comparing the effectiveness of the established and substitute procedures against a satisfactory battery of potential contaminating microorganisms.

(1) Blood derivatives of equine origin shall be heated at 58.0–59.0°C for 60 minutes, and blood derivatives of bovine, porcine, or other origin shall be heated at 58.0–59.0°C for 30 minutes. In lieu of heat treatment, blood derivatives of any origin may be treated with at least 2.5 megarads of ionizing radiation, with a maximum radiation dosage specified in the filed Outline of Production for the product.

(2) Lacteal secretions shall be heated as described in paragraph (e)(1) of this section, or shall be pasteurized at either 72°C for 15 seconds or 89°C for 1 second using appropriate equipment. In lieu of the heat treatment regimens prescribed, lacteal secretions may be treated with at least 2.5 megarads of ionizing radiation, with a maximum radiation dosage specified in the Outline of Production for the product.

(3) Egg material shall be heated at 58.0–59.0°C for 30 minutes, or treated with at least 2.5 megarads of ionizing radiation, with a maximum radiation dosage specified in the filed Outline of Production for the product.

(4) Blood derivatives, lacteal secretions, and egg material shall not contain preservatives at the time of heat treatment, and immediately after heat treatment shall be cooled to 7°C or lower.

(5) Licensees shall keep detailed records as to each batch treated and each serial of product prepared for marketing. Recording charts shall bear full information concerning the material treated and tests made of the equipment used for treatment.

(f) *Preservatives.* Liquid antibody products, except those immediately frozen following preparation and maintained in a frozen state until time of use, shall contain at least one preservative from the following list, within the range of concentration set forth:

(1) Phenol 0.25 to 0.55 percent, or

(2) Cresol 0.10 to 0.30 percent, and/or

(3) Thimerosal 0.01 to 0.03 percent, or

(4) Other preservative(s) specified in the filed Outline of Production for the product.

(g) *Antigens for hyperimmunization.* If animals are hyperimmunized to generate antibodies for a product for the prevention and/or alleviation of a specific infectious disease, and a USDA-licensed veterinary biological product is not employed for this purpose, the following shall apply:

(1) For each antigen, a Master Seed shall be established.

(i) Bacterial Master Seeds shall be tested for purity and identity as prescribed for live bacterial vaccines in §113.64.

(ii) Viral Master Seeds shall be tested for purity and identity as prescribed for live virus vaccines in §113.300.

(2) The maximum allowable passage level of the hyperimmunizing antigen shall be the passage level of the antigen used to generate product shown to be efficacious and shall not exceed 10 passages from the Master Seed.

(h) *Purity tests.* Final container samples of each serial and each subserial shall be tested for viable bacteria and fungi as follows:

(1) Dried products for parenteral administration and liquid products shall be tested as prescribed in §113.26.

(2) For dried products for oral administration, 10 final container samples shall be reconstituted with sterile water at the volume recommended on the label and tested for the following contaminants:

(i) *Coliforms.* One milliliter of each rehydrated sample shall be pipetted into a 100×15 mm petri dish and 10–15 ml of violet red bile agar at 45–50°C added. The plate shall be manipulated to coat its entirety with the agar-sample mixture and allowed to stand until the mixture solidifies. The plate shall then be incubated at 35°C for 24 hours. A positive control plate and a negative control plate shall be prepared at the same time and in the same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If characteristic growth is observed on the negative control plate, or no characteristic growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If characteristic growth is observed on any of the 10 plates containing samples of the serial, one

retest to rule out faulty technique may be conducted on samples from 20 final containers. If characteristic growth is observed on any of the retest plates, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(ii) *Salmonellae.* One milliliter of each rehydrated sample shall be pipetted into a 100×15 mm petri dish and 10–15 ml of brilliant green agar at 45–50°C added. The dish shall be manipulated to coat its entirety with the agar-sample mixture and allowed to stand until the mixture solidifies. The plate shall then be incubated at 35°C for 24 hours. A positive control plate and a negative control plate shall be prepared at the same time and in the same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If characteristic growth is observed on the negative control plate, or no characteristic growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If characteristic growth is observed on any of the 10 plates containing samples of the serial, one retest to rule out faulty technique may be conducted on samples from 20 final containers. If characteristic growth is observed on any of the retest plates, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(iii) *Fungi.* One milliliter of each rehydrated sample shall be pipetted into a 100×15 mm petri dish and 10–15 ml of appropriately acidified potato dextrose agar at 45–50°C added. The plate shall be manipulated to coat its entirety with the agar-sample mixture and allowed to stand until the mixture solidifies. The plate shall then be incubated at 20–25°C for 5 days. A positive control plate and a negative control plate shall be prepared at the same time and in the same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If growth is observed on the negative control plate, or no growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If growth is observed on any of the 10 plates containing samples of the serial, one retest to rule out faulty technique may be conducted on samples from 20 final containers. If growth is observed on any of the retest plates, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(iv) *Total bacterial count.* One milliliter of each rehydrated sample, undiluted or diluted as prescribed in the Outline of Production, shall be pipetted into a 100×15 mm petri dish and 10–15 ml of tryptone glucose extract agar at 45–50°C added. The plate shall be manipulated to coat its entirety with the agar-sample mixture and allowed to stand until the mixture solidifies. The plate shall then be incubated at 35°C for 48 hours. A positive control plate and a negative control plate shall be prepared at the same time and in the same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If growth is observed on the negative control plate, or no growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If the average number of bacterial colonies on the 10 plates containing samples of the serial exceeds that specified in the filed Outline of Production for the product, one retest to rule out faulty technique may be conducted on samples from 20 final containers. If the average number of bacterial colonies on the retest plates exceeds that specified in the filed Outline of Production for the product, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(i) *Safety tests.* Bulk or final container samples of each serial shall be tested as prescribed in § 113.33(b). Dried product shall be reconstituted as indicated on the label and 0.5 ml injected per mouse.

[61 FR 51774, Oct. 4, 1996]

§ 113.451 Tetanus Antitoxin.

Tetanus Antitoxin is a specific antibody product containing antibodies directed against the toxin of *Clostridium tetani.* Each serial shall meet the applicable general requirements provided in

§ 113.450 and paragraph (a) of this section, and be tested for potency as provided in paragraph (b) of this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *General requirements.* The amount of antitoxin in a final container shall be the amount which is delivered from such container when opened and inverted until the flow stops. A graduated volumetric cylinder which conforms to the National Institute of Standards and Technology requirements shall be used. The reading shall be made at the bottom of the meniscus. Volumes of 10 ml or less shall be recorded to the nearest 0.1 and volumes over 10 ml shall be recorded to the nearest ml.

(1) All final containers of Tetanus Antitoxin shall yield not less than the labeled unitage of antitoxin throughout the dating period. The minimum package size permitted for marketing in the United States shall be a 1,500 unit vial.

(2) The expiration date of Tetanus Antitoxin shall be not more than 3 years after the date of a potency test which demonstrates that the recoverable antitoxin from the final container provides at least 20 percent excess over the number of units claimed on the label or not more than 1 year after the date of a potency test which demonstrates that the recoverable antitoxin from the final container provides 10 to 19 percent excess over the number of units claimed on the label.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be assayed to calculate the units of Tetanus Antitoxin in each final container. A comparative toxin-antitoxin neutralization test shall be conducted using a standard antitoxin and a standard toxin. All dilutions shall be made in M/15 phosphate buffered (pH) 7.4 physiological saline with 0.2 percent gelatin.

(1) One ml of the Standard Antitoxin shall be diluted before use so the final volume contains 0.1 unit per ml. The dilution shall be held at 20° to 25 ° C for 30 minutes prior to combination with a test does of toxin.

(2) The Standard Toxin test dose is that amount which when mixed with 0.1 unit of Standard Antitoxin, incubated at 20° to 25 ° C for 1 hour, and injected subcutaneously into a 340 to 380 gram guinea pig, results in death of that guinea pig within 60 to 120 hours with clinical signs of tetanus. The toxin shall be diluted so the test dose shall be in 2.0 ml.

(3) A mixture of diluted Standard Toxin and diluted Standard Antitoxin shall be made so that 0.1 unit of antitoxin in 1 ml is combined with a test dose of toxin. This Standard Toxin-Antitoxin mixture shall be held at 20° to 25 ° C for 1 hour before injections of guinea pigs are made.

(4) A sample from each serial of antitoxin shall be prepared as was the Standard Toxin-Antitoxin mixture; except the amount of antitoxin shall be based on an estimation of the expected potency. When testing is done on bulk material, the final container fill shall reflect the endpoint value plus 10 percent overage for 1 year dating and 20 percent overage for 3 year dating.

(5) Normal guinea pigs weighing within a range of 340 to 380 grams shall be used. Pregnant guinea pigs must not be used.

(i) Each of two guinea pigs (controls) shall be injected subcutaneously with a 3 ml dose of the Standard Toxin-Antitoxin mixture. Injections shall be made in the same order that toxin is added to the dilutions of antitoxins. These shall be observed parallel with the titration of one or more unknown antitoxins.

(ii) Two guinea pigs shall be used as test animals for each dilution of the unknown antitoxin. A 3.0 ml dose shall be injected subcutaneously into each animal.

(6) Controls shall be observed until they are down and are unable to rise or stand under their own power. At this time they are euthanized and the time of death is recorded in hours. For a satisfactory test, the controls must reach this point with clinical signs of tetanus within 24 hours of each other and within an overall time of 60 to 120 hours. The clinical signs to be observed are increased muscle tonus, curvature of the spine, asymmetry of the body outline when the resting animal is viewed from above, generalized spastic paralysis, particularly of the extensor muscles, inability to rise from a smooth surface

when the animal is placed on its side, or any combination of these signs. If the control guinea pigs do not respond in this manner, the entire test shall be repeated.

(7) Potency of an unknown antitoxin is determined by finding the mixture which will protect the test animal the same as the Standard Toxin-Antitoxin mixture. Test animals dying sooner than the controls indicate the unit value selected in that dilution was not present, whereas those living longer indicate a greater unit value.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, and amended at 40 FR 760, Jan. 3, 1975; 40 FR 41996, Sept. 10, 1975; 43 FR 1479, Jan. 10, 1978; 50 FR 24905, June 14, 1985. Redesignated at 55 FR 35561, Aug. 31, 1990; 61 FR 51776, Oct. 4, 1996; 64 FR 43045, Aug. 9, 1999]

§ 113.452 Erysipelothrix Rhusiopathiae Antibody.

Erysipelothrix Rhusiopathiae Antibody is a specific antibody product containing antibodies directed against one or more somatic antigens of *Erysipelothrix rhusiopathiae.* Each serial shall be tested as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Each serial shall meet the applicable general requirements provided in § 113.450.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested using the two-stage test provided in this section.

(1) In the first stage, each of 40 Swiss mice, each weighing 16 to 20 grams, shall be injected subcutaneously with 0.1 ml of product (dried product shall be rehydrated according to label directions). Twenty-four hours postinjection, the injected mice and 10 additional mice designated controls shall be challenged subcutaneously with the same culture of *Erysipelothrix rhusiopathiae.*

(2) If less than eight of the 10 controls die from erysipelas within 7 days post-challenge, the test is invalid. All dead mice shall be examined to determine if the cause of death was *Erysipelothrix rhusiopathiae* infection.

(3) The mice injected with product shall be observed for 10 days postchallenge and all deaths recorded. The second stage shall be required when 7–10 of the mice injected with product die in the first stage. The second stage shall be conducted in a manner identical to the first stage.

(4) The results of the test shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of deaths for a satisfactory test	Cumulative total number of deaths for an unsatisfactory test
1	40	40	6 or less	11 or more.
2	40	80	12 or less	13 or more.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, as amended at 40 FR 20067, May 8, 1975; 40 FR 23989, June 4, 1975. Redesignated at 55 FR 35561, Aug. 31, 1990; 61 FR 51776, Oct. 4, 1996; 64 FR 43045, Aug. 9, 1999]

§ 113.453 [Reserved]

§ 113.454 Clostridium Perfringens Type C Antitoxin.

Clostridium Perfringens Type C Antitoxin is a specific antibody product containing antibodies directed against the toxin of *Clostridium perfringens* Type C. Each serial shall be tested as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Each serial shall meet the applicable general requirements provided in § 113.450.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested using the toxin-neutralization test for Beta Antitoxin provided in this section. Dried products shall be rehydrated according to label directions.

(1) When used in this test, the following words and terms shall mean:

(i) *International antitoxin unit.* (I.U.) That quantity of Beta Antitoxin which reacts with L_0 and L_+ doses of Standard Toxin according to their definitions.

(ii) *L_0dose.* The largest quantity of toxin which can be mixed with one unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) *L_+dose.* The smallest quantity of toxin which can be mixed with one unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.

(iv) *Standard antitoxin.* The Beta Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International *Clostridium perfringens* Beta Antitoxin Standard and which is either supplied by or acceptable to Animal and Plant Health Inspection Service. The antitoxin unit value shall be stated on the label.

(v) *Standard toxin.* The Beta toxin preparation which is supplied by or is acceptable to Animal and Plant Health Inspection Service.

(vi) *Diluent.* The solution used to make proper dilutions prescribed in this test. Such solution shall be made by dissolving 1 gram of peptone and 0.25 gram of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 250 °F. for 25 minutes; and storing at 4 °C. until used.

(2) The antitoxin content of the test sample shall be determined as follows:

(i) Make a dilution of Standard Antitoxin to contain 10 International Units of antitoxin per ml.

(ii) Make one dilution of Standard Toxin to contain 10 L_0 doses per ml and make a second dilution of Standard Toxin to contain 10 L_+ doses per ml.

(iii) Dilute 1 ml of the test sample with 49 ml of diluent and combine 1 ml of this dilution with 1 ml of the Standard Toxin diluted to contain 10 L_0 doses.

(iv) Combine 10 International Units of Standard Antitoxin with 10 L_0 doses of diluted Standard Toxin and combine 10 International Units of Standard Antitoxin with 10 L_+ doses of diluted Standard Toxin.

(v) Neutralize all toxin-antitoxin mixtures at room temperature for 1 hour and hold in ice water until injections of mice can be made.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 24 hours post-injection and record all deaths.

(3) *Test Interpretation.* (i) If any mice inoculated with the mixture of 10 International Units of Standard Antitoxin and 10 L_0 doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) If less than 80 percent of the mice inoculated with the mixture of 10 International Units of Standard Antitoxin and 10 L_+ doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(iii) If any mice inoculated with the mixture of Clostridium Perfringens Type C Antitoxin diluted 1:50 and 10 L_0 doses of Standard Toxin die, the antitoxin is considered to contain less than 500 International Unit per ml and the serial is unsatisfactory.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991; 61 FR 51777, Oct. 4, 1996]

§ 113.455 Clostridium Perfringens Type D Antitoxin.

Clostridium Perfringens Type D Antitoxin is a specific antibody product containing antibodies directed against the toxin of *Clostridium perfringens* Type D. Each serial shall be tested as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Each serial shall meet the applicable general requirements provided in § 113.450.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested using the toxin-neutralization test for Epsilon Antitoxin provided in this section. Dried products shall be rehydrated according to label directions.

(1) When used in this test, the following words and terms shall mean:

(i) *International antitoxin unit.* (I.U.) That quantity of Epsilon Antitoxin which reacts with L_0 and L_+ doses of Standard Toxin according to their definitions.

(ii) *L_0dose.* The largest quantity of toxin which can be mixed with one-tenth unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) *L_+dose.* The smallest quantity of toxin which can be mixed with one-tenth unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.

(iv) *Standard antitoxin.* The Epsilon Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International *Clostridium perfringens* Epsilon Antitoxin Standard and which is either supplied by or acceptable to Animal and Plant Health Inspection Service. The antitoxin unit value shall be stated on the label.

(v) *Standard toxin.* The Epsilon toxin preparation which is supplied by or is acceptable to Animal and Plant Health Inspection Service.

(vi) *Diluent.* The solution used to make proper dilutions prescribed in this test. Such solution shall be made by dissolving 1 gram of peptone and 0.25 gram of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 250 °F. for 25 minutes; and storing at 4 °C. until used.

(2) The antitoxin content of the test sample shall be determined as follows:

(i) Make a dilution of Standard Antitoxin to contain 1 International Unit of antitoxin per ml.

(ii) Make one dilution of Standard Toxin to contain 10 L_0 doses per ml and make a second dilution of Standard Toxin to contain 10 L_+ doses per ml.

(iii) Dilute 1 ml of the test sample with 33 ml of diluent and combine 1 ml of this dilution with 1 ml of the Standard Toxin diluted to contain 10 L_0 doses.

(iv) Combine 1 International Unit of Standard Antitoxin with 10 L_0 doses of Standard Toxin and combine 1 International Unit of Standard Antitoxin with 10 L_+ doses of Standard Toxin.

(v) Neutralize all toxin-antitoxin mixtures at room temperature for 1 hour, and hold in ice water until injections of mice can be made.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 24 hours post-injection and record all deaths.

(3) Test Interpretation. (i) If any mice inoculated with the mixture of 1 International Unit of Standard Antitoxin and 10 L_0 doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) If less than 80 percent of the mice inoculated with mixture of 1 International Unit of Standard Antitoxin and 10 L_+ doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided,* That, if the test is not repeated, the serial shall be declared unsatisfactory.

(iii) If any mice inoculated with the mixture of Clostridium Perfringens Type D Antitoxin diluted 1:34 and 10 L_0 doses of Standard Toxin die, the antitoxin is considered to contain less than 34 International Units per ml and the serial is unsatisfactory.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, as amended at 40 FR 760, Jan. 3, 1975. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991; 61 FR 51777, Oct. 4, 1996]

§§ 113.456–113.498 [Reserved]

§ 113.499 Products for treatment of failure of passive transfer.

A product for the treatment of failure of passive transfer (FPT) shall contain a specified minimum quantity of IgG per dose and shall be recommended for use only in neonates of the same species as that of antibody origin. A product for oral administration shall not be recommended for use in animals more than 24 hours of age, while one for parenteral administration shall only be recommended for use in neonatal animals. Each serial shall meet the applicable general requirements provided in § 113.450 and be tested for potency as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Qualification of an IgG Reference Product.* An IgG Reference Product (reference) shall be a serial of product that is manufactured according to the filed Outline of Production, properly qualified, and used to assess the potency of

subsequent product serials, as described in paragraph (c) below. The reference shall be qualified as follows:

(1) At least 20 newborn, colostrum-deprived animals of the species for which the product is recommended shall be randomly selected.

(2) Blood samples shall be taken from each animal.

(3) Each animal shall be administered one dose of reference by the recommended route and shall be observed for 24 hours.

(i) Any adverse reactions shall be recorded.

(ii) The dosage of reference administered to each animal shall be in accordance with label directions. Label directions may indicate a single dosage regardless of weight, in which case the animals in the study shall be at or near the maximum weight for neonates of the species.

(4) After 24 hours, blood samples shall be taken from each animal.

(5) Pretreatment and post treatment serum IgG concentrations shall be concurrently determined for each animal using a radial immunodiffusion (RID) method acceptable to APHIS and described in the filed Outline of Production for the product.

(6) Concurrently, using the same method, five IgG measurements shall be made on an IgG Species Standard supplied or approved by APHIS. The IgG Species Standard shall be a preparation that contains IgG specific for the species in question at a concentration acceptable to APHIS.

(7) For an IgG Reference Product to be satisfactory, all animals used to qualify the reference must remain free of unfavorable product-related reactions and at least 90 percent of the paired serum samples must reflect an increase in IgG concentration (posttreatment minus pretreatment concentration) equal to or greater than the IgG concentration of the IgG Species Standard.

(b) *Antibody functionality.* Prior to licensure, the prospective licensee shall perform a neutralization study, or another type of study acceptable to APHIS, to demonstrate functionality of product antibody.

(c) *Potency.* Bulk or final container samples of completed product from each serial shall be tested for IgG content as provided in this paragraph. Samples of the test serial and of an IgG Reference Product established in accordance with paragraph (a) of this section shall be concurrently tested for IgG content by the RID method referred to in paragraph (a)(5) of this section. Five IgG measurements shall be made on each. If the IgG level per dose of the test serial does not meet or exceed that of the reference, one complete retest, involving five IgG measurements on both the reference and two samples of the test serial, may be conducted. If, upon retest, the average IgG level per dose of the two samples of the test serial does not meet or exceed that of the reference, or if a retest is not conducted, the serial is unsatisfactory.

[61 FR 51777, Oct. 4, 1996]

PART 114—PRODUCTION REQUIREMENTS FOR BIOLOGICAL PRODUCTS

Sec.

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AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 39 FR 16869, May 10, 1974, unless otherwise noted.

§ 114.1 Applicability.

Unless exempted by regulation or otherwise authorized by the Administrator, all biological products prepared, sold, bartered or exchanged, shipped or delivered for shipment in or from the

United States, the District of Columbia, any Territory of the United States, or any place under the jurisdiction of the United States shall be prepared in accordance with the regulations in this part. The licensee or permittee shall adopt and enforce all necessary measures and shall comply with all directions the Administrator prescribes for carrying out such regulations.

[52 FR 11026, Apr. 7, 1987, as amended at 56 FR 66784, Dec. 26, 1991]

§ 114.2 Products not prepared under license.

(a) When an establishment license is issued, if biological products which were not prepared in compliance with the regulations are in the establishment, such products shall not be shipped or delivered for shipment or otherwise dealt with as having been prepared under such regulations.

(b) Except as provided in 9 CFR part 103, a biological product shall not be prepared in a licensed establishment unless the person to whom the establishment license is issued holds an unexpired, unsuspended, and unrevoked product license issued by the Administrator to prepare such biological product, or unless the products prepared are subject to the provisions of § 107.2 of this subchapter.

(c) A biological product produced in a USDA-licensed establishment shall be produced under a U.S. Veterinary Biological Product License or a license granted by a State under § 107.2 (referred to as a State biological product license and the products prepared pursuant thereto as State-licensed biological products, including autogenous biologics), but not under both a U.S. Veterinary Biological Product License and a State biological product license. Before a U.S. Veterinary Biological Product License (including a conditional license) is issued, the licensee shall relinquish its State license for that product: *Provided,* That autogenous biologics shall not be subject to this provision when they are prepared in accordance with the provisions of paragraph (c)(5) of this section.

(1) State-licensed biological products (including autogenous biologics) shall only be distributed or shipped intrastate, must not bear a U.S. Veterinary Biologics Establishment License Number, and must not otherwise be represented in any manner as having met the requirements for a U.S. Veterinary Biological Product license. Labeling of State- and USDA-licensed biological products produced in the same establishment must be distinctly different in color and design.

(2) All biological products in USDA-licensed establishments, whether licensed by USDA or by the State, shall be prepared only in locations indicated in legends filed in accordance with 9 CFR part 108. A description of each State-licensed product must be filed with the Animal and Plant Health Inspection Service as part of the blueprint legends and must be sufficient for Animal and Plant Health Inspection Service to determine any risk to the production of other products in the licensed establishment and to determine that adequate procedures are followed to prevent contamination during production.

(3) Records in such establishments must be maintained in accordance with §§ 116.1 and 116.2 of this subchapter and shall include all products licensed by the State or USDA.

(4) Reports prescribed in § 116.5 of this subchapter for USDA-licensed establishments shall be submitted for all veterinary biological products in the establishment.

(5) Under the following conditions, an autogenous biologic may be produced in a USDA-licensed establishment under either a State or U.S. Veterinary Biological Product License:

(i) When a culture of microorganisms, isolated from a herd in a State, is received at a USDA-licensed establishment that is in the same State but that holds both a State and a U.S. Veterinary Biological Products License for autogenous biologics, the isolate shall be designated by the licensee for use in the production of an autogenous biological product under either the State product license, or the U.S. Veterinary Biological Product License: *Provided,* That the isolate meets the requirements of the respective regulatory authority for an autogenous biologic. If, after producing the product pursuant to one license, the licensee elects to produce an autogenous biologic from

the same isolate under provisions of the other license, the licensee may do so only with the approval of the other licensing authority.

(ii) The true name of a State-licensed autogenous biologic shall specify the State of licensure: e.g.

''_____ Autogenous Bacterin''
(State)

or _____ Autogenous Vaccine''.
(State)

[39 FR 16869, May 10, 1974, as amended at 60 FR 48021, Sept. 21, 1995]

§ 114.3 Separation of establishments.

(a) Each licensed establishment shall be separate and distinct from any other establishment in which a biological product is prepared.

(b) No biological products authorized to be prepared in a licensed establishment shall be prepared in whole or in part by another licensed establishment except as provided in paragraphs (c) and (d) of this section.

(c) When a partially prepared biological product cannot be completed at a licensed establishment due to failure of essential equipment, the Administrator may authorize the use of similar equipment at another licensed establishment: *Provided,* That, such authorization shall be limited to the duration of the emergency and to the phase of production affected by the equipment failure.

(d) Partially prepared products or serials of completed products for further manufacture may be moved from one licensed establishment to another licensed establishment, imported under the provisions of § 104.5, or moved from a licensed establishment for purpose of being exported under conditions prescribed in an Outline of Production filed with Animal and Plant Health Inspection Service. Licensed products or products imported for distribution and sale may be prepared and recommended for final use, for further manufacturing purposes, or both. All serials shall be subject to the requirements for testing and release specified in § 113.5 or § 113.10 and to the requirements for identification specified in § 114.4.

[39 FR 16869, May 10, 1974, as amended at 40 FR 46093, Oct. 6, 1975; 49 FR 45846, Nov. 21, 1984; 56 FR 66784, Dec. 26, 1991]

§ 114.4 Identification of biological products.

Suitable tags or labels of a distinct design shall be used for identifying all ingredients used in the preparation of biological products, all component parts to be combined to form a biological product, all biological products while in the course of preparation and all completed biological products held in storage at licensed establishments: *Provided,* That, if such ingredients, components, or biological products are not so identified, they shall be disposed of as provided in § 114.15.

§ 114.5 Micro-organisms used as seed.

Micro-organisms used in the preparation of biological products at licensed establishments shall be free from the causative agents of other diseases or conditions. A complete record of such micro-organisms shall be kept currently correct and a list submitted to Animal and Plant Health Inspection Service upon request of the Administrator.

(Approved by the Office of Management and Budget under control number 0579–0059)

[39 FR 16869, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991]

§ 114.6 Mixing biological products.

Each biological product, when in liquid form, shall be mixed thoroughly in a single container. During bottling operations, the product shall be constantly mixed sufficient to maintain physical uniformity of the entire fill. A serial number, with any other markings that may be necessary for ready identification of the serial, shall be applied to identify it with the records of preparation and labeling.

§ 114.7 Personnel at licensed establishments.

(a) Each licensee shall designate a person(s) to make all official contacts with Animal and Plant Health Inspection Service on matters pertaining to the preparation of biological products under the Virus-Serum-Toxin Act. The licensee shall file three copies of biographical summary with Animal and Plant Health Inspection Service for such designated person and for each

person responsible for any phase of preparation of a biological product.

(b) All personnel employed in the preparation of biological products at a licensed establishment shall be competent in good laboratory techniques through education or training, or both, so as to consistently prepare high quality products.

(c) All biological products prepared at licensed establishments shall be prepared and handled with due sanitary precautions. Good sanitary measures shall be practiced at all times by all personnel involved in such preparation and handling of biological products.

(1) The clothing worn by persons while preparing biological products shall be clean. All persons, immediately before entering laboratory rooms of a licensed establishment, shall change their outer clothing or effectively cover the same with gowns or other satisfactory clean garments.

(2) Unsanitary practices such as, but not limited to, eating, smoking, or expectorating on the floors or otherwise creating a nuisance in any room, compartment, or place in which biological products are prepared, handled, or stored at licensed establishments are prohibited.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16869, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991]

§ 114.8 Outline of Production required.

An Outline of Production shall be on file with Animal and Plant Health Inspection Service for each licensed biological product or for each biological product authorized to be imported into the United States for Distribution and Sale. Preparation of a biological product in a licensed establishment shall be in accordance with the Outline of Production for such product filed with Animal and Plant Health Inspection Service as provided in this section, but subject to changes as may be required under § 114.8(f).

(a) The Outline of Production shall be prepared as prescribed in § 114.9 and submitted to Animal and Plant Health Inspection Service for filing. When objectionable features, if any, are corrected and no further exceptions are taken by Animal and Plant Health Inspection Service to an Outline of Production for a biological product, such Outline of Production shall be approved for filing.

(b) Each page shall be stamped as filed on the date such action was taken in the bottom right hand corner. Although the filed outline may be referred to as an approved outline, approval for filing constitutes no endorsement by Animal and Plant Health Inspection Service of such biological product or the methods and procedures used to prepare such biological product.

(c) The original and two copies shall be retained by Animal and Plant Health Inspection Service and the remaining copies returned.

(d) Each licensee shall review each Outline of Production for accuracy and sufficiency not less frequently than once a year. Revisions necessary to bring an Outline of Production into compliance with the regulations shall be submitted to Animal and Plant Health Inspection Service.

(e) When a list of licensed products to be continued in production at a licensed establishment is requested by the Administrator in accordance with § 102.5(d) of this subchapter, the licensee shall supplement the list with information for each product as follows:

(1) The Outline of Production currently being used shall be identified as to the date when last revised and filed with Animal and Plant Health Inspection Service and the date of the last review made by the licensee.

(2) The Outline of Production to be kept in the active file shall be designated. If more than one has been filed for a product, only the Outline of Production currently being used shall be included.

(f) The Administrator may, upon the basis of information not available to him at the time the current Outline of Production for a biological product was filed, object to the methods or procedures being used in the preparation of such biological product and notify the licensee to modify the filed Outline of Production to eliminate such objections. If the licensee does not comply with the notice, the Administrator

may, after affording opportunity for a hearing to the licensee, suspend the product license for the biological product involved; in which case, the licensee shall not prepare such product until subsequent notice of withdrawal of the suspension is given to the licensee.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16869, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991]

§ 114.9 Outline of Production guidelines.

Each Outline of Production shall be prepared in accordance with the applicable directions provided in this section.

(a) *General requirements.* (1) The original and not more than four copies of each Outline of Production or special outline or revised pages of either shall be prepared on heavy paper (8.5″ x 11″) of a type receptive to permanent stamp ink.

(2) The name of the biological product (or component), the establishment license number, and the date prepared shall appear on a front cover page and each page of the Outline of Production or special outline. The name of the licensee (or foreign manufacturer) shall appear on the front cover page.

(3) The pages shall be numbered in the upper center. At least 1½ inch margin shall be left at the top of the first page and a 2 inch margin at the bottom of each page for the Animal and Plant Health Inspection Service stamp.

(4) Amended pages shall be numbered the same as those being superseded. They shall bear the date prepared and refer to the date on the pages being superseded. If one replacement page supersedes more than one page, the new page shall indicate same, but if several replacement pages are added to supersede one page, the page number followed by letters shall be used.

(5) The last page of the original and one copy of either a new or a completely rewritten Outline of Production and each page revised separately shall be signed in the lower left corner by the authorized representative of the licensee (or foreign producer). Stamped or facsimile signatures are not acceptable.

(6) A summary of changes shall appear on an attached page and refer to each page, paragraph, or subparagraph being changed.

(7) Transmittal forms shall be used for the original and subsequent revisions. Blank forms shall be available upon request to Animal and Plant Health Inspection Service.

(b) *Special outline.* An outline describing the preparation of a component of a biological product or an operation performed in the preparation of a biological product may be required if such special outline could be referred to in Outlines of Production to eliminate repetition. Each special outline shall be identified by number and shall not be used until accepted and filed by Animal and Plant Health Inspection Service.

(c) Outline of Production for antiserum, antitoxin, and normal serum shall be written according to the following:

OUTLINE GUIDE FOR PRODUCTION OF ANTISERUM AND ANTITOXIN AND NORMAL SERUM

License No. Name of Product Date

I. *Serum animals.* A. Species, conditions, age, and general health.

B. Examination, preparation, care, quarantine, tests, and treatment of animals before injections are started.

C. Holding, handling, exercising, and monitoring the condition of animals after injections are started.

II. *Antigens.* A. Composition and character of the antigen.

1. Micro-organisms.

2. Source and date of accession of each micro-organism.

3. Strains.

4. Proportions of each micro-organism and strain.

B. Identification methods used for each micro-organism and frequency with which these methods are applied.

C. Virulence and purity of cultures or antigen and the determination and maintenance thereof. Range of subcultures or passages to be used in production.

D. Attenuation, if any, before use for production purposes.

E. Character, size, and shape of containers used for growing micro-organisms.

F. Media used for stock, seed, and antigen cultures (composition and reaction of). May refer to a special outline by number.

G. Preparation of the antigen or toxin and toxoid. Complete and full description of each

step and its manner of accomplishment and number these steps in sequence. Include all tests for each antigen, and the specifications for character, identity, virulence, concentration, and standardization.

III. *Immunization of animals.* A. Outline fully with special attention given to the following:

1. Character and dose of the antigen.
2. Method and frequency of injections.
3. Time required for immunization or hyperimmunization.
4. Preliminary bleedings and tests, if any, to ascertain quality of serum.
5. All other similar matters, including treatments between bleedings.

B. Period of time elapsing between last injection and first bleeding; and between bleedings.

C. Technique of bleeding operations; volume of blood collected at each bleeding; and period of rest.

IV. *Preparation of the biological product.* A. Describe fully and show each step of preparation from the first bleeding to the completion of the preserved product in bulk containers prior to filling of final containers.

B. Composition of the preservative and proportions used. Indicate at which step of production, and the method used in adding the preservative.

C. Agglutination and complement-fixation titers and the methods of their determinations.

D. Disposition of unsatisfactory biological products and infective materials not used in production.

E. Assembly of units to make a serial; volume of the average serial; and the volume of the maximum serial.

V. *Testing.* Indicate the stages in the preparation of the biological product at which samples are collected. Refer to all applicable Standard Requirements. Outline all additional tests in detail and state minimum requirements for each satisfactory test.

A. Purity.
B. Safety.
C. Potency.
D. Other tests.

VI. *Post preparatory steps.*

A. Form and size of final containers in which the product is to be distributed.

B. Methods and techniques of filling final containers. Volume of fill for each size final container.

C. Collection, storage, and submission of representative samples. Indicate at which steps in the production these samples are taken.

D. Expiration date based on the earliest date of harvest and the date of the last satisfactory potency test.

E. Use, dosage, and route of administration for each animal species for which it is recommended.

F. Include any additional pertinent information.

(d) Outline of Production for *vaccines, bacterins, antigens,* and *toxoids* shall be written according to the following:

OUTLINE GUIDE FOR VACCINES, BACTERINS, ANTIGENS, AND TOXOIDS

License No. Name of Product Date

I. *Composition, etc., of the product.* A. Micro-organisms used. Give the isolation and passage history.

B. Source and date of accession of each micro-organism.

C. Strains.

D. Proportions of each strain.

II. *Cultures.* A. Brief description of methods of identifying each micro-organism and the frequency with which these methods are applied.

B. Virulence and purity of cultures and the determination and maintenance thereof. Range of subcultures or passages to be used in production.

C. Composition and reaction of media used for seed and production cultures. Include the source of eggs, tissue, or cell cultures, and the tests to determine that eggs, tissues, and cells are free of contamination.

D. Character, size, and shape of containers used for growing cultures.

E. Storage conditions of seed cultures.

F. Methods of preparing suspensions for seeding or inoculation.

G. Technique of inoculating (1) seed media; (2) production media. Titer or concentration of inoculum, and the volume of medium for each size and type of culture container.

H. Period of time and conditions for incubation and degree of temperature used for each micro-organism or group of micro-organisms.

I. Character and amount of growth; observation as to contamination of growth.

J. Method of attenuation, if any, before used for production purposes.

III. *Harvest.* A. Handling and preparation of cultures and media (including eggs) before removal of micro-organisms or tissues for production purposes.

B. Minimum and maximum period of time elapsing from time of inoculation until harvest.

C. Technique of harvesting micro-organisms or tissues (specify) for production purposes.

D. Specifications for acceptable harvest material.

E. Handling of discarded material not used in production.

F. Include any additional pertinent information.

IV. *Preparation of the product.* Describe fully and show each step of preparation from harvest of antigen containing tissues or production cultures to the completion of the

finished product in final containers. In describing the preparation of the product, emphasize the following:

A. Method of inactivation, attenuation, or detoxification.

B. Composition of preservative, adjuvant or stabilizer, and proportions used stated in such a manner that the concentration can be calculated; stage and method of addition.

C. Method and degree of concentration.

D. If product is standardized to give concentration of antigen, show procedures and calculations.

E. 1. Assembly of units to make a serial (illustrate by example).

2. Volume of average serial.

3. Volume of maximum serial.

4. Any other pertinent information.

F. Volume of fill for each size vial. Type of vial if unusual.

G. Method and technique of filling and sealing of final containers.

H. Desiccation, including moisture control. Give maximum percent moisture.

I. Amount of antigenic material per dose or doses in final container.

V. *Testing.* Indicate the stages in the preparation of the biological product at which the samples are collected. Refer to all applicable Standard Requirements. Outline all additional tests in detail and state the minimum requirement for each satisfactory test.

A. Purity.

B. Safety.

C. Potency.

D. Moisture, if desiccated.

E. Any other tests.

VI. *Post preparatory steps.* A. Form and size of final containers in which the product is to be distributed.

B. Collection, storage, and submission of representative samples. Indicate at which steps in the production these samples are taken.

C. Expiration date based on the earliest date of harvest and the date of the last satisfactory potency test. If applicable, give the date of lyophilization.

D. Use, dosage, and route of administration for each animal species for which the biological product is recommended.

(e) Outlines of Production for allergenic extracts shall be written according to the following:

OUTLINE GUIDE FOR ALLERGENIC EXTRACTS

License No. Name of Product Date

I. *Composition of the product.* A. Source and type of raw material.

B. Weight/volume concentration.

II. *Preparation of the product.* A. Describe fully and show each step of preparation to the completion of the finished product in true containers. In describing the preparation of the product, emphasize the following:

1. Method of extraction.

2. Composition of preservative, adjuvant or stabilizer, and proportions used; stage and method of addition.

3. Method and degree of concentration.

4. Standardization of the product.

5. (a) Assembly of units to make a serial.

(b) Volume of average serial.

(c) Maximum serial.

6. Volume of fill for each size vial.

7. Method and technique of filling and sealing of final containers.

8. Amount material per dose or doses in final container.

III. *Testing.* Indicate the stages in the preparation of the biological product at which the samples are collected. Refer to all applicable Standard Requirements. Outline all additional tests in detail and state the minimum requirement for each satisfactory test.

A. Purity.

B. Safety.

C. Potency.

D. Any other tests.

E. Include any additional pertinent information.

IV. *Post preparatory steps.* A. Form and size of final containers in which the product is to be distributed.

B. Collection, storage, and submission of representative samples. Indicate at which steps in the production these samples are taken.

C. Expiration date based on the earliest date of harvest and the date of the last satisfactory potency test.

D. Use, dosage, and route of administration for each animal species for which the biological product is recommended.

(f) Outlines of Production for diagnostic test kits based on antigen-antibody reactions, and other diagnostics whose production methods are amenable to description as described herein shall be written according to the following requirements:

OUTLINE GUIDE FOR DIAGNOSTIC TEST KITS

License No. Name of product Date

Introduction

Provide a brief description of the kit as follows:

1. Principle of the test (ELISA, latex agglutination, etc.).

2. Antigen or antibody detection test.

3. Sample(s) used for testing (serum, whole blood, tears, etc.).

4. List reagents, references, and equipment included.

5. Identify materials obtained under split manufacturing agreements.

6. General description of test interpretations and their limitations, including followup tests.

I. *Antibody Components*

A. Production of polyclonal antibody components.

1. If purchased, list suppliers, criteria for acceptability, and describe all tests performed after receipt to determine that specifications have been met.

2. If produced in-house, describe the species, age, weight, conditions, and general health of all animals used in antiserum production.

a. Preinjection considerations:

Describe the examination, preparation, care, quarantine procedures, and treatments administered before immunization(s). Describe all tests used to determine suitability for use. Describe the preparation of any standard negative serum(s) collected prior to immunization.

b. Immunization of animals.

i. Describe the character and dose of the antigen; if adjuvant is used provide details on its preparation. If commercial product is used include its true name as shown on the label, the manufacturer, serial number, and expiration date.

ii. Describe the method and schedule for immunizations.

iii. Describe the method for harvesting and evaluating the immunization product, including tests for acceptability.

iv. Provide number and intervals between harvests, volume obtained, and any other pertinent information.

B. Production of Monoclonal Antibody Components.

1. Hybridoma components:

a. If hybridoma components are purchased, list suppliers and criteria for acceptability; if tests are performed after receipt, describe fully.

b. If hybridomas are prepared inhouse, identify the antigen(s) used, describe the immunization scheme, and the species of animal used.

c. Identify the tissue of origin, and the procedures for harvesting, isolating, and identifying the immune cells.

d. Describe the source, identity, and the product secreted (light or heavy chain) by the parent Myeloma Cell Line.

e. Summarize cloning and recloning procedures, including clone characterization and propagation, if appropriate.

f. If appropriate, describe procedures for establishing and maintaining seed lots.

g. Describe any other pertinent tests or procedures performed on the hybridoma cell line.

2. Antibody production:

a. Describe the production method. If produced in cell culture, animal serum additives must conform to 9 CFR 113.53. If produced in animals, describe fully including husbandry practices and passage procedures.

b. Provide the criteria for acceptable monoclonal antibody, including tests for purity.

c. Describe all tests or other methods used to ensure uniformity between production lots of monoclonal antibody. Include all reaction conditions, equipment used, and reactivity of the component.

d. Describe all characterization procedures and include the expected reactivity of all reference monoclonal antibodies.

II. *Antigen Preparation*

A. Identify the microorganism(s) or antigen being used. If previously approved Master Seed virus, bacteria, or antigen derived therefrom is used, provide pertinent information on the testing performed, and details of dates of United States Department of Agriculture confirmatory tests and approval, as appropriate.

B. Describe all propagation steps, including identification of cell cultures, media ingredients, cell culture conditions, and harvest methods. For antigen produced in eggs, give the egg source, age, and route of inoculation. If cell lines are being used, give dates of testing and approval as specified in 9 CFR 113.52.

C. Describe procedures used for extracting and characterizing the antigen.

D. Describe the method used to standardize the antigen.

E. If the antigen is purchased, identify the supplier and describe the criteria for acceptable material, including all tests performed by the producer and/or the recipient to determine acceptability.

III. *Preparation of Standard Reagents*

A. Describe the positive and negative controls included in the kit. If purchased, list suppliers and criteria for acceptance.

B. Describe the preparation and standardization of the conjugate(s). If purchased, list suppliers and criteria for acceptance.

C. Describe the preparation and standardization of the substrate(s). If purchased, list suppliers and criteria for acceptance.

D. Identify buffers, diluents, and other reagents included in the kit. The preparation of these components may be described in this section or in filed Special Outlines.

IV. *Preparation of the Product*

Fully describe methods used to standardize antigens, reference standards, positive control serum, negative control serum, and standard reagents from production/purchase to completion of finished product in final containers, including the following:

1. Composition and quantity of preservative in each.

2. Method of filling, plating, or attaching the antigen or antibody component to a solid phase.

3. Minimum and maximum acceptable fill volumes for each final container of reagent included in the kit.

4. The disposition of unsatisfactory material.

V. *Testing*

Refer to all applicable standard requirements.

A. Purity.

Describe all tests of the kit for purity or specify the exemption as provided in 9 CFR 113.4.

B. Safety.

In vitro products are exempt from safety tests.

C. Potency.

Provide details of tests used to determine the relative reactivity of the kit including minimum requirements for a satisfactory test. Reference standards and control serum used for this purpose should be identified by unique codes or lot numbers.

VI. *Postpreparatory Steps*

A. Describe the form and size of final containers of each reagent/component included in the kit.

B. Describe the collection, storage, and submission of representative samples. Refer to 9 CFR 113.3(b)(7).

C. Specify the expiration date. Refer to 9 CFR 114.13.

D. Provide details of recommendations for use, including all limitations, qualifications, and interpretation of results.

E. Submit confidentiality statement identifying specific parts of the outline containing information, the release of which would cause harm to the submitter.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16869, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 20124, May 2, 1991; 56 FR 66784, Dec. 26, 1991]

§ 114.10 Antibiotics as preservatives.

Antibiotics are authorized for use as preservatives for biological products if used within the limitations as to kinds and amounts prescribed in this section.

(a) When an antibiotic or combination of antibiotics, with or without a fungistat is to be used in the preparation of a biological product, the kind(s) and amount(s) of each shall be specified in the outline for such product in such a way that the concentration in the final product may be calculated. Except as may be approved by the Administrator, only those individual antibiotics or combinations of antibiotics listed in paragraphs (b) and (c) of this section shall be used.

(b) Permitted individual antibiotics:

(1) The antibiotic level of a specified individual antibiotic in one ml. of a biological product, when prepared as recommended for use, shall not exceed the amounts listed in this paragraph: *Provided,* That in the case a desiccated biological product is to be used with an indefinite quantity of water or other menstruum, the determination shall be based on 30 ml. per 1,000 dose vial or equivalent.

(2) Except as prescribed in paragraph (c) of this section, only one antibiotic shall be used as a preservative in a biological product. The kind and maximum amount per ml. of such antibiotic shall be restricted to:

Amphotericin B	2.5 mcg.
Nystatin (Mycostatin)	30.0 units
Tetracyclines	30.0 mcg.
Penicillin	30.0 units
Streptomycin	30.0 mcg.
Polymyxin B	30.0 mcg.
Neomycin	30.0 mcg.
Gentamicin	30.0 mcg.

(c) Permitted combinations:

(1) Penicillin and streptomycin.

(2) Either amphotericin B or nystatin, but not both, may be used with one of the other antibiotics listed in paragraph (b) of this section, or with a combination of penicillin and streptomycin, or with a combination of polymyxin B and neomycin.

(3) The maximum amount of each antibiotic in a combination shall be the amount prescribed for such antibiotic in paragraph (b) of this section.

(d) Antibiotics used in virus seed stock purification are not restricted as to kind or amounts provided carryover into the final product is controlled and specified in outlines of production.

[39 FR 16869, May 10, 1974, as amended at 56 FR 66784, Dec. 26, 1991

§ 114.11 Storage and handling.

Biological products at licensed establishments shall be protected at all times against improper storage and handling. Completed product shall be kept under refrigeration at 35 °to 45 °F. (2 °to 7 °C.) unless the inherent nature of the product makes storage at a different temperature advisable, in which

case, the proper storage temperature shall be specified in the filed Outline of Production. All biological products to be shipped or delivered shall be securely packed.

§ 114.12 Expiration date required.

Each serial or subserial of biological product prepared in a licensed establishment shall be given an expiration date determined in accordance with the requirements provided in § 114.13 or § 114.14. A licensed biological product shall be considered worthless under the Virus-Serum-Toxin Act subsequent to the expiration date appearing on the label.

[41 FR 44687, Oct. 12, 1976]

§ 114.13 Expiration date determination.

Unless otherwise provided for in a Standard Requirement of filed Outline of Production, the expiration date for each product shall be computed from the date of the initiation of the potency test. Prior to licensure, stability of each fraction shall be determined by methods acceptable to Animal and Plant Health Inspection Service. Expiration dates based on this stability data shall be confirmed as follows:

(a) *Products consisting of viable organisms.* Each serial shall be tested for potency at release and at the approximate expiration date until a statistically valid stability record has been established.

(b) *Nonviable biological products.* Each serial presented in support of licensure shall be tested for potency at release and at or after the dating requested.

(c) Subsequent changes in the dating period for a product may be granted, based on statistically valid data submitted to support a revision of the Outline of Production.

[50 FR 24903, June 14, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

§ 114.14 Extension of expiration date for a serial or subserial.

(a) Unless otherwise provided for in a filed Outline of Production for the product, the expiration date shall not be extended:

(1) If all fractions of the product are not evaluated for potency by tests designated in the filed Outline of Production for such product in accordance with § 113.4(b) of this subchapter.

(2) For any serial or portion of any serial which has left licensed premises: *Provided,* That product which has been shipped from one licensed premises to another licensed premises shall be exempt from this requirement.

(3) For a serial or portion of a serial if the expiration date has been extended previously, unless otherwise authorized in accordance with § 114.1.

(b) An extension of the expiration date may be granted by Animal and Plant Health Inspection Service if a request from the licensee is substantiated by valid test data which demonstrate the potency of the product meets or exceeds the requirements for release. The new expiration date shall be calculated from the date the latest satisfactory potency test was initiated. The extension of the expiration date shall not exceed the maximum dating allowed in the filed Outline of Production.

(1) Serials are approved for redating under the condition that Animal and Plant Health Inspection Service may require the firm to retest the redated serial for potency during the extended dating period and if found unsatisfactory require it be removed from the market by the licensee.

(2) [Reserved]

[50 FR 24903, June 14, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

§ 114.15 Disposal of unsatisfactory products and byproducts.

All biological products found to be unsatisfactory for marketing, all biological products which have become worthless subsequent to the expiration date, all refuse, other materials deemed unsatisfactory for production purposes, all carcasses (part or whole) of production or test animals, and any undesirable byproducts of manufacture shall be disposed of as may be required by the Administrator.

[41 FR 44687, Oct. 12, 1976, as amended at 56 FR 66784, Dec. 26, 1991]

§ 114.16 Producing subsidiaries.

A serial or subserial of a biological product may be produced jointly by a

licensee and one or more subsidiaries, or by two or more subsidiaries. The exact amount of each serial or subserial credited to each participating producer shall be determined at the time of labeling and packaging and shall be noted in the records for such serial or subserial.

[40 FR 46093, Oct. 6, 1975]

§ 114.17 Rebottling of biological products.

The Administrator may authorize the rebottling of a completed product in liquid form subject to the conditions prescribed in this section.

(a) All or part of a serial which has not left the licensed establishment may be aseptically returned to the mixing tank, thoroughly mixed, and rebottled in new final containers.

(b) The rebottled product shall be adequately identified by serial number or subserial number, as the case may be.

(c) Required purity tests for final container samples of the product shall be conducted on new samples selected from the rebottled product (serial or subserials). Rebottled product found to be unsatisfactory by such tests shall not be released.

(d) New test samples from each serial or subserial and copies of test reports of all tests conducted on the rebottled product shall be submitted to Animal and Plant Health Inspection Service.

(e) The licensee shall not release the rebottled product unless notified by Animal and Plant Health Inspection Service that such product is eligible for release. Production records shall show the results of all tests conducted and shall accurately reflect the actions taken.

[39 FR 16869, May 10, 1974, as amended at 56 FR 66784, Dec. 26, 1991

§ 114.18 Reprocessing of biological products.

The Administrator may authorize a licensee to reprocess a serial of completed product subject to the conditions prescribed in this section.

(a) Reprocessing shall not include any method or procedure which would be deleterious to the product.

(b) All appropriate tests for purity, safety, potency, and efficacy for the product shall be conducted on the reprocessed product. A serial found unsatisfactory by a required test shall not be released.

(c) The reprocessed serial shall be identified by a new serial number and the records for the serial shall accurately reflect the action taken.

(d) Test samples of the reprocessed serial and test reports for all tests conducted shall be submitted to Animal and Plant Health Inspection Service. The licensee shall not release the serial until notified by Animal and Plant Health Inspection Service that the serial is eligible for release.

[50 FR 24904, June 15, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

PART 115—INSPECTIONS

Sec.
115.1 Inspections of establishments.
115.2 Inspections of biological products.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 115.1 Inspections of establishments.

(a) Any inspector shall be permitted to enter any establishment where any biological product is prepared, at any hour during the day or night, and shall be permitted to inspect, without previous notification, the entire premises of the establishment, including all buildings, compartments, and other places, all biological products, and organisms and vectors in the establishment, and all materials and equipment, such as chemicals, instruments, apparatus, and the like, and the methods used in the manufacture of, and all records maintained relative to, biological products produced at such establishment.

(b) Each inspector will have in his or her possession a numbered USDA badge or identification card. Either shall be sufficient identification to entitle him/her to admittance at all regular entrances and to all parts of such establishment and premises and to any place at any time for the purpose of making an inspection pursuant to paragraph (a) of this section.

[52 FR 30134, Aug. 13, 1987]

§ 115.2 Inspections of biological products.

Any biological product, the container of which bears a United States veterinary license number or a United States veterinary permit number or other mark required by these regulations may be inspected at any time or place. If, as a result of such inspection, it appears that any such product is worthless, contaminated, dangerous or harmful, the Secretary shall give notice thereof to the manufacturer or importer and to any jobbers, wholesalers, dealers or other persons known to have any of such product in their possession, and may proceed against such product pursuant to the provisions of part 118 of this subchapter. Unless and until the Secretary shall otherwise direct, no persons so notified shall thereafter sell, barter, or exchange any such product in any place under the jurisdiction of the United States or ship or deliver for shipment any such product in or from any State, Territory, or the District of Columbia. However, failure to receive such notice shall not excuse any person from compliance with the Virus-Serum-Toxin Act.

[52 FR 30134, Aug. 13, 1987]

PART 116—RECORDS AND REPORTS

Sec.

116.1 Applicability and general considerations.
116.2 Inventory and disposition records.
116.3 Label records.
116.4 Sterilization and pasteurization records.
116.5 Reports.
116.6 Animal records.
116.7 Test records.
116.8 Completion and retention of records.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 116.1 Applicability and general considerations.

(a) Each licensee, permittee, and foreign manufacturer of biological products imported into the United States shall maintain, at the licensed or foreign establishment in which the products are prepared, detailed records of information necessary to give a complete accounting of all the activities within each establishment. Such records shall include, but shall not be limited to, the items enumerated in this part.

(1) Records shall be made concurrently with the performance of successive steps in the development and preparation of biological products, including new products under development. Such records shall include the date and where critical, the time that each essential step was taken, the identity and quantity of ingredients added or removed at each step, and any gain or loss of product from the beginning to the end of product preparation.

(2) Records shall be legible and indelible; shall be as detailed as necessary for a clear understanding of each step by one experienced in the preparation of biological products; and shall be verified by initials or signature of the person immediately responsible for the action taken.

(3) Records (other than disposition records) required by this part shall be completed by the licensee or the foreign manufacturer, as the case may be, before any portion of a serial of any product shall be marketed in the United States or exported.

(b) If, at any time, there are indications that raise questions regarding the purity, safety, potency, or efficacy of a product, or if it appears that there may be a problem regarding the preparation, testing, or distribution of a product, the licensee, permittee, or foreign manufacturer must immediately notify the Animal and Plant Health Inspection Service concerning the circumstances and the action taken, if any. Notification may be made by mail to Director, Center for Veterinary Biologics, Inspection and Compliance, 510 South 17th Street, Suite 104, Ames, IA 50010–8197; by electronic mail to cvb@usda.gov; by fax to (515) 232–7120; or by telephone to (515) 232–5785.

(c) When authorized by the Administrator, the licensee, permittee, or foreign manufacturer may maintain and retain records required under this part at an alternative location. Such authorization shall be confirmed by the filing of an addendum to the plot plan legend. The addendum shall list the location of the records and the condition of their storage and shall permit the

inspection of the records by APHIS inspectors, or foreign inspectors acting on behalf of APHIS.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16872, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 61 FR 52874, Oct. 9, 1996; 64 FR 43045, Aug. 9, 1999; 66 FR 21064, Apr. 27, 2001]

§ 116.2 Inventory and disposition records.

(a) Records shall show the quantity and location of each biological product being prepared, in storage, and in distribution channels.

(b) Detailed disposition records, in a form satisfactory to the Administrator, shall be maintained by each licensee, each distributor, and each permittee showing the sale, shipment, or other disposition made of the biological products handled by such person.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16872, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991; 61 FR 52874, Oct. 9, 1996; 66 FR 21064, Apr. 27, 2001]

§ 116.3 Label records.

(a) Each licensee and permittee shall maintain a list of all approved labels currently being used. Each label shall be identified as to:

(1) Name and product code number as it appears on the product license or permit for the product;

(2) Where applicable, the size of the package (doses, ml, cc, or units) on which the label shall be used;

(3) Label number and date assigned; and

(4) Name of licensee or subsidiary appearing on the label as the producer.

(b) All labels printed shall be accounted for and an inventory maintained.

Records shall include the disposition of such labels including those not used in labeling a product.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16872, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 61 FR 52874, Oct. 9, 1996; 66 FR 21064, Apr. 27, 2001]

§ 116.4 Sterilization and pasteurization records.

Records shall be made by means of automatic recording devices or an equivalent accurate and reliable system. Such records shall be identified with the ingredients, equipment, or biological product subjected to sterilization or pasteurization.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16872, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 61 FR 52874, Oct. 9, 1996; 66 FR 21064, Apr. 27, 2001]

§ 116.5 Reports.

(a) When required by the Administrator, reports containing accurate and complete information concerning biological products, including but not limited to, product development and preparation, and market suspensions and recalls, shall be prepared and submitted to the Animal and Plant Health Inspection Service by the licensee, permittee, or foreign manufacturer (whose products are being imported or offered for importation). Unless otherwise authorized by the Administrator, records necessary to make such reports shall be maintained in each establishment.

(b) If, at any time, there are indications that raise questions regarding the purity, safety, potency, or efficacy of a product, or if it appears that there may be a problem regarding the preparation, testing, or distribution of a product, the licensee, permittee, or foreign manufacturer must immediately notify the Animal and Plant Health Inspection Service concerning the circumstances and the action taken, if any. Notification may be made by mail to Director, Center for Veterinary Biologics, Inspection and Compliance, 510 South 17th Street, Suite 104, Ames, IA 50010–8197; by electronic mail to cvb@usda.gov; by fax to (515) 232–7120; or by telephone to (515) 232–5785.

(Approved by the Office of Management and Budget under control number 0579–0013)

[61 FR 52874, Oct. 9, 1996, as amended at 64 FR 43045, Aug. 9, 1999]

§ 116.6 Animal records.

Complete records shall be kept for all animals at a licensed establishment. Results of tests performed, antigens or

treatment administered, maintenance and production records, disposition records, necropsy records, if any, and all other pertinent records shall be included.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16872, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 61 FR 52874, Oct. 9, 1996; 66 FR 21064, Apr. 27, 2001]

§ 116.7 Test records.

Detailed records of all tests conducted on each serial and each subserial shall be maintained by the licensee. Summaries of such tests shall be prepared from such records and submitted to the Animal and Plant Health Inspection Service using APHIS Form 2008 or an acceptable equivalent form prior to release of the serial or subserial. Blank forms for such summaries shall be available from Animal and Plant Health Inspection Service upon request.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16872, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991; 61 FR 52874, Oct. 9, 1996]

§ 116.8 Completion and retention of records.

All records (other than disposition records) required by this part shall be completed by the licensee, permittee, or foreign manufacturer before any portion of a serial of any product may be marketed in the United States or exported. All records shall be retained at the licensed or foreign establishment or permittee's place of business for a period of two years after the expiration date of a product, or for such longer period as may be required by the Administrator.

(Approved by the Office of Management and Budget under control number 0579–0013)

[61 FR 52874, Oct. 9, 1996]

PART 117—ANIMALS AT LICENSED ESTABLISHMENTS

Sec.

117.1 Applicability.
117.2 Animal facilities.
117.3 Admittance of animals.
117.4 Test animals.
117.5 Segregation of animals.
117.6 Removal of animals.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 38 FR 15499, June 13, 1973, unless otherwise noted.

§ 117.1 Applicability.

(a) All animals used in licensed establishments in the preparation or testing of biological products shall meet the regulations in this subchapter and special requirements as may be prescribed by the Administrator to prevent the preparation, sale, and distribution of worthless, contaminated, dangerous, or harmful biological products.

(b) Unless otherwise authorized or directed by the Administrator, animals used in the preparation or testing of biological products shall be admitted to and maintained at the licensed establishment and ultimately disposed of in accordance with the regulations in this part, and with the Act of August 24, 1966 (Pub. L. 89–544) as amended by the Animal Welfare Act of 1970 (Pub. L. 91–579) and the regulations in parts 1, 2, and 3 of this chapter. Personnel who supervise the care and welfare of such animals shall be qualified by education, training, and experience to carry out the regulations in this part.

[38 FR 15499, June 13, 1973, as amended at 56 FR 66784, Dec. 26, 1991]

§ 117.2 Animal facilities.

Animal facilities shall comply with the requirements provided in part 108 of this chapter.

§ 117.3 Admittance of animals.

(a) No animal which shows clinical signs or other evidence of disease shall be admitted to the premises of licensed establishments, except as provided in paragraphs (d) and (e) of this section. The health status of all animals offered for admission shall be determined by or under the direction of a veterinarian prior to admission. If the determination cannot be made prior to admission, the animals shall be kept separate from animals already on the premises and in a quarantine area to be provided by the licensee for this purpose

until the animal's health status is determined.

(b) If special test requirements for admittance of the animals are specified in the Outline of Production for the product to be produced, the animals shall remain in the quarantine area until such tests have been performed and the results obtained. Animals which do not meet the requirements shall not be admitted to the production area or allowed to contact production animals.

(c) All animals admitted to the premises of a licensed establishment shall be permanently identified either collectively or individually by the licensee with tags, marks, or other means acceptable to the Administrator.

(d) When an animal which has a disease is to be used to prepare a biological product for control of such disease, the animal shall be admitted directly to the processing facilities in which the product is to be prepared but shall not be permitted contact with other animals on the premises.

(e) The Administrator may authorize the maintenance of diagnostic facilities at the licensed establishment: *Provided,* That safeguards proposed by the licensee are adequate to prevent diseased or dead animals brought into such facilities from being a threat to biological products prepared in such establishment or to other animals on the premises used in the preparation of biological products.

[38 FR 15499, June 13, 1973, as amended at 56 FR 66784, Dec. 26, 1991]

§ 117.4 Test animals.

(a) All test animals shall be examined for clinical signs of illness, injury, or abnormal behavior prior to the start of a test and throughout the observation period specified in the test protocol.

(b) All animals used for test purposes shall be identified either collectively or individually in a manner conducive to an accurate interpretation of the results of the test.

(c) No test animals shall be given a biological product during the preconditioning period which would affect its eligibility according to the test requirements. No treatment, with a biological product or otherwise, shall be administered to a test animal during a test period which could interfere with a true evaluation of the biological product being tested.

(d) During the course of a test, animals that are injured or show clinical signs of illness or unfavorable reactions that are not due to the test may be removed from the test and treated or humanely destroyed. If sufficient animals do not remain for the test to be evaluated, the test shall be declared inconclusive and may be repeated.

(e) Test animals that show clinical signs of illness that are due to the test may be treated or humanely destroyed if the illness has progressed to a point (defined in the filed Outline of Production) when death is certain to occur without therapeutic intervention. When interpreting the results of the test, the animals that were treated or humanely destroyed because of illness due to the test and the animals that have died from illness due to the test prior to being humanely destroyed shall be combined into a common statistic of mortality due to the test.

[38 FR 15499, June 13, 1973, as amended at 60 FR 43356, Aug. 21, 1995]

§ 117.5 Segregation of animals.

Animals which have been infected with or exposed to a dangerous, infectious, contagious, or communicable disease shall be kept effectively segregated at a licensed establishment until such time as they are humanely destroyed or successfully treated and removed as healthy animals.

§ 117.6 Removal of animals.

Production animals or ex-test animals which are no longer useful at the licensed establishment may be removed from the premises of the licensed establishment; provided, such removal is accomplished in a manner as shall preclude the dissemination of disease and in accordance with the following conditions:

(a) Meat-producing animals which received a biological product containing inactivated microorganisms and adjuvants within 21 days shall not be removed; or

(b) Animals which received virulent microorganisms within 30 days shall not be removed; or

(c) Only animals that are in a healthy condition as determined by a veterinarian shall be removed, except as provided in paragraph (d) of this section.

(d) Other animals that are injured or otherwise unhealthy, except when affected with a communicable disease, may be removed for immediate slaughter to an abattoir operated in accordance with the Federal Meat Inspection Act of March 4, 1907, 34 Stat. 1260, as amended by the Wholesome Meat Act of 1967, 81 Stat. 585 (21 U.S.C. sec. 601 et seq.): *Provided,* That such animals shall be properly marked for identification and the inspector in charge of slaughter operations is given due notice in advance.

(e) All animals on the premises shall be disposed of in accordance with the provisions of the regulations in this part and where specific provision is not made therefor shall be disposed of as required by the Administrator.

[38 FR 15499, June 13, 1973, as amended at 56 FR 66784, Dec. 26, 1991]

PART 118—DETENTION; SEIZURE AND CONDEMNATION

Sec.

118.1 Administrative detention.
118.2 Method of detention; Notifications.
118.3 Movement of detained biological products; Termination of detention.
118.4 Seizure and condemnation.

AUTHORITY: 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 52 FR 30135, Aug. 13, 1987, unless otherwise noted.

§ 118.1 Administrative detention.

Whenever any biological product which is prepared, sold, bartered, exchanged, or shipped in violation of the Act or regulations is found by any authorized representative of the Administrator upon any premises, it may be detained by such representative for a period not to exceed 20 days, pending action under §118.4, and shall not be moved by any person from the place at which it is located when so detained, until released by such representative.

[52 FR 30135, Aug. 13, 1987, as amended at 56 FR 66784, Dec. 26, 1991]

§ 118.2 Method of detention; Notifications.

An authorized representative of the Administrator shall detain any biological product subject to detention under this part by:

(a) Giving oral notification to the owner of the biological product if such owner can be ascertained, and, if not, to the agent representing the owner or to the immediate custodian of the biological product; and

(b) Promptly furnishing the person so notified with a preliminary notice of detention which shall include identity and quantity of the product detained, the location where detained, the reason for the detention, and the name of the authorized representative of the Administrator.

(c) Within 48 hours after the detention of any biological product, an authorized representative of the Administrator shall, if the detention is to continue, give written notification to the owner of the biological product detained by furnishing a written statement which shall include the identity and quantity of the product detained, the location where detained, specific description of the alleged noncompliance including reference to the provisions in the Act or the regulations which have resulted in the detention, and the identity of the authorized representative of the Administrator; or, if such owner cannot be ascertained and notified within such period of time, furnish such notice to the agent representing such owner, or the carrier or other person having custody of the biological product detained. The notification, with a copy of the preliminary notice of detention shall be served by either delivering the notification to the owner or to the agent or to such other person, or by certifying and mailing the notification, addressed to such owner, agent, or other person, at the last known residence or principal office or place of business.

[52 FR 30135, Aug. 13, 1987, as amended at 56 FR 66784, Dec. 26, 1991]

§ 118.3 Movement of detained biological products; Termination of detention.

Except as provided in paragraphs (a) and (b) of this section, no biological product detained in accordance with the provisions in this part shall be moved by any person from the place at which such product is located when it is detained.

(a) A detained biological product may be moved from the place at which it is located when so detained for the purpose of providing proper storage conditions if such movement has been approved by an authorized representative of the Administrator; *Provided,* That, the biological product so moved shall be detained by an authorized representative of the Administrator after such movement.

(b) A detained biological product may be moved from the place at which it is detained on written notification by an authorized representative of the Administrator that the detention is terminated; *Provided,* That, the conditions under which the detained biological product may be moved will be specified in the written notification of the termination. The notification of termination shall be served by either personally delivering the notification, or by certifying and mailing the notification addressed to such person at the last known residence or principal office or place of business of the owner, agent, or other person having custody of the biological product.

[52 FR 30135, Aug. 13, 1987, as amended at 56 FR 66784, Dec. 26, 1991]

§ 118.4 Seizure and condemnation.

Any biological product which is prepared, sold, bartered, exchanged, or shipped in violation of the Act or regulations shall be liable to be proceeded against and seized and condemned, at any time, on a libel of information in any United States district court or other proper court within the jurisdiction of which the product is found. If the product is condemned, it shall, after entry of the decree, be disposed of by destruction or sale as the court may direct, and the proceeds, if sold, less the court costs and fees, and storage and other proper expenses, shall be paid into the Treasury of the United States, but the product shall not be sold contrary to the provisions of the Act or the laws of the jurisdiction in which it is sold; *Provided,* That, upon the execution and delivery of a good and sufficient bond conditioned that the product shall not be sold or otherwise disposed of contrary to the provisions of the Act or the laws or jurisdiction in which disposal is made, the court may direct that such product be delivered to the owner thereof subject to such supervision by authorized representatives of the Administrator as is necessary to ensure compliance with the applicable laws. When a decree of condemnation is entered against the product and it is released under bond, or destroyed, court costs and fees, and storage and other proper expenses shall be awarded against the person, if any, intervening as claimant of the product. The proceedings in such libel cases shall conform, as nearly as may be practicable, to the proceedings in admiralty, except that either party may demand trial by jury of any issue of fact joined in any case, and all such proceedings shall be at the suit of and in the name of the United States.

[52 FR 30135, Aug. 13, 1987, as amended at 56 FR 66784, Dec. 26, 1991]

PART 121—POSSESSION, USE, AND TRANSFER OF BIOLOGICAL AGENTS AND TOXINS

Sec.

121.0 Effective and applicability dates.
121.1 Definitions.
121.2 Purpose and scope.
121.3 List of biological agents and toxins.
121.4 Exemptions for overlap agents or toxins.
121.5 Exemptions for animal agents and toxins.
121.6 Registration; who must register.
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121.9 Registration; how to register.
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121.11 Restricting access to biological agents and toxins.
121.12 Biosafety and security plan.
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121.14 Transfer of biological agents and toxins.
121.15 Records.
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121.17 Notification in the event of theft, loss, or release of a biological agent or toxin.
121.18 Administrative review.

AUTHORITY: Secs. 211–213, Title II, Pub. L. 107–188, 116 Stat. 647 (7 U.S.C. 8401).

SOURCE: 67 FR 76931, Dec. 13, 2002, unless otherwise noted.

§ 121.0 Effective and applicability dates.

(a) The regulations in this part are effective on February 11, 2003. On and after that date, any person possessing, using, or transferring any agent or toxin listed in § 121.3 must be in compliance with the provisions of this part. However, so as not to disrupt research or educational projects involving listed agents or toxins that were underway as of the effective date of this part, any person possessing such agents or toxins as of the effective date (current possessors) will be afforded additional time to reach full compliance with this part. Any provision not specifically cited in paragraphs (a)(1) through (a)(6) of this section will be applicable as of February 11, 2003. In addition, any person who does not possess listed agents or toxins by the effective date of this part, but who wishes to initiate a research or educational project prior to November 12, 2003, must be in compliance with the provisions of this part that are applicable for current possessors at the time of application, as provided in paragraphs (a)(1) through (a)(5) of this section.

(1) During the period from February 11, 2003, to November 12, 2003, biological agents or toxins listed in § 121.3 may only be transferred to an individual or entity that is not registered under this part if:

(i) The individual or entity is registered by CDC for that specific overlap agent or toxin in accordance with 42 CFR part 72; or

(ii) The individual or entity has been issued a permit by the Administrator under part 122 of this subchapter to import or move interstate that specific agent or toxin. If an individual or entity has not been issued a permit under part 122 of this subchapter, the individual or entity may apply for a permit. To receive an agent or toxin, an individual or entity will also be required to submit APHIS Form 2041, in accordance with § 121.14(c). Because USDA permits do not cover intrastate movement, unless registered by CDC under 42 CFR part 72, an individual or entity may not receive a listed agent or toxin that is being moved intrastate until that individual or entity is registered in accordance with this part.

(2) By March 12, 2003, the responsible official must submit the registration application package as required in § 121.9. In addition, the responsible official must submit to the Attorney General the names and identifying information for the responsible official; alternate responsible official, where applicable; entity; and, where applicable, the individual who owns or controls the entity.

(3) By April 11, 2003, the responsible official must submit to the Attorney General the names and identifying information for all individuals whom the responsible official has identified as having a legitimate need to handle or use listed agents or toxins, and who have the appropriate training and skills to handle such agents or toxins, as required in § 121.11.

(4) By June 12, 2003, the responsible official must submit the security section of the Biosafety and Security Plan required in § 121.12 to APHIS or, for overlap agents or toxins, to APHIS or CDC.

(5) By September 12, 2003, the responsible official must implement the security section of the Biosafety and Security Plan, as required in § 121.12, and provide security training in accordance with 9 CFR 121.13.

(6) By November 12, 2003, the registration application process must be complete and the entity in full compliance with the regulations in this part, except as otherwise provided in paragraphs (b) and (c) of this section.

(b) *Provisional registration*. (1) Notwithstanding the provisions in paragraph (a) of this section, APHIS may issue a provisional registration certificate to current possessors if, as of November 12, 2003:

(i) The Attorney General has received all of the information, including fingerprint cards, required by the Attorney General to conduct a security risk assessment of the entity, including any

individual who owns or controls the entity; and

(ii) The entity otherwise meets all of the requirements of this part.

(2) Notwithstanding the provisions in paragraph (a) of this section, APHIS may issue a provisional registration certificate to individuals and entities that did not possess listed biological agents or toxins as of February 11, 2003, if, as of November 12, 2003:

(i) The Attorney General has received all of the information, including fingerprint cards, required by the Attorney General to conduct a security risk assessment of the entity, including any individual who owns or controls the entity;

(ii) The entity otherwise meets all of the requirements of this part; and

(iii) The Administrator finds that circumstances warrant such action in the interest of the health of plants or plant products or national security.

(3) A provisional registration certificate will be effective until APHIS either issues a certificate of registration or suspends or revokes the provisional registration.

(c) Notwithstanding the provisions in paragraph (a) of this section, APHIS may issue a provisional grant of access for individuals identified by an entity as having a legitimate need to handle or use agents or toxins listed in §121.3 if, as of November 12, 2003, the Attorney General has received all of the information, including fingerprint cards, required by the Attorney General to conduct a security risk assessment of that individual. A provisional grant of access will be effective until APHIS grants or denies access to biological agents or toxins listed in §121.3.

[68 FR 62220, Nov. 3, 2003]

§121.1 Definitions.

Administrator. The Administrator, Animal and Plant Health Inspection Service, or any person authorized to act for the Administrator.

Animal and Plant Health Inspection Service (APHIS). The Animal and Plant Health Inspection Service of the United States Department of Agriculture.

Attorney General. The Attorney General of the United States or any person authorized to act for the Attorney General.

Biological agent. Any microorganism (including, but not limited to, bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing:

(1) Death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism;

(2) Deterioration of food, water, equipment, supplies, or material of any kind; or

(3) Deleterious alteration of the environment.

Centers for Disease Control and Prevention (CDC). The Centers for Disease Control and Prevention of the United States Department of Health and Human Services.

Clinical laboratory. A laboratory facility that receives patients and collects specimens for processing or shipping to another laboratory.

Diagnostic laboratory. A laboratory facility that receives specimens for the purpose of determining the identities of pests, pathogens, contaminants, or causes of disease.

Entity. Any government agency (Federal, State, or local), academic institution, corporation, company, partnership, society, association, firm, sole proprietorship, or other legal entity.

Import. To move into, or the act of movement into, the territorial limits of the United States.

Interstate. From one State into or through any other State, or within the District of Columbia, Guam, the Virgin Islands of the United States, or any other territory or possession of the United States.

Overlap agent or toxin. Any microorganism (including, but not limited to, bacteria, viruses, fungi, rickettsiae, or protozoa) or toxin that poses a risk to both human and animal health and that is listed in §121.3(b).

Permit. A written authorization by the Administrator to import or move interstate biological agents or toxins, under conditions prescribed by the Administrator.

Proficiency testing. A sponsored, time-limited analytical trial whereby one or more analytes, previously confirmed by

the sponsor, are submitted to the testing laboratory for analysis and where final results are graded, scores are recorded and provided to participants, and scores for participants are evaluated for acceptance.

Responsible official. The individual designated by an entity to act on its behalf. This individual must have the authority and control to ensure compliance with the regulations in this part.

Specimen. A sample of material collected for use in testing, such as tissues, gastrointestinal contents, feces, bodily fluids (blood, serum, etc.), soil, water, feed or feed ingredients, swabs, cultures, and suspensions.

State. Any of the several States of the United States, the Commonwealth of the Northern Mariana Islands, the Commonwealth of Puerto Rico, the District of Columbia, Guam, the Virgin Islands of the United States, or any other territory or possession of the United States.

Toxin. The toxic material or product of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes:

(1) Any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or

(2) Any poisonous isomer or biological product, homolog, or derivative of such a substance.

United States. All of the States.

USDA. The United States Department of Agriculture.

§ 121.2 Purpose and scope.

(a) This part sets forth the requirements for possession, use, and transfer of biological agents or toxins that have been determined to have the potential to pose a severe threat to both human and animal health, to animal health, or to animal products. The purpose of this part is to ensure the safe handling of such agents or toxins, and to protect against the use of such agents or toxins in domestic or international terrorism or for any other criminal purpose.

(b) Accordingly, this part provides that any individual or entity that possesses, uses, or transfers any agent or toxin listed in § 121.3 must register in accordance with § 121.7. To register, each entity must designate an individual who has the authority and control to ensure compliance with the regulations to be the responsible official. The responsible official must complete and submit the registration application package to APHIS or, for overlap agents or toxins, to APHIS or CDC. As part of registration, the responsible official, the entity, and, where applicable, the individual who owns or controls such entity will be subject to a security risk assessment by the Attorney General.

(c) The responsible official is responsible for ensuring compliance with the safety procedures in this part, including implementing the Biosafety and Security Plan in accordance with § 121.12, providing the proper training to individuals who handle or use agents or toxins listed in § 121.3, and providing proper laboratory facilities to contain and dispose of such agents or toxins. In addition, the responsible official is responsible for ensuring compliance with the safeguard and security measures in this part, including restricting access to only those individuals who have a legitimate need to handle or use agents or toxins and who have been approved in accordance with § 121.11, and transferring such agents or toxins only to registered individuals or entities in accordance with § 121.13.

§ 121.3 List of biological agents and toxins.

(a) Except as provided in paragraphs (f) and (g) of this section, the Administrator has determined that the biological agents and toxins listed in this section have the potential to pose a severe threat to both human and animal health, to animal health, or to animal products.

(b) *Overlap agents and toxins.*

Bacillus anthracis
Botulinum neurotoxins
Botulinum neurotoxin producing species of *Clostridium*
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei

Burkholderia pseudomallei
Clostridium botulinum
Clostridium perfringens epsilon toxin
Coccidioides immitis
Coxiella burnetii
Eastern equine encephalitis virus
Francisella tularensis
Hendra virus
Nipah virus
Rift Valley fever virus
Shigatoxin
Staphylococcal enterotoxins
T–2 toxin
Venezuelan equine encephalitis virus

(c) Genetic elements, recombinant nucleic acids, and recombinant organisms of overlap agents or toxins:

(1) Biological agent viral nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the biological agent viruses.

(2) Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the toxins listed in paragraph (b) of this section, if the nucleic acids:

(i) Are in a vector or host chromosome;

(ii) Can be expressed *in vivo* or *in vitro*; or

(iii) Are in a vector or host chromosome and can be expressed in *vivo* or *in vitro*.

(3) Viruses, bacteria, fungi, and toxins listed in paragraph (b) of this section that have been genetically modified.

(d) *Animal agents and toxins.*

African horse sickness virus
African swine fever virus
Akabane virus
Avian influenza virus (highly pathogenic)
Bluetongue virus (exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine fever virus
Cowdria ruminantium (Heartwater)
Foot-and-mouth disease virus
Goat pox virus
Japanese encephalitis virus
Lumpy skin disease virus
Malignant catarrhal fever virus (exotic)
Menangle virus
Mycoplasma capricolum/M. F38/*M. mycoides capri* (contagious caprine pleuropneumonia)
Mycoplasma mycoides mycoides (contagious bovine pleuropneumonia)
Newcastle disease virus (VVND)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Vesicular stomatitis virus (exotic)

(e) The Administrator has determined that it would be impractical to regulate a biological agent or toxin that is in its naturally occurring environment. Therefore, any biological agent or toxin listed in this section that is in its naturally occurring environment will not be subject to the requirements of this part, provided that the biological agent or toxin has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.

(f) The Administrator has determined that biological agents or toxins that meet any of the following criteria do not have the potential to pose a severe threat to both human and animal health, or to animal health or animal products. Therefore, an individual or entity that only possesses, uses, or transfers an agent or toxin that meets any of the following criteria will not be subject to the requirements of this part:

(1) Nonviable agents or fixed tissues that are, bear, or contain agents or toxins listed in this section.[1]

(2) Genetic elements or subunits of agents or toxins listed in paragraph (d) of this section, if the genetic elements or subunits are not capable of causing disease.[2]

(3) Overlap toxins under the control of a principal investigator (or equivalent), if the total aggregate amount does not, at any time, exceed the following amounts: 0.5 mg of Botulinum neurotoxins (types A–G), 100 mg of *Clostridium perfringens* epsilon toxin, 100 mg of Shigatoxin, 5 mg of Staphylococcal enterotoxins, and 1,000 mg of T–2 toxin.

(g) *Attenuated strains.* Attenuated strains of biological agents listed in this section may not have the potential to pose a severe threat to both human and animal health, to animal health, or

[1] However, the importation and interstate movement of these genetic elements or subunits of listed agents or toxins are still subject to the permit requirements under part 122 of this subchapter.

[2] See footnote 1.

to animal products. Thus, an individual or entity may request review by the Administrator to determine whether a specific attenuated strain poses a severe threat to both human and animal health, or to animal health or animal products. For overlap agents, an individual or entity may request review by APHIS or CDC.

(1) If APHIS or CDC determines that a specific attenuated strain does not pose a severe threat to human and animal health, or to animal health or animal products, an individual or entity will not be subject to the requirements of this part. This determination will be limited to the specific attenuated strain and to the specific activities involving that attenuated strain.

(2) An individual or entity may request a review by writing to the Administrator or, for overlap agents, by writing to the Administrator or CDC.[3]

(3) If it is determined that a specific attenuated strain does not pose a severe threat, APHIS or CDC will notify the applicant and publish a notice in the FEDERAL REGISTER.

(4) An individual or entity may request reconsideration of an adverse decision in writing to the Administrator. The request for reconsideration must state all of the facts and reasons upon which the individual or entity relies upon to show the decision was incorrect. The Administrator will grant or deny the request for reconsideration as promptly as circumstances allow and will state, in writing, the reasons for the decision. If there is a conflict as to any material fact, the individual or entity may request a hearing to resolve the conflict.

§ 121.4 Exemptions for overlap agents or toxins.

(a) Clinical or diagnostic laboratories and other entities possessing, using, or transferring overlap agents or toxins that are contained in specimens presented for diagnosis or verification will be exempt from the requirements of this part, provided that:

(1) The identification of such agents or toxins is immediately reported to APHIS or CDC, and to other appropriate authorities when required by Federal, State, or local law; and

(2) Within 7 days after identification, the agents or toxins are transferred or inactivated, and APHIS Form 2040 is submitted to APHIS or CDC.[4] During agricultural emergencies or outbreaks, or in endemic areas, the Administrator may require less frequent reporting. A copy of the completed form must be maintained for 3 years.

(b) Clinical or diagnostic laboratories and other entities possessing, using, or transferring overlap agents or toxins that are contained in specimens presented for proficiency testing will be exempt from the requirements of this part, provided that:

(1) The identification of such agents or toxins, and their derivatives, is immediately reported to the APHIS or CDC, and to other appropriate authorities when required by Federal, State, or local law; and

(2) Within 90 days of receipt, the agents or toxins are transferred or inactivated, and APHIS Form 2040 is submitted to APHIS or CDC. A copy of the completed form must be maintained for 3 years.

(c) Unless the Administrator by order determines that additional regulation of a specific product is necessary to protect animal or plant health, or animal or plant products, an individual or entity possessing, using, or transferring products that are, bear, or contain overlap agents or toxins will be exempt from the requirements of this part if the products have been cleared, approved, licensed, or registered pursuant to:

[3] A request to review an attenuated strain may be mailed to National Center for Import and Export, VS, APHIS, 4700 River Road Unit 40, Riverdale, MD 20737–1231; or faxed to (301) 734–3652. For overlap agents, a request for review may be mailed to the above address or to Select Agent Program, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mail Stop E 79, Atlanta, GA 30333; or faxed to (404) 498–2265.

[4] A clinical or diagnostic laboratory, or other entity, may immediately notify APHIS by faxing (301) 734–3652. APHIS Form 2040 may be obtained by calling APHIS at (301) 734–3277 or by calling CDC at (404) 498–2265. The form is also available on the Internet at *http://www.aphis.usda.gov/vs/ncie.bta.html* or *http://www.cdc.gov/od/ohs/lrsat.htm.* The completed form may be mailed or faxed to APHIS or CDC, as provided in footnote 3.

(1) The Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 *et seq.*);

(2) Section 351 of Public Health Service Act (42 U.S.C. 262);

(3) The Virus-Serum-Toxin Act (21 U.S.C. 151–159); or

(4) The Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. 131 *et seq.*).

(d) An individual or entity possessing, using, or transferring investigational products that are, bear, or contain overlap agents or toxins may be exempt from the requirements of this part if such product is being used in an investigation authorized by any Federal law and the Administrator determines that additional regulation under this part is not necessary to protect animal or plant health, and animal or plant products.

(1) An individual or entity possessing, using, or transferring such investigational products may apply for an exemption from the requirements of this part by submitting APHIS Form 2042 to APHIS or CDC.

(2) For investigational products authorized under any of the Federal laws specified in paragraph (c) of this section, the Administrator shall make a determination regarding an exemption within 14 days after receipt of the application and notification that the investigation has been authorized under a Federal law.

(e) The Administrator may exempt an individual or entity from the requirements of this part, in whole or in part, for 30 days if it is necessary to respond to a domestic or foreign agricultural emergency involving an overlap agent or toxin. The Administrator may extend the exemption once for an additional 30 days.

(f) Upon request of the Secretary of Health and Human Services, the Administrator may exempt an individual or entity from the requirements of this part, in whole or in part, for 30 days if the Secretary of Health and Human Services has granted an exemption for a public health emergency involving an overlap agent or toxin. The Administrator may extend the exemption once for an additional 30 days.

§ 121.5 Exemptions for animal agents and toxins.

(a) Diagnostic laboratories and other entities possessing, using, or transferring agents or toxins that are contained in specimens presented for diagnosis or verification will be exempt from the requirements of this part, provided that:

(1) The identification of such agents or toxins is immediately reported to the Administrator and to other appropriate authorities when required by Federal, State, or local law; and

(2) Within 7 days after identification, the agents or toxins are transferred or inactivated, and APHIS Form 2040 is submitted to the Administrator.[5] During agricultural emergencies or outbreaks, or in endemic areas, the Administrator may require less frequent reporting. A copy of the completed form must be maintained for 3 years.

(b) Diagnostic laboratories and other entities possessing, using, or transferring agents or toxins that are contained in specimens presented for proficiency testing will be exempt from the requirements of this part, provided that:

(1) The identification of such agents or toxins, and their derivatives, is immediately reported to the Administrator, and to other appropriate authorities when required by Federal, State, or local law; and

(2) Within 90 days of receipt, the agent or toxins are transferred or inactivated, and APHIS Form 2040 is submitted to the Administrator. A copy of the completed form must be maintained for 3 years.

(c) An individual or entity receiving diagnostic reagents and vaccines that are, bear, or contain listed agents or toxins, also known as high consequence livestock pathogens or toxins, that are produced at USDA diagnostic facilities

[5] A diagnostic laboratory or other entity must immediately notify APHIS by faxing (301) 734–3652. APHIS Form 2040 may be obtained by calling (301) 734–3277. The form is also available on the Internet at *http://www.aphis.usda.gov/vs/ncie.bta.html.* The completed form may be mailed to National Center for Import and Export, VS, APHIS, 4700 River Road Unit 40, Riverdale, MD 20737–1231; or faxed to (301) 734–3652.

will be exempt from the requirements of this part.

(d) Unless the Administrator by order determines that additional regulation is necessary to protect animal health or animal products, an individual or entity possessing, using, or transferring products that are, bear, or contain listed agents or toxins will be exempt from the requirements of this part if the products have been cleared, approved, licensed, or registered pursuant to:

(1) The Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 *et seq.*);

(2) Section 351 of Public Health Service Act (42 U.S.C. 262);

(3) The Virus-Serum-Toxin Act (21 U.S.C. 151–159); or

(4) The Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. 131 *et seq.*).

(e) An individual or entity possessing, using, or transferring experimental products that are, bear, or contain listed agents or toxins may be exempt from the requirements of this part if such product is being used in an investigation authorized by any Federal law and the Administrator determines that additional regulation under this part is not necessary to protect animal or plant health, and animal or plant products. An individual or entity possessing, using, or transferring such experimental products may apply for an exemption from the requirements of this part by submitting APHIS Form 2042 to APHIS.

(f) In addition to the exemptions provided in paragraphs (a) through (e) of this section, the Administrator may grant a specific exemption upon a showing of good cause and upon his or her determination that such exemption is consistent with protecting animal health and animal products. An individual or entity that possesses, uses, or transfers agents or toxins may request in writing an exemption from the requirements of this part. If granted, such exemptions are valid for a maximum of 3 years; thereafter, an individual or entity must request a new exemption. If a request for exemption is denied, an individual or entity may request reconsideration in writing to the Administrator. The request for reconsideration must state all of the facts and reasons upon which the individual or entity relies to show that the exemption was wrongfully denied. The Administrator will grant or deny the request for reconsideration as promptly as circumstances allow and will state, in writing, the reasons for the decision. If there is a conflict as to any material fact, the individual or entity may request a hearing to resolve the conflict.[6]

§ 121.6 Registration; who must register.

(a) Unless exempted under §§ 121.4 or 121.5, any individual or entity that possesses, uses, or transfers any agent or toxin listed in § 121.3 must register with APHIS or, for overlap agents or toxins, APHIS or CDC.

(b) Each entity must designate an individual to be its responsible official. The responsible official must have the authority and control to ensure compliance with the regulations. The responsible official must complete and sign the registration application package, and will be the individual contacted by APHIS or CDC if any questions arise concerning the application or subsequent compliance with the regulations in this part. As part of registration, the responsible official and the entity will be subject to a security risk assessment by the Attorney General. While most registrants are likely to be entities, in the event that an individual applies for and is granted a certificate of registration, APHIS will consider the individual to be the responsible official.

(c) An entity may designate one or more individuals to be an alternate responsible official, who may act for the responsible official when he/she is unavailable. These individuals must have the authority and control to ensure compliance with the regulations when acting as the responsible official. These individuals will also be subject to a security risk assessment by the Attorney General as part of registration.

[6] A request for exemption may be mailed to National Center for Import and Export, VS, APHIS, 4700 River Road Unit 40, Riverdale, MD 20737–1231; or faxed to (301) 734–3652.

§ 121.7 Registration; general provisions.

(a) Unless exempted under §§ 121.4 or 121.5, an individual or entity shall not possess, use, or transfer any agent or toxin listed in § 121.3 without a certificate of registration issued by APHIS or CDC.

(b) A certificate of registration may be issued upon:

(1) Approval of the responsible official; the alternate responsible official, where applicable; the entity; and, where applicable, the individual who owns or controls the entity following a security risk assessment by the Attorney General;[7] and

(2) Approval of the biosafety, containment, and security of the entity. The entity's biosafety, containment, and security procedures must be commensurate with the risk of the agent or toxin, given its intended use. APHIS or CDC will review the Biosafety and Security Plan, and may inspect and evaluate the premises and records to determine compliance with the regulations and the biosafety, containment, and security requirements; and

(3) A determination by the Administrator that the individual or entity seeking to register has a lawful purpose to possess, use, or transfer such agents or toxins.

(c) For overlap agents, APHIS and CDC will review applications for registration and amendments to a certificate of registration, and a certificate of registration or amendment to a certificate of registration will only be issued if APHIS and CDC concur.

(d) A certificate of registration will be valid for only the specific agents or toxins listed in the certificate and specific activities and locations. A certificate of registration may cover more than one listed agent or toxin, and it may be amended to cover additional listed agents or toxins.

(e) A certificate of registration may be amended to reflect changed circumstances (*e.g.*, replacement of the responsible official, changes in ownership or control of the entity,[8] changes in the activities involving the agent or toxin). The responsible official must immediately notify the agency that issued the certificate of registration, either APHIS or CDC, of such changes in circumstances that occur after submission of the application for registration or after receipt of a certificate of registration.

(f) If a responsible official wishes to discontinue possessing, using, or transferring a particular agent or toxin, the responsible official may inactivate the agent or toxin or he/she may transfer the agent or toxin to a registered individual or entity in accordance with § 121.13. The responsible official must notify APHIS or, for overlap agents or toxins, APHIS or CDC, 5 business days prior to the planned inactivation so that we may have the opportunity to observe the inactivation of the agents or toxins. APHIS or CDC will notify the responsible official if we wish to observe the inactivation of the agents or toxins.

(g) A certificate of registration will be valid for a maximum of 3 years.

§ 121.8 Denial, revocation, or suspension of registration.

(a) APHIS may deny an application for registration or revoke registration if:

(1) The Attorney General identifies the responsible official, entity, or individual who owns or controls the entity as within any of the categories described in 18 U.S.C. 175b; or

(2) The Attorney General identifies the responsible official, entity, or individual who owns or controls the entity as reasonably suspected by any Federal law enforcement or intelligence agency of:

(i) Committing a crime set forth in 18 U.S.C. 2332b(g)(5); or

(ii) Knowing involvement with an organization that engages in domestic or international terrorism (as defined in 18 U.S.C. 2331) or with any other organization that engages in intentional crimes of violence; or

[7] The security risk assessment of the entity and the individual who owns or controls such entity may be waived for Federal, State, or local governmental agencies.

[8] Any change in ownership or control of an entity will require a security risk assessment for the new individual(s) who owns or controls the entity.

(iii) Being an agent of a foreign power as defined in 50 U.S.C. 1801; or

(3) The responsible official does not have a lawful purpose to possess, use, or transfer agents or toxins listed in § 121.3; or

(4) The responsible official is an individual who handles or uses agents or toxins listed in § 121.3 and he/she does not have the necessary training or skills to handle such agents or toxins; or

(5) The entity does not meet the biosafety, containment, and security requirements prescribed by the Administrator;[9] or

(6) There are egregious or repeated violations of the biosafety, containment, or security requirements; or

(7) The Administrator determines that such action is necessary to protect animal or plant health, and animal or plant products.

(b) For overlap agents or toxins, APHIS or CDC shall deny an application for registration or revoke registration if the Attorney General identifies the responsible official, entity, or individual who owns or controls the entity as within any of the categories described in 18 U.S.C. 175b. APHIS or CDC may also deny registration or revoke registration for the reasons set forth in paragraphs (a)(2) through (a)(7) of this section.

(c) APHIS may summarily revoke or suspend registration for any of the reasons set forth in paragraphs (a) and (b) of this section.

(d) APHIS will notify the responsible official in writing if an application for registration is denied or a certificate of registration is revoked or suspended. For overlap agents or toxins, APHIS or CDC will notify the responsible official in writing if an application for registration is denied or a certificate of registration is revoked or suspended.

(e) Denial of an application for registration, revocation of registration, and suspension of registration may be appealed under § 121.17.

[9] If registration is denied for this reason, we may provide technical assistance and guidance.

§ 121.9 Registration; how to register.

(a) To apply for a certificate of registration, the responsible official must submit all of the information and documentation required in the registration application package to APHIS, including the name, source, and characterization data for each agent or toxin to be registered. For overlap agents or toxins, the responsible official must submit all of the information and documentation required in the registration package to either APHIS or CDC. The responsible official must submit the registration application package to APHIS in cases where he/she is seeking registration for both animal and overlap agents and toxins.

(b) For animal agents and toxins, the registration application package may be obtained by calling (301) 734–3277 or faxing a request to (301) 734–3652. It is also available on the Internet at *http://www.aphis.usda.gov/vs/ncie.bta.html.* The completed registration application package must be mailed to National Center for Import and Export, VS, APHIS, 4700 River Road Unit 40, Riverdale, MD 20737–1231. Assistance in completing the registration application may be requested by calling (301) 734–3277.

(c) For overlap agents and toxins, the registration application package may be obtained by contacting APHIS, as set forth in paragraph (b) of this section, or by calling CDC at (404) 498–2255; faxing a request to (404) 498–2265; or writing to Select Agent Program, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mail Stop E 79, Atlanta, GA 30333. It is also available on the Internet at *http://www.cdc.gov/od/ohs/lrsat.htm.* The completed registration application package may be mailed to APHIS at the address provided in paragraph (b) of this section or to CDC's Select Agent Program at the address provided in this paragraph. Assistance in completing the registration application may be requested by calling APHIS or CDC at the telephone numbers provided in this section.

§ 121.10 Responsibilities of the responsible official.

(a) The responsible official is responsible for ensuring compliance with the regulations, including:

(1) Developing and implementing a Biosafety and Security Plan in accordance with § 121.12;

(2) Allowing only approved individuals within the entity to have access to any agents or toxins listed in § 121.3 in accordance with § 121.11;

(3) Providing appropriate training in biosafety, containment, and security procedures for all personnel in accordance with § 121.13;

(4) Transferring agents or toxins only to registered individuals or entities in accordance with § 121.14;

(5) Ensuring that all visitors are informed of and follow the entity's security requirements and procedures;

(6) Notifying APHIS or, for overlap agents or toxins, APHIS or CDC, of changes in circumstances in accordance with § 121.7;

(7) Providing timely notice of any theft, loss, or release of a biological agent or toxin in accordance with § 121.17;

(8) Maintaining detailed records of information necessary to give a complete accounting of all of the activities related to agents or toxins listed in § 121.3 in accordance with § 121.15.

(b) In addition to the requirements in paragraph (a) of this section, the responsible official for a diagnostic laboratory or other entities possessing, using, or transferring agents or toxins listed in § 121.3 that are contained in specimens presented for diagnosis must immediately report the identification of such agents or toxins to the Administrator and to other appropriate authorities when required by Federal, State, or local law.[10] During agricultural emergencies or outbreaks, or in endemic areas, the Administrator may require less frequent reporting.

(c) In addition to the requirements in paragraph (a) of this section, the responsible official must ensure that the following experiments are not conducted unless approved by the Administrator, after consultation with experts:

(1) Experiments utilizing recombinant DNA that involve the deliberate transfer of a pathogenic trait or drug resistance trait to biological agents that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.

(2) Experiments involving the deliberate formation of recombinant DNA containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50}<100 ng/kg body weight.

§ 121.11 Restricting access to biological agents and toxins.

(a) An individual may not have access to biological agents or toxins listed in § 121.3 unless approved by APHIS or CDC. APHIS will grant, limit, or deny access of individuals to listed agents or toxins. APHIS or CDC will grant, limit, or deny access of individuals to overlap agents or toxins.

(b) The responsible official is responsible for ensuring that only approved individuals within the entity have access to any agents or toxins listed in § 121.3. The responsible official must request such access for only those individuals who have a legitimate need to handle or use agents or toxins, and who have the appropriate training and skills to handle such agents or toxins.

(c) The responsible official must provide appropriate training in biosafety, containment, and security procedures to all individuals with access to agents and toxins listed in § 121.3.

(d) For each individual identified by the responsible official as having a legitimate need to handle or use agents or toxins, the responsible official must submit that individual's name and identifying information to APHIS and the Attorney General. For overlap agents or toxins, the responsible official must submit this information to either APHIS or CDC and the Attorney General.

(e) In addition, the responsible official must submit information about the individual's training and skills to APHIS or, for overlap agents or toxins, APHIS or CDC (*e.g.*, curriculum vitae

[10] A diagnostic laboratory or other entity must immediately notify APHIS by faxing (301) 734–3652.

for principal investigators and researchers, and a description of training completed by support personnel).

(f) APHIS may expedite the access approval process for individuals upon request by the responsible official and a showing of good cause (*e.g.*, public health or agricultural emergencies, national security, impending expiration of a research grant, a short-term visit by a prominent researcher).

(g) APHIS will notify the responsible official if an individual is granted full or limited access, or denied access to listed agents or toxins. APHIS will also notify the individual if he/she is denied access or granted only limited access. For overlap agents or toxins, APHIS or CDC will provide the necessary notification.

(h) APHIS may deny or limit access of an individual to listed agents or toxins if:

(1) The Attorney General identifies the individual as within any of the categories described in 18 U.S.C. 175b;

(2) The Attorney General identifies the individual as reasonably suspected by any Federal law enforcement or intelligence agency of committing a crime set forth in 18 U.S.C. 2332b(g)(5); knowing involvement with an organization that engages in domestic or international terrorism (as defined in 18 U.S.C. 2331) or with any other organization that engages in intentional crimes of violence; or being an agent of a foreign power as defined in 50 U.S.C. 1801;

(3) The individual does not have a legitimate need to handle listed agents or toxins;

(4) The individual does not have the necessary training or skills to handle listed agents or toxins;

(5) The Administrator determines that such action is necessary to protect animal health or animal products.

(i) For overlap agents or toxins, APHIS or CDC will deny an individual access to such agents or toxins if the Attorney General identifies the individual as within any of the categories described in 18 U.S.C. 175b. APHIS or CDC may also deny or limit access of an individual for the reasons set forth in paragraphs (f)(2) through (f)(5) of this section.

(j) An individual may appeal the Administrator's decision to deny or limit access under § 121.17.

(k) Access approval is valid for 5 years; thereafter, the responsible official shall request renewal of access approval every 5 years for as long as the individual needs access to agents or toxins listed in § 121.3.

(l) The responsible official must immediately notify APHIS or, for overlap agents or toxins, APHIS or CDC, when an individual's access to agents or toxins listed in § 121.3 is terminated by the entity and the reasons therefore.

§ 121.12 Biosafety and security plan.

(a) As a condition of registration, the responsible official must develop and implement a Biosafety and Security Plan.[11] The Biosafety and Security Plan must contain sufficient information and documentation to describe the biosafety and containment procedures, and the security systems and procedures. The plan must be commensurate with the risk of the agent or toxin, given its intended use.

(1) *Biosafety and containment procedures.*[12] The biosafety and containment procedures must be sufficient to contain the agent or toxin (*e.g.*, physical structure and features of the entity, and operational and procedural safeguards). At a minimum, the plan must address containment, personnel safety and health, and inventory control.

(2) *Security systems and procedures.*[13] The security systems and procedures

[11] Technical assistance and guidance may be obtained by calling (301) 734–3277.

[12] For guidance on biosafety and containment procedures, see the CDC/NIH publication, ''Biosafety in Microbiological and Biomedical Laboratories'' (4th ed. 1999).

[13] For guidance, see the USDA Departmental Manual No. 9610–001, ''USDA Security Policies and Procedures for Biosafety Level-3 Facilities'' (August 30, 2002). The manual may be obtained by calling (301) 734–3277. The manual is also available on the Internet at *http://www.usda.gov/ocio/directives/DM/DM9610-001.htm.* See also Appendix F, ''Biosafety in Microbiological and Biomedical Laboratories,'' in Morbidity and Mortality Weekly Report (2002). This document may be obtained by writing to Select Agent Program, Centers for Disease Control and Prevention,

Continued

must be designed according to a site-specific risk assessment and must provide graded protection in accordance with the threat posed by the agent or toxin.

(i) The site-specific risk assessment should involve a threat assessment and risk analysis in which threats are defined, vulnerabilities examined, and risks associated with those vulnerabilities are identified.

(ii) The security systems and procedures must be tailored to address site-specific characteristics and requirements, ongoing programs, and operational needs and must mitigate the risks identified under paragraph (a)(2)(i) of this section.

(iii) The plan must describe inventory control procedures, personnel suitability for those individuals with access to agents or toxins listed in §121.3, physical security, and cybersecurity. The plan must also contain provisions for routine cleaning, maintenance, and repairs; provisions for securing the area (*e.g.*, card access, key pads, locks) and protocols for changing access numbers or locks following staff changes; procedures for loss or compromise of keys, passwords, combinations, etc.; procedures for reporting suspicious persons or activities, loss or theft of listed agents or toxins, release of listed agents or toxins, or alteration of inventory records; provisions for the control of access to containers where listed agents and toxins are stored; and procedures for reporting and removing unauthorized persons.

(iv) With respect to areas containing listed agents or toxins, an entity or individual must adhere to the following security requirements or implement measures to achieve an equivalent or greater level of security as the provisions below:

(A) Allow unescorted access only to approved individuals who are performing a specifically authorized function during hours required to perform that job;

(B) Allow individuals not approved under §121.11 to conduct routine cleaning, maintenance, repairs, and other non-laboratory functions only when escorted and continually monitored by approved individuals;

(C) Provide for the control of access to containers where listed agents and toxins are stored by requiring that such containers be locked when not in the direct view of an approved individual and by using other monitoring measures, as needed;

(D) Require the inspection of all packages upon entry and exit;

(E) Establish a protocol for intra-entity transfers, including provisions for ensuring that the packaging and movement, is conducted under the supervision of an approved individual;

(F) Require that approved individuals do not share with any other person their unique means of accessing the area or listed agents or toxins; and

(G) Require that approved individuals immediately report any of the following to the responsible official:

(*1*) Any loss or compromise of keys, passwords, combinations, etc.;

(*2*) Any suspicious persons or activities;

(*3*) Any loss or theft of listed agents or toxins;

(*4*) Any release of a listed agent or toxin; and

(*5*) Any sign that inventory and use records for listed agents and toxins have been altered or otherwise compromised.

(3) *Incident response procedures.*[14] The Biosafety and Security Plan must also include incident response plans for containment breach, security breach, inventory violations, non-biological incidents such as workplace violence, and cybersecurity breach. The incident response plans must address personnel safety and health, containment, inventory control, and notification of managers and responders. The incident response plans must also address such events as bomb threats, severe weather (floods, hurricanes, tornadoes), earthquakes, power outages, and other natural disasters or emergencies.

(b) The Biosafety and Security Plan must be reviewed, performance tested,

1600 Clifton Road, NE, Mail Stop E 79, Atlanta, GA 30333. It is also available on the Internet at *http://www.cdc.gov/mmwr.*

[14] The requirements in this paragraph do not supercede or preempt the enforcement of emergency response requirements imposed by other statutes or regulations.

and updated annually. The plan must also be reviewed and revised, as necessary, after any incident.

§ 121.13 Training.

(a) The responsible official must provide appropriate training in biosafety, containment, and security procedures to all individuals with access to agents and toxins listed in § 121.3.

(b) The responsible official must provide information and training to an individual at the time the individual is assigned to work with a listed agent or toxin. The responsible official must provide refresher training annually.

§ 121.14 Transfer of biological agents and toxins.

Biological agents and toxins listed in § 121.3 may only be transferred to individuals or entities registered to possess, use, or transfer that particular agent or toxin. However, the sender of an agent or toxin may be an individual or entity that has a certificate of registration for the agent or toxin, an individual or entity that is exempt from the requirements of this part, or an individual or entity located outside of the United States. Biological agents or toxins may only be transferred under the conditions of this section and must be authorized by APHIS or, for overlap agents or toxins, by APHIS or CDC, prior to the transfer.

(a) *Importation and interstate movement.* In addition to the permit required under part 122 of this subchapter, biological agents or toxins listed in § 121.3 may be imported or moved interstate only with the prior authorization of APHIS or, for overlap agents or toxins, APHIS or CDC. To obtain such authorization, the sender and the responsible official for the recipient must complete and submit APHIS Form 2041 to APHIS or CDC, in accordance with paragraph (c) of this section.

(b) *Intrastate movement.* Biological agents or toxins listed in § 121.3 may be moved intrastate only with the prior authorization of APHIS or, for overlap agents or toxins, APHIS or CDC. To obtain such authorization, the sender and the responsible official for the recipient must complete and submit APHIS Form 2041 to APHIS or CDC, in accordance with paragraph (c) of this section.

(c) *APHIS Form 2041; process and procedures.* (1) Prior to each transfer, the responsible official for the recipient and sender must complete APHIS Form 2041, and the sender must submit the form to APHIS or, for overlap agents or toxins, to APHIS or CDC.[15]

(2) APHIS or CDC will authorize the transfer based on a finding that the recipient has a certificate of registration covering the transfer of the listed agent or toxin.

(3) The responsible official for the recipient must notify the agency authorizing the transfer (either APHIS or CDC) and the sender upon receipt of the agent or toxin by mailing or faxing a completed APHIS Form 2041 to APHIS or CDC within 2 business days.

(4) The responsible official for the recipient must notify APHIS or CDC immediately if the agent or toxin has not been received within 48 hours after the expected delivery or if the package containing the agent or toxin is leaking or has been damaged.

(d) The sender must comply with all applicable laws governing packaging and shipping.

§ 121.15 Records.

(a) The responsible official must maintain complete, up-to-date records of information necessary to give an accounting of all of the activities related to agents or toxins listed in § 121.3. Such records must include the following:

(1) The Biosafety and Security Plan;

(2) A current list of all individuals with access to agents or toxins listed in § 121.3;

(3) Training records for individuals with access to such agents or toxins;

[15] APHIS Form 2041 may be obtained by calling APHIS at (301) 734–3277 or by calling CDC at (404) 498–2265. The form is also available on the Internet at *http://www.aphis.usda.gov/vs/ncie.bta.html* or *http://www.cdc.gov/od/ohs/lrsat.htm.* APHIS Form 2041 may be mailed to National Center for Import and Export, VS, APHIS, 4700 River Road Unit 40, Riverdale, MD 20737–1231; or faxed to (301) 734–3652. For overlap agents and toxins, it may be mailed to the above address or to Select Agent Program, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mail Stop E 79, Atlanta, GA 30333; or faxed to (404) 498–2265.

(4) Accurate and current inventory records (including source and characterization data);

(5) Permits and transfer documents (APHIS Form 2041) issued by APHIS and CDC;

(6) Security records (*e.g.*, transactions from automated access control systems, testing and maintenance of security systems, visitor logs);

(7) Biosafety, containment, and security incident reports.

(b) The responsible official must maintain such records for 3 years.

(c) All records must be produced upon request to APHIS or CDC inspectors, and appropriate Federal, State, or local law enforcement authorities.

§ 121.16 Inspections.

(a) To ensure compliance with the regulations, any APHIS or CDC inspector must be allowed, without previous notification, to enter and inspect the entire premises, all materials and equipment, and all records required to be maintained by this part.

(b) Prior to issuing a certificate of registration to an entity or individual, APHIS or CDC may inspect and evaluate the premises and records to ensure compliance with the regulations and the biosafety, containment, and security requirements.

§ 121.17 Notification in the event of theft, loss, or release of a biological agent or toxin.

(a) The responsible official must orally notify APHIS and appropriate Federal, State, or local law enforcement agencies immediately upon discovery of the theft or loss of agents or toxins listed in §121.3. The oral notification must be followed by a written report (APHIS Form 2043) within 7 days.

(b) The responsible official must orally notify APHIS immediately upon discovery that a release of an agent or toxin has occurred outside of the biocontainment area. The oral notification shall be followed by a written report (APHIS Form 2043) within 7 days. Upon notification and a finding that the release poses a threat to animal or plant health, or animal or plant products, APHIS will notify relevant Federal, State, and local authorities, and the public, if necessary. If the release involves an overlap agent or toxin, APHIS will also notify the Secretary of Health and Human Services.

(c) The responsible official must orally notify APHIS of a theft, loss, or release of an agent or toxin by calling (866) 994–5698. A copy of APHIS Form 2043 may be obtained by writing to National Center for Import and Export, VS, APHIS, 4700 River Road Unit 40, Riverdale, MD 20737–1231; or by calling (301) 734–3277. The form is also available on the Internet at *http://www.aphis.usda.gov/vs/ncie.bta.html.* APHIS Form 2043 may be mailed to the same address or faxed to (301) 734–3652.

§ 121.18 Administrative review.

An individual or entity may appeal a denial or revocation of registration under this part. An individual who has been denied access to listed agents or toxins or who has been granted only limited access to listed agents or toxins under this part may appeal that decision.[16] The appeal must be in writing and submitted to the Administrator within 30 days of the decision. The appeal must state all of the facts and reasons upon which the individual or entity disagrees with the decision. Where the denial or revocation of registration or the denial or limitation of an individual's access approval is based solely upon an identification by the Attorney General, APHIS will forward the request for review to the Attorney General. The Administrator's decision constitutes final agency action.

PART 122—ORGANISMS AND VECTORS

Sec.

122.1 Definitions.
122.2 Permits required.
122.3 Application for permits.
122.4 Suspension or revocation of permits.

AUTHORITY: 7 U.S.C. 8301–8317; 21 U.S.C. 151–158; 7 CFR 2.22, 2.80, and 371.4.

§ 122.1 Definitions.

The following words, when used in the regulations in this part 122, shall be construed, respectively, to mean:

[16] An entity may not appeal the denial or limitation of an individual's access to listed agents or toxins.

(a) *Department.* The U.S. Department of Agriculture.

(b) *Secretary.* ''Secretary'' means the Secretary of Agriculture of the United States, or any officer or employee of the Department to whom authority has heretofore been delegated, or to whom authority may hereafter be delegated, to act in his stead.

(c) *Administrator.* The Administrator, Animal and Plant Health Inspection Service, United States Department of Agriculture, or any person authorized to act for the Administrator.

(d) *Organisms.* All cultures or collections of organisms or their derivatives, which may introduce or disseminate any contagious or infectious disease of animals (including poultry).

(e) *Vectors.* All animals (including poultry) such as mice, pigeons, guinea pigs, rats, ferrets, rabbits, chickens, dogs, and the like, which have been treated or inoculated with organisms, or which are diseased or infected with any contagious, infectious, or communicable disease of animals or poultry or which have been exposed to any such disease.

(f) *Permittee.* A person who resides in the United States or operates a business establishment within the United States, to whom a permit to import or transport organisms or vectors has been issued under the regulations.

(g) *Person.* Any individual, firm, partnership, corporation, company, society, association, or other organized group of any of the foregoing, or any agent, officer, or employee of any thereof.

[31 FR 81, Jan. 5, 1966, as amended at 57 FR 30899, July 13, 1992]

§ 122.2 Permits required.

No organisms or vectors shall be imported into the United States or transported from one State or Territory or the District of Columbia to another State or Territory or the District of Columbia without a permit issued by the Secretary and in compliance with the terms thereof: *Provided,* That no permit shall be required under this section for importation of organisms for which an import permit has been issued pursuant to part 102 of this subchapter or for transportation of organisms produced at establishments licensed under part 102 of this subchapter. As a condition of issuance of permits under this section, the permittee shall agree in writing to observe the safeguards prescribed by the Administrator for public protection with respect to the particular importation or transportation.

(Approved by the Office of Management and Budget under control number 0579–0015)

[28 FR 7896, Aug. 2, 1963. Redesignated at 31 FR 81, Jan. 5, 1966 and amended at 48 FR 57473, Dec. 30, 1983; 57 FR 30899, July 13, 1992; 59 FR 67134, Dec. 29, 1994]

§ 122.3 Application for permits.

The Secretary may issue, at his discretion, a permit as specified in § 122.2 when proper safeguards are set up as provided in § 122.2 to protect the public. Application for such a permit shall be made in advance of shipment, and each permit shall specify the name and address of the consignee, the true name and character of each of the organisms or vectors involved, and the use to which each will be put.

(Approved by the Office of Management and Budget under control number 0579–0015)

[23 FR 10065, Dec. 23, 1958. Redesignated at 31 FR 81, Jan. 5, 1966 and amended at 48 FR 57473, Dec. 30, 1983; 59 FR 67134, Dec. 29, 1994]

§ 122.4 Suspension or revocation of permits.

(a) Any permit for the importation or transportation of organisms or vectors issued under this part may be formally suspended or revoked after opportunity for hearing has been accorded the permittee, as provided in part 123 of this subchapter, if the Secretary finds that the permittee has failed to observe the safeguards and instructions prescribed by the Administrator with respect to the particular importation or transportation or that such importation or transportation for any other reason may result in the introduction or dissemination from a foreign country into the United States, or from one State, Territory or the District of Columbia to another, of the contagion of any contagious, infectious or communicable disease of animals (including poultry).

(b) In cases of wilfulness or where the public health, interest or safety so requires, however, the Secretary may

without hearing informally suspend such a permit upon the grounds set forth in paragraph (a) of this section, pending determination of formal proceedings under part 123 of this subchapter for suspension or revocation of the permit.

[23 FR 10065, Dec. 23, 1958. Redesignated at 31 FR 81, Jan. 5, 1966, and amended at 57 FR 30899, July 13, 1992]

PART 123—RULES OF PRACTICE GOVERNING PROCEEDINGS UNDER THE VIRUS-SERUM-TOXIN ACT

AUTHORITY: 7 U.S.C. 8301–8317; 21 U.S.C. 151–159; 7 CFR 2.22, 2.80, and 371.4.

§ 123.1 Scope and applicability of rules of practice.

The Uniform Rules of Practice for the Department of Agriculture promulgated in subpart H of part 1, subtitle A, title 7, Code of Federal Regulations, are the Rules of Practice applicable to adjudicatory, administrative proceedings under the Virus-Serum-Toxin Act.

[42 FR 10960, Feb. 25, 1977]

PART 124—PATENT TERM RESTORATION

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AUTHORITY: 35 U.S.C. 156; 7 CFR 2.22, 2.80, and 371.4.

SOURCE: 58 FR 11369, Feb. 25, 1993, unless otherwise noted.

Subpart A—General Provisions

§ 124.1 Scope.

(a) This parts sets forth procedures and requirements for APHIS review of applications for the extension of the term of certain patents for veterinary biological products pursuant to 35 U.S.C. 156—Extension of patent term. Responsibilities of APHIS include:

(1) Assisting PTO in determining eligibility for patent term restoration;

(2) Determining the length of a product's regulatory review period;

(3) If petitioned, reviewing and ruling on due diligence challenges to APHIS's regulatory review period determinations; and

(4) Conducting hearings to review initial APHIS findings on due diligence challenges.

(b) The regulations in this part are designed to be used in conjunction with regulations issued by PTO concerning patent term extension which may be found at 37 CFR 1.710 through 1.791.

[58 FR 11369, Feb. 25, 1993, as amended at 64 FR 43045, Aug. 9, 1999]

§ 124.2 Definitions.

Animal and Plant Health Inspection Service (APHIS). The agency in the Department of Agriculture responsible for licensing veterinary biological products under the Virus-Serum-Toxin Act.

Applicant. Any person who submits an application or an amendment or supplement to an application under 35 U.S.C. 156 seeking extension of the term of a patent.

Due diligence petition. A petition submitted under § 124.30 of this part.

Informal hearing. A hearing that is not subject to the provisions of 5 U.S.C. 554, 556, and 557 and that is conducted as provided in 21 U.S.C. 321(x).

License applicant. Any person who, in accordance with part 102 of this chapter, submits an application to the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture for a U.S. Veterinary Biological Product License.

Patent. A patent issued by the Patent and Trademark Office of the United States Department of Commerce.

Person. Any individual, firm, partnership, corporation, company, association, educational institution, State or local government agency, or other organized group of any of the foregoing, or any agent, officer, or employee of any thereof.

PTO. The Patent and Trademark Office of the United States Department of Commerce.

[58 FR 11369, Feb. 25, 1993, as amended at 68 FR 6346, Feb. 7, 2003]

Subpart B—Eligibility Assistance

§ 124.10 APHIS liaison with PTO.

Upon receipt of a copy of an application for extension of the term of a veterinary biologic patent from PTO, APHIS will assist PTO in determining whether a patent related to a biological product is eligible for patent term extension by:

(a)(1) Verifying whether the product was subject to a regulatory review period before its commercial marketing or use;

(2) Determining whether the permission for commercial marketing or use of the product after the regulatory review period was the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred, and, if so, whether it was the first permitted commercial marketing or use of the veterinary biological product for administration to a food-producing animal;

(3) Ascertaining whether the patent term restoration application was submitted within 60 days after the product was approved for marketing or use; and

(4) Providing such other information as may be necessary and relevant to PTO's determination of whether a patent related to a product is eligible for patent term restoration.

(b) APHIS will notify PTO of its findings in writing, send a copy of this notification to the applicant, and make a copy available for public inspection in room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays.

Subpart C—Regulatory Review Period

§ 124.20 Patent term extension calculation.

(a) As provided in 37 CFR 1.779 of PTO's regulations, in order to determine a product's regulatory review period, APHIS will review the information in each application to determine the lengths of the following phases of the review period, and will then find their sum:

(1) The number of days in the period beginning on the date authorization to prepare an experimental biological product under the Virus-Serum-Toxin Act became effective and ending on the date an application for a license was initially submitted under the Virus-Serum-Toxin Act; and

(2) The number of days in the period beginning on the date an application for a license was initially submitted for approval under the Virus-Serum-Toxin Act and ending on the date such license was issued.

(b) A license application is ''initially submitted'' on the date it contains sufficient information to allow APHIS to commence review of the application. A product license is issued on the date of the APHIS letter informing the applicant of the issuance. The issuance of a license releases the product for commercial marketing or use.

§ 124.21 Regulatory review period determination.

(a) Not later than 30 days after the receipt of an application from PTO, APHIS shall determine the regulatory review period. Once the regulatory review period for a product has been determined, APHIS will notify PTO in writing of the determination, send a copy of the determination to the applicant, and make a copy available for public inspection in room 1141, South

Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays.

(b) APHIS will also publish a notice of the regulatory review period determination in the FEDERAL REGISTER. The notice will include the following:

(1) The name of the applicant;

(2) The trade name and true name of the product;

(3) The number of the patent for which an extension of the term is sought;

(4) The approved indications or uses for the product;

(5) The regulatory review period determination, including a statement of the length of each phase of the review period and the dates used in calculating each phase.

§ 124.22 Revision of regulatory review period determination.

(a) Any interested person may request a revision of the regulatory review period determination within the 30 day period beginning on its publication in the FEDERAL REGISTER. The request must be sent to the Director, Center for Veterinary Biologics, Licensing and Policy Development, 510 South 17th Street, Suite 104, Ames, IA 50010—8197. The request must specify the following:

(1) The identity of the product;

(2) The identity of the applicant for patent term restoration;

(3) The docket number of the FEDERAL REGISTER notice announcing the regulatory review period determination; and

(4) The basis for the request for revision, including any documentary evidence.

(b) If APHIS decides to revise its prior determination, APHIS will notify PTO of the decision, and will send a copy of notification to the applicant and the person requesting the revision (if different from the applicant) with a request for comments within 10 days of notification. If no comment on the proposed revision is received, APHIS will publish the revision in the FEDERAL REGISTER, and include a statement giving the reasons for the revision. If comment is received, APHIS will make a final determination regarding the revision based on such comment and will then publish the revision in the FEDERAL REGISTER, giving reasons for its determination.

[59 FR 11369, Feb. 25, 1993, as amended at 59 FR 67617, Dec. 30, 1994; 64 FR 43045, Aug. 9, 1999]

§ 124.23 Final action on regulatory review period determination.

APHIS will consider its regulatory review period determination to be final upon expiration of the 180-day period for filing a due diligence petition under § 124.30 unless it receives:

(a) New information from PTO records, or APHIS records, that affects the regulatory review period determination;

(b) A request under § 124.22 for revision of the regulatory review period determination;

(c) A due diligence petition filed under § 124.30; or

(d) A request for a hearing filed under § 124.40.

[58 FR 11369, Feb. 25, 1993; 58 FR 29028, May 18, 1993]

Subpart D—Due Diligence Petitions

§ 124.30 Filing, format, and content of petitions.

(a) Any interested person may file a petition with APHIS, no later than 180 days after the publication of a regulatory review period determination under § 124.21, alleging that a license applicant did not act with due diligence in seeking APHIS approval of the product during the regulatory review period.

(b) The petition must be filed with APHIS under the docket number of the FEDERAL REGISTER notice of the agency's regulatory review period determination. The petition must contain any additional information required by this subpart.

(c) The petition must allege that the applicant failed to act with due diligence sometime during the regulatory review period and must set forth sufficient facts to merit an investigation by APHIS of whether the applicant acted with due diligence.

(d) The petition must contain a certification that the petitioner has served a true and complete copy of the petition on interested parties by certified or registered mail (return receipt requested) or by personal delivery.

§ 124.31 Applicant response to petition.

(a) The applicant may file with APHIS a written response to the petition no later than 20 days after the applicant's receipt of a copy of the petition.

(b) The applicant's response may present additional facts and circumstances to address the assertions in the petition, but shall be limited to the issue of whether the applicant acted with due diligence during the regulatory review period. The applicant's response may include documents that were not in the original patent term extension application.

(c) If the applicant does not respond to the petition, APHIS will decide the matter on the basis of the information submitted in the patent term restoration application, the due diligence petition, and APHIS records.

§ 124.32 APHIS action on petition.

(a) Within 90 days after APHIS receives a petition filed under § 124.30, the Under Secretary for Marketing and Regulatory Programs shall make a determination under paragraphs (b) or (c) of this section or under § 124.33 whether the applicant acted with due diligence during the regulatory review period. APHIS will publish its determination in the FEDERAL REGISTER together with factual and legal basis for the determination, notify PTO of the determination in writing, and send copies of the determination to PTO, the applicant, and the petitioner.

(b) APHIS may deny a due diligence petition without considering the merits of the petition if:

(1) The petition is not filed in accordance with § 124.30;

(2) The petition does not contain information or allegations upon which APHIS may reasonably determine that the applicant did not act with due diligence during the applicable regulatory review period; or

(3) The petition fails to allege a sufficient total amount of time during which the applicant did not exercise due diligence so that, even if the petition were granted, the petition would not affect the maximum patent term extension which the applicant is entitled to under 35 U.S.C. 156.

[59 FR 11369, Feb. 25, 1993, as amended at 64 FR 43045, Aug. 9, 1999]

§ 124.33 Standard of due diligence.

(a) In determining the due diligence of an applicant, APHIS will examine the facts and circumstances of the applicant's actions during the regulatory review period to determine whether the applicant exhibited the degree of attention, continuous directed effort, and timeliness as may reasonably be expected from, and are ordinarily exercised by, a person during a regulatory review period. APHIS will take into consideration all relevant factors, such as the amount of time between the approval of an experimental use permit and licensure of the veterinary biological product.

(b) For purposes of this Part, the actions of the marketing applicant shall be imputed to the applicant for patent term restoration. The actions of an agent, attorney, contractor, employee, licensee, or predecessor in interest of the marketing applicant shall be imputed to the applicant for patent term restoration.

Subpart E—Due Diligence Hearing

§ 124.40 Request for hearing.

(a) Any interested person may request, within 60 days beginning on the date of publication of a due diligence determination by APHIS in accordance with § 124.32, that APHIS conduct an informal hearing on the due diligence determination.

(b) The request for a hearing must:

(1) Be in writing;

(2) Contain the docket number of the FEDERAL REGISTER notice of APHIS's regulatory review period determination;

(3) Be delivered to the Director, Center for Veterinary Biologics, Licensing and Policy Development, 510 South 17th Street, Suite 104, Ames, IA 50010—8197.

(4) Contain a full statement of facts upon which the request for hearing is based;

(5) Contain the name, the address, and the principal place of business of the person requesting the hearing; and

(6) Contain a certification that the person requesting the hearing has served a true and complete copy of the request upon the petitioner of the due diligence determination and the applicant for patent term extension by certified or registered mail (return receipt requested) or by personal service.

(c) The request must state whether the requesting party seeks a hearing not later than 30 days after the date APHIS receives the request, or, at the request of the person making the request, not later than 60 days after such date.

[58 FR 11369, Feb. 25, 1993, as amended at 59 FR 67617, Dec. 30, 1994; 64 FR 43045, Aug. 9, 1999]

§ 124.41 Notice of hearing.

No later than ten days before the hearing, APHIS will notify the requesting party, the applicant, the petitioner, and any other interested person of the date, time, and location of the hearing.

§ 124.42 Hearing procedure.

(a) The presiding officer shall be appointed by the Administrator of APHIS from officers and employees of the Department who have not participated in any action of the Secretary which is the subject of the hearing and who are not directly responsible to an officer or employee of the Department who has participated in any such action.

(b) Each party to the hearing shall have the right at all times to be advised and accompanied by an attorney.

(c) Before the hearing, each party to the hearing shall be given reasonable notice of the matters to be considered at the hearing, including a comprehensive statement of the basis for the action taken or proposed by the Secretary which is the subject of the hearing and any general summary of the information which will be presented at the hearing in support of such action.

(d) At the hearing the parties to the hearing shall have the right to hear a full and complete statement of the action which is the subject of the hearing together with the information and reasons supporting such action, to conduct reasonable questioning, and to present any oral and written information relevant to such action.

(e) The presiding officer in such hearing shall prepare a written report of the hearing to which shall be attached all written material presented at the hearing. The participants in the hearing shall be given the opportunity to review and correct or supplement the presiding officer's report of the hearing.

(f) The Secretary may require the hearing to be transcribed. A party to the hearing shall have the right to have the hearing transcribed at his expense. Any transcription of a hearing shall be included in the presiding officer's report of the hearing.

(g) The due diligence hearing will be conducted in accordance with rules of practice adopted for the proceeding. APHIS will provide the requesting party, the applicant, and the petitioner with an opportunity to participate as a party in the hearing. The standard of due diligence set forth in §124.33 will apply at the hearing. The party requesting the due diligence hearing will have the burden of proof at the hearing.

§ 124.43 Administrative decision.

Within 30 days after completion of the due diligence hearing, the Under Secretary for Marketing and Regulatory Programs, taking into consideration the recommendation of the Administrator, will affirm or revise the determination made under §124.32. APHIS will publish the due diligence redetermination in the FEDERAL REGISTER, notify PTO of the redetermination, and send copies of the notice to PTO and the requesting party, the applicant, and the petitioner.

[59 FR 11369, Feb. 25, 1993, as amended at 64 FR 43045, Aug. 9, 1999]