

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 shall 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. The following information must be submitted to the Administrator by the establishment seeking authorization (in c/o the Director, Center for Veterinary Biologics, Inspection and Compliance, 510 South 17th Street, Suite 104, Ames, IA 50010-8197) to request authorization to prepare a product for use in herds adjacent to the herd of origin. (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) Name 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 pre-

pare 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) Summaries of test results shall be submitted to APHIS in accordance with §116.7 within 4 days after the completion of required testing.

(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 require-

ments 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

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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]

§ 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 se-

rial 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.