1. Probable public support and methods of its determination.
2. Practicability of service operations.
3. Interference encountered.
4. Pertinent information relative to merits of the proposed service.
5. Propagation characteristics of frequencies used, particularly with respect to the service objective.
6. Frequencies, if any, believed to be more suitable and reasons therefore.
7. Type of signals or communications employed in the experimental work.

c. Normally, developmental reports will be made a part of the Commission's public records. However, an applicant may request that the Commission withhold from the public certain reports and associated material relative to the accomplishments achieved under developmental authorization, and, if it appears that such information should be withheld, the Commission will so direct.

[FR Doc.74-10598 Filed 5-9-74;8:45 am]

Title 5—Administrative Personnel

CHAPTER I—CIVIL SERVICE COMMISSION

PART 213—EXCEPTED SERVICE

Department of Housing and Urban Development

Section 213.3384 is amended to show that two positions of Special Assistant to the Under Secretary are excepted under Schedule C.

Effective on May 10, 1974, § 213.3384 (a) (9) is added as set out below.

§ 213.3384 Department of Housing and Urban Development.

(a) *Office of the Secretary.* * * *

(9) Two Special Assistants to the Under Secretary.

* * * * *

((5 U.S.C. secs. 3301, 3302); E.O. 10577, 3 CFR 1954-58 Comp. p. 218)

UNITED STATES CIVIL SERVICE COMMISSION,
[SEAL] JAMES C. SPRY,
Executive Assistant to the Commissioners.

[FR Doc.74-10870 Filed 5-9-74;8:45 am]

PART 213—EXCEPTED SERVICE

Executive Office of the President; Correction

In the FEDERAL REGISTER of May 3, 1974 (FR Doc. 74-10212) on page 15383 § 213.3303(k) (2) was added, it should be § 213.3303(k) (3) as set out below.

§ 213.3303 Executive Office of the President.

* * * * *

(k) *Federal Energy Office.* * * *

(3) One Secretary to the Deputy General Counsel.

((5 U.S.C. secs. 3301, 3302); E.O. 10577, 3 CFR 1954-58 Comp., p. 218)

UNITED STATES CIVIL SERVICE COMMISSION,
[SEAL] JAMES C. SPRY,
Executive Assistant, to the Commissioners.

[FR Doc.74-10871 Filed 5-9-74;8:45 am]

PART 733—POLITICAL ACTIVITY OF FEDERAL EMPLOYEES

Residence of Howard County, Maryland

Section 733.124 is amended to include political activity privileges to employee residents of Howard County, Maryland.

Section 733.124(b) is amended by adding "Howard County (April 25, 1974)" between "Greenbelt (Oct. 4, 1940)" and "Hayattsville (Sept. 20, 1940)" under the heading "In Maryland".

((5 U.S.C. 1308, 3301, 3302, 7301, 7324, 7325, 7327; 42 U.S.C. 2729); E.O. 10577, 3 CFR 1954-58 Comp. p. 218)

UNITED STATES CIVIL SERVICE COMMISSION
[SEAL] JAMES C. SPRY,
Executive Assistant to the Commissioners.

[FR Doc.74-10869 Filed 5-9-74;8:45 am]

Title 7—Agriculture

CHAPTER II—FOOD AND NUTRITION SERVICE, DEPARTMENT OF AGRICULTURE

[Amdt. 20]

PART 220—SCHOOL BREAKFAST AND NONFOOD ASSISTANCE PROGRAMS AND STATE ADMINISTRATIVE EXPENSES

Apportionment of Funds to States

The purpose of this amendment to the regulations governing the School Breakfast and Nonfood Assistance Programs and State Administrative Expenses is to provide the authority for State educational agencies and FNSROs to approve applications for nonfood assistance to be financed over a period of three consecutive fiscal years. This authority is also applicable with respect to reserve funds withheld to assist schools without food service. The authority may be appropriately utilized in those situations where a school or school district makes application and is approved to purchase extensive equipment in an amount which exceeds the funds available therefor in the hands of the State or FNSRO for the fiscal year in which the school's application is made. Likewise, the school or school district which acquires partial and/or inadequate equipment with reserve funds available for one fiscal year may still be considered as a school(s) without food service and qualify for additional monies from the no-food-service reserve in the next two fiscal years. However, in either situation, the obligation of funds for three fiscal years is contingent upon future availability of appropriated monies.

Since this amendment affects internal management of the program funds and does not adversely affect any participating school(s), notice and public participation procedure is impracticable and unnecessary.

Accordingly, in 7 CFR Chapter II, Part 220 is amended by adding a new paragraph to § 220.12, reading as follows:

§ 220.12 Apportionment of funds to States.

* * * * *

(d) If, in any fiscal year, the amount of funds made available under paragraph (a) or paragraph (a-1) of this section to any State agency, or FNSRO where applicable, is insufficient to pay the entire amount of funds requested and justified in the application of a School Food Authority, the State agency, or FNSRO where applicable, may approve the entire amount justified on condition that such amount will be paid to the School Food Authority, to the extent that sufficient funds are available therefor, over a period not to exceed three consecutive fiscal years beginning with the fiscal year in which the application was approved. If the application was for equipment for a school which, at the time of the application, was without a food service, the State agency, or FNSRO where applicable, may use funds available under paragraph (a-1) during the three year period to pay the entire amount of the funds approved.

(Catalog of Federal Domestic Assistance Program No. 10.553 National Archives Reference Services)

Effective date: This amendment will become effective on July 1, 1974.

Dated: May 8, 1974.

RICHARD L. FELTNER,
Assistant Secretary.

[FR Doc.74-10902 Filed 5-9-74;8:45 am]

CHAPTER VII—AGRICULTURAL STABILIZATION AND CONSERVATION SERVICE (AGRICULTURAL ADJUSTMENT), DEPARTMENT OF AGRICULTURE

SUBCHAPTER C—SPECIAL PROGRAMS

[Amdt. 8]

PART 780—APPEAL REGULATIONS

Requests for Reconsideration and Appeals Requiring Special Handling

Section 780.11 of the appeal regulations, 7 CFR Part 780, is amended by revising paragraph (a) to read as follows:

§ 780.11 Requests for reconsideration and appeals requiring special handling.

(a) Determinations made by a State committee with respect to (1) the establishment of farm yields for wheat, feed grain, and cotton, (2) the establishment of wheat allotments, (3) the establishment of farm feed grain allotments, (4) the establishment of upland cotton base acreage allotments, (5) the establishment of conserving bases, (6) matters rising under the tobacco discount variety program, and (7) eligibility provisions of the livestock feed program are not appealable to the Deputy Administrator.

Effective date. The changes in the foregoing amendment of Part 780 relate to its applicability to the 1974-77 programs for feed grains, wheat, and upland cotton. Farmers are completing their plans for the 1974 crop year, and it is essential that the foregoing amendment of Part 780 be made effective as soon as possible. It is hereby found and determined that compliance with the notice and public procedure provisions of 5 U.S.C. 553 is impracticable and contrary to the public interest. Accordingly, this

amendment shall become effective May 10, 1974.

Signed at Washington, D.C., May 3, 1974.

GLENN A. WEIR,
Acting Administrator.

[FR Doc.74-10828 Filed 5-9-74;8:45 am]

CHAPTER IX—AGRICULTURAL MARKETING SERVICE (MARKETING AGREEMENTS AND ORDERS; FRUITS, VEGETABLES, NUTS), DEPARTMENT OF AGRICULTURE

[Lemon Reg. 638]

PART 910—LEMONS GROWN IN CALIFORNIA AND ARIZONA

Limitation of Handling

This regulation fixes the quantity of California-Arizona lemons that may be shipped to fresh market during the weekly regulation period May 12–18, 1974. It is issued pursuant to the Agricultural Marketing Agreement Act of 1937, as amended, and Marketing Order No. 910. The quantity of lemons so fixed was arrived at after consideration of the total available supply of lemons, the quantity of lemons currently available for market, the fresh market demand for lemons, lemon prices, and the relationship of season average returns to the parity price for lemons.

§ 910.938 **Lemon Regulation 638.**

(a) *Findings.* (1) Pursuant to the marketing agreement, as amended, and Order No. 910, as amended (7 CFR Part 910), regulating the handling of lemons grown in California and Arizona, effective under the applicable provisions of the Agricultural Marketing Agreement Act of 1937, as amended (7 U.S.C. 601–674), and upon the basis of the recommendations and information submitted by the Lemon Administrative Committee, established under the said amended marketing agreement and order, and upon other available information, it is hereby found that the limitation of handling of such lemons, as hereinafter provided, will tend to effectuate the declared policy of the act.

(2) The need for this regulation to limit the quantity of lemons that may be marketed during the ensuing week stems from the production and marketing situation confronting the lemon industry.

(i) The committee has submitted its recommendation with respect to the quantity of lemons it deems advisable to be handled during the ensuing week. Such recommendation resulted from consideration of the factors enumerated in the order. The committee further reports the demand for lemons is about unchanged.

Average f.o.b. price was $5.87 per carton the week ended May 4, 1974 compared to $5.73 per carton the previous week.

Track and rolling supplies at 140 cars were down 6 cars from last week.

(ii) Having considered the recommendation and information submitted by the committee, and other available information, the Secretary finds that the quantity of lemons which may be handled should be fixed as hereinafter set forth.

(3) It is hereby further found that it is impracticable and contrary to the public interest to give preliminary notice, engage in public rule-making procedure, and postpone the effective date of this regulation until 30 days after publication hereof in the FEDERAL REGISTER (5 U.S.C. 553) because the time intervening between the date when information upon which this regulation is based became available and the time when this regulation must become effective in order to effectuate the declared policy of the act is insufficient, and a reasonable time is permitted, under the circumstances, for preparation for such effective time; and good cause exists for making the provisions hereof effective as hereinafter set forth. The committee held an open meeting during the current week, after giving due notice thereof, to consider supply and market conditions for lemons and the need for regulation; interested persons were afforded an opportunity to submit information and views at this meeting; the recommendation and supporting information for regulation during the period specified herein were promptly submitted to the Department after such meeting was held; the provisions of this regulation, including its effective time, are identical with the aforesaid recommendation of the committee, and information concerning such provisions and effective time has been disseminated among handlers of such lemons; it is necessary, in order to effectuate the declared policy of the act, to make this regulation effective during the period herein specified; and compliance with this regulation will not require any special preparation on the part of persons subject hereto which cannot be completed on or before the effective date hereof. Such committee meeting was held on May 7, 1974.

(b) *Order.* (1) The quantity of lemons grown in California and Arizona which may be handled during the period May 12, 1974, through May 18, 1974, is hereby fixed at 265,000 cartons.

(2) As used in this section, "handled", and "carton(s)" have the same meaning as when used in the said amended marketing agreement and order.

(Secs. 1–19, 48 Stat. 31, as amended (7 U.S.C. 601–674))

Dated: May 8, 1974.

CHARLES R. BRADER,
Acting Director, Fruit and Vegetable Division, Agricultural Marketing Service.

[FR Doc.74-11043 Filed 5-9-74;11:47 am]

Title 9—Animals and Animal Products

CHAPTER I—ANIMAL AND PLANT HEALTH INSPECTION SERVICE, DEPARTMENT OF AGRICULTURE

SUBCHAPTER C—INTERSTATE TRANSPORTATION OF ANIMALS (INCLUDING POULTRY) AND ANIMAL PRODUCTS; EXTRAORDINARY EMERGENCY REGULATION OF INTRASTATE ACTIVITIES

PART 76—HOG CHOLERA AND OTHER COMMUNICABLE SWINE DISEASES

Hog Cholera Eradication and Free States

This amendment deletes the State of Texas from the list of Hog Cholera Eradication States in 9 CFR 76.2(f), as amended, and adds said State to the list of Hog Cholera Free States in § 76.2(g) upon the basis of a determination that such State qualifies for hog cholera free status under § 76.2(g). The special provisions in 9 CFR Part 76, as amended, pertaining to the interstate movement of swine and swine products from such Eradication or Free States remain applicable to Texas.

The removal of the State of Texas from the list of Hog Cholera Eradication States and the addition of this State to the list of Hog Cholera Free States affects the Federal indemnities payable under other regulations (9 CFR Part 56, as amended) for swine slaughtered because of hog cholera in the State of Texas.

Accordingly, Part 76, Title 9, Code of Federal Regulations, as amended, restricting the interstate movement of swine and certain products because of hog cholera and other communicable swine diseases, is hereby amended in the following respects:

In § 76.2, the reference to the State of Texas in paragraph (f) is deleted, and paragraph (g) is amended by adding thereto the name of said State.

(Secs. 4–7, 23 Stat. 32, as amended; secs. 1 and 2, 32 Stat. 791–792, as amended; secs. 1–4, 3[illegible] Stat. 1264, 1265, as amended; sec. 1, 75 Stat. 481; secs. 3 and 11, 76 Stat. 130, 132 (21 U.S.C. 111–113, 114g, 115, 117, 120, 121, 123–126, 134b, 134f); 37 FR 28464, 28477; 38 FR 19141.)

Effective date. The foregoing amendment shall become effective May 10, 1974.

The amendment does not change the requirements under the regulations in 9 CFR Part 76 with respect to the interstate movement of swine or swine products. It has the effect of relieving restrictions on indemnity payments under the regulations in 9 CFR Part 56 and should be made effective promptly in order to be of maximum benefit to affected persons. It does not appear that public participation in this rulemaking proceeding would make additional relevant information available to the Department.

Accordingly, under the administrative procedure provisions in 5 U.S.C. 553, it is found upon good cause that notice and other public procedure with respect to

the amendment are impracticable, unnecessary, and contrary to the public interest, and good cause is found for making it effective less than 30 days after publication in the FEDERAL REGISTER.

Done at Washington, D.C., this 7th day of May 1974.

PIERRE A. CHALOUX,
Acting Deputy Administrator, Veterinary Services, Animal and Plant Health Inspection Service

[FR Doc.74-10881 Filed 5-9-74;8:45 am]

SUBCHAPTER D—EXPORTATION AND IMPORTATION OF ANIMALS (INCLUDING POULTRY) AND ANIMAL PRODUCTS

PART 92—IMPORTATION OF CERTAIN ANIMALS AND POULTRY AND CERTAIN ANIMAL AND POULTRY PRODUCTS; INSPECTION AND OTHER REQUIREMENTS FOR CERTAIN MEANS OF CONVEYANCE AND SHIPPING CONTAINERS THEREON

Importation of Slaughter Animals; Clarification

Statement of considerations: The purpose of this amendment is to clarify and bring up to date with present practice the requirements contained in 9 CFR Part 92, to provide that all livestock imported for slaughter go direct to a recognized slaughtering establishment operating under Federal or State inspection and approved by Veterinary Services to receive such animals for slaughter.

Accordingly, 9 CFR Part 92, is hereby amended in the following respects:

1. In § 92.1 paragraphs (m) and (n) are amended to read:

§ 92.1 Definitions.

* * * * *

(m) *Recognized slaughtering establishment.*[10] An establishment where slaughtering operations are regularly carried on under Federal or State inspection and which has been approved by Veterinary Services to receive animals for slaughter under this Part.

(n) *Immediate slaughter.* Consignment directly from the port of entry to a recognized slaughtering establishment [10] and slaughter thereat within two weeks from the date of entry.

* * * * *

§§ 93.23, 92.28, 92.40 [Amended]

2. In §§ 92.23, 92.28(c), and 92.40 in the second sentence the term "recognized slaughtering center" is deleted and the term "recognized slaughtering establishment" [10] is substituted therefor.

3. In § 92.40 the first sentence is amended by inserting after the reference to "§ 92.33" the word "and" and by deleting the phrase "and 92.39(a)."

(Sec. 2, 32 Stat. 792, as amended; secs. 2, 3, 4, and 11, 76 Stat. 129, 130, 132 (21 U.S.C. 111, 134a, 134b, 134c, 134f); 37 FR 28464, 28477; 38 FR 19141)

[10] The name of recognized slaughtering establishments approved under this Part may be obtained from the Area Veterinarian in Charge, Veterinary Services, for the State of destination of the shipment.

Effective date. The foregoing amendment shall become effective May 10, 1974.

This amendment clarifies and brings up to date with present practice the requirements for importation of slaughter animals under the regulations of this Part 92 and does not impose or relieve any restrictions. The amendment should be made effective promptly to be of maximum benefit to persons affected. It does not appear that public participation in this rulemaking proceeding would make additional relevant information available to the Department.

Accordingly, under the administrative procedure provisions in 5 U.S.C. 553, it is found upon good cause that notice and other public procedure with respect to the amendment are impracticable and unnecessary and good cause is found for making it effective less than 30 days after publication in the FEDERAL REGISTER.

Done at Washington, D.C., this 6th day of May 1974.

J. M. HEJL,
Acting Deputy Administrator, Veterinary Services, Animal and Plant Health Inspection Service.

[FR Doc.74-10826 Filed 5-9-74;8:45 am]

VIRUSES, SERUMS, TOXINS, AND ANALOGOUS PRODUCTS: ORGANISMS AND VECTORS

Miscellaneous Amendments

On September 18, 1973, a notice of proposed amendments to Part 113 was published in the FEDERAL REGISTER, Volume 38, Number 180, page 26118.

On December 18, 1973, a notice of proposed amendments to Part 108, Part 114, and Part 116 was published in the FEDERAL REGISTER, Volume 38, Number 242, page 34736.

On January 25, 1974, a notice of proposed amendments to Part 112 was published in the FEDERAL REGISTER, Volume 39, Number 18, page 3275.

The basic requirements included in these amendments have been in effect for several years and have been utilized by Veterinary Services and by industry during that time. These amendments would codify in the regulations such requirements as have been issued in administrative memorandums. The publication of these amendments is done to make more readily available to the general public the nature of these requirements.

These amendments to Part 112 were proposed to update the current label requirements for biological products. They include requirements found essential for proper labeling. They also include authorization for the issuance of export certificates to licensees interested in foreign commerce. A proposed new label requirement in the form of a caution statement for bacterins authorized for use as diluents was published in § 113.85(d)(2). This requirement has been redesignated as § 112.7(h).

These amendments codify in Part 113 some test methods, procedures, and criteria established by Veterinary Services for evaluating biological products to be pure, safe, potent, and efficacious and not to be worthless, contaminated, dangerous, or harmful. All products are required to meet the applicable requirements before marketing release is authorized. Regulations proposed as § 114.-12, "Biological products prepared from animal blood" have been combined with the regulations in § 113.75 under the caption of "General requirements for biological products of animal blood origin."

These amendments contain a complete revision of Part 114. The regulations in this part pertain to the production of biological products in a licensed establishment. They include guidelines for preparing an Outline of Production in addition to the requirements to be met. Among the items added are personnel requirements. The designation of an official representative of each licensee to act as contact with Veterinary Services is required. "Gentamicin" is added to the list of permitted antibiotics.

These amendments expand the regulations in Part 116 by codifying detailed recordkeeping requirements now published in administrative memorandums.

After due consideration of all revelant matters, including the proposals set forth in the aforesaid notices of rulemaking, and the comments and views submitted by interested persons, and pursuant to the authority contained in the Virus-Serum-Toxin Act of March 4, 1913 (U.S.C. 151–158), the amendments of Parts 108, 112, 113, 114, and 116 of Subchapter E, Chapter 1, Title 9 of the Code of Federal Regulations, as contained in the aforesaid notices are hereby adopted and are set forth herein, subject to the following noted modifications:

1. Section 108.2 has been reworded by deleting "and shipment" and substituting "a brief description" for "detailed explanation" as being more desirable. Section 108.5 lead paragraph has been changed to "A brief description" also.

2. Section 108.6 lead paragraph has been reworded to permit submission of preliminary drawings for comment. Section 108.6(a) has been reworded for clarity.

3. Section 108.8 (a) and (b) have been reworded for clarity.

4. Section 108.9(b) has been reworded for clarity.

5. Section 108.10(a) spelling of "establishment" is corrected.

6. Section 112.2(a)(11) is changed to limit the requirement to cartons containing more than one final container.

7. Section 113.85(d)(2) has been reworded for clarity and redesignated as a new paragraph 112.7(h) as being more appropriately codified.

8. Section 112.7(d)(5) has been reworded for clarity.

9. The proposed mouse test in § 113.39 was revised to permit subcutaneous injection as an alternate to intraperitoneal injection, to limit its use to liquid products and to redesignate it as § 113.33 (b). Where § 113.39 appears, § 113.33(b) is substituted. Section 113.39 is reserved.

10. The proposed guinea pig test in § 113.38 is revised to permit subcutaneous injection.

11. Section 113.65 is revised to establish Veterinary Services as the source of seeds for production of tuberculin; to permit the testing of bulk samples; and minor changes for clarification.

12. Section 113.66 and § 113.67 are revised for clarity and correctness.

13. "Salmonella" is spelled out in § 113.66 and "serum" corrected.

14. The general requirements for products of blood origin proposed in § 113.75 are combined with the requirements for preparing such products proposed as § 114.12.

15. Section 113.76 is revised for clarity, specificity, and accuracy. Bulk testing has been added as an alternate. "Twenty-25° C" is substituted for "room temperature."

16. Section 113.77 has been changed to permit a tolerance of two controls and observation period reduced to 10 days.

17. Section 113.78 has been changed for clarification and correctness.

18. Section 113.79 and § 113.80 has been changed to permit the use of animals other than horses and to correct minor errors.

19. Section 113.85 has been revised by rewording the definitions for bacterin, toxoid, and bacterin-toxoid as being more meaningful and complete. Reference to previous publications has been deleted and label requirements for bacterins authorized for use as diluents moved to § 112.7(h). Redesignation of paragraphs made and text corrected where indicated for clarification and accuracy.

20. The lead paragraphs of §§ 113.86, 113.87, 113.88, 113.91, 113.92, 113.93, 113.94, 113.95, 113.96, 113.97, 113.98, 113.99, 113.100, 113.101, and 113.104 which pertain to products subject to requirements in § 113.85 have been rewritten to include a reference to § 113.85 for completeness and clarification. Also, where the phrase "has been rendered sterile and nontoxic" appeared, the phrase "has been inactivated and is nontoxic" has been substituted as being more meaningful. Minor changes in these sections have been made for clarification and accuracy of the test procedure prescribed. In addition, the following changes have been made:

21. The potency test in § 113.94 has been deleted in favor of tests written into the filed Outline of Production until further data on the proposed test can be developed. Section 113.98 is reserved. "Room temperature" is changed to "20 to 25° C" in § 113.99.

22. The caption for § 113.100 has been changed to "§ 113.100 *Staphylococcus Aureaus Bacterin-Toxoid*" to limit its application to that product. Minor changes made for accuracy.

23. The captions of § 113.101, § 113.102, and § 113.103 have been changed to include "Multocida (avicida)." "Approved Outline of Production" has been changed in § 113.101 to "filed Outline of Production" for accuracy. Minor changes made for clarification. Age of turkeys allowed in § 113.103 reduced to 6 weeks.

24. Swine protection test in proposed § 113.112 redesignated as § 113.104(e). Reference to its use has been made in § 113.112. Other minor changes made in § 113.104 for clarification.

25. Sections 113.105 to 113.109 are reserved.

26. Minor changes have been made in § 113.110 to clarify the conditions of the tests. The upper limit of viable *Brucella abortus* organisms has been deleted as being unnecessary. Redesignation of paragraphs made as indicated.

27. Minor changes also have been made in § 113.111 for clarity and accuracy.

28. The table of contents for Part 114 is corrected to show the proper caption for §§ 114.5 and 114.12. A printing error is corrected in § 114.5. Section 114.6 is reworded for clarity. Proposed § 114.7(b) is deleted; § 114.7(c) is renumbered § 114.7(b) and "competent" is substituted for "well versed;" § 114.7(d) is renumbered § 114.7(c) and subparagraph § 114.7(c)(2) is reworded for clarity.

29. Section 114.8(e)(1) is reworded for clarity.

30. Paragraph V of the Outline Guide in § 114.9(c) is clarified by deleting the words "by number" and to be consistent with the other Outline Guide presented. Paragraph VI of the Outline Guide in § 114.9(c) is amended to conform with the other Outline Guides presented by adding the volume of fill.

31. Paragraph III B of the Outline Guide in § 114.9(d) is amended by substituting the word "and" for "or" for completeness. Paragraph IV B of the Outline Guide in § 114.9(d) is amended for clarity and completeness.

32. Section 114.10 is amended to permit the use of a penicillin and streptomycin combination.

33. Section 114.11 is changed to include centigrade readings of temperatures. The word "such" is deleted in the last sentence as too restrictive.

34. Material in § 114.12 has been recodified as part of § 113.75. Section 114.12 is reserved.

35. Section 114.13(a)(2) has been reworded for clarity. The lead paragraph in § 114.13(c) has been reworded to provide for antitoxins separately.

36. A new § 114.13(c)(2) has been added and § 114.13(c)(2) renumbered § 114.13(c)(3).

37. Section 114.14(a)(2) and § 114.14(b)(2) have been reworded for clarity.

38. Section 114.18(d) has been reworded to remove the "R" requirement, but substitute a new serial number for the records.

39. Section 116.1(a) has been rewritten to require the "time" where critical.

40. Section 116.8 the word "such" has been deleted and "all" substituted for clarification of disposition records requirements.

1. Part 108—*Sanitation Requirements for Licensed Establishments*—is amended to read:

PART 108—FACILITY REQUIREMENTS FOR LICENSED ESTABLISHMENTS

Sec.

AUTHORITY: 37 Stat. 832–833; 21 U.S.C. 151–158.

§ 108.1 Applicability.

Unless otherwise authorized by the Deputy 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.

§ 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 product code numbers for products prepared in whole or in part in each room. Exceptions may be listed for general purpose areas or rooms. Functions performed in each area and room shall be described.
(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.

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

Preliminary drawings may be submitted to Veterinary Services 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 Veterinary Services 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.

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

Three copies of all plot plans, blueprints, and legends, including revisions, shall be submitted to Veterinary Services 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 Veterinary Services files.

§ 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 Veterinary Services for each licensed establishment.

PART 112—PACKAGING AND LABELING

2. Section 112.2(a)(8) is amended; (a)(11) is added; and (e) is amended to read:

§ 112.2 Final container label, carton label and enclosure.

(a) * * *

(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 Deputy Administrator. Very small final container labels are exempted from this requirement.

* * * * *

(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.

* * * * *

(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 Veterinary Services upon request of the licensee.

3. Section 112.3 is amended by adding a new paragraph (g) to read:

§ 112.3 Diluent labels.

* * * * *

(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).

4. Section 112.7 is amended by adding a lead paragraph; amending paragraphs (d)(4) and (e)(5) and adding paragraphs (g) and (h).

§ 112.7 Special additional requirements.

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

* * * * *

(d) * * *

(4) * * *

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

* * * * *

(5) A statement containing the recommended action to be taken in cases of exposure to the vaccine virus shall be prominently placed on all enclosures and also placed on carton labels for all cartons containing more than one final container of biological product. Satisfactory recommendations may be found in the U.S. Department of Health, Education, and Welfare. Public Health Service, Center for Disease Control Weekly Report, June 24, 1972.

* * * * *

(g) In the case of autogenous bacterins and autogenous vaccines, labels shall include the recommended dose, the number of repeat doses, if any, and the interval between doses: *Provided,* That the label shall not show:

(1) The identity of the herd or flock from which the cultures were isolated; or

(2) The name(s) of the person(s) responsible for making the isolations; or

(3) A recommendation for use of the product in a herd or flock other than the one from which the cultures were isolated.

(h) In the case of bacterins authorized in § 113.85 of this subchapter to be used as diluents in combination packages, the carton labels and enclosures used with serials of bacterin which are either not tested by the viricidal activity test provided in § 113.35 of this subchapter or have been found unsatisfactory by such viricidal activity test shall contain the statement: "CAUTION: DO NOT USE AS DILUENT FOR LIVE VIRUS VACCINES."

5. The table of contents for Part 113—Standard Requirements is amended; § 113.33 is amended by adding a new paragraph (b); §113.38–§ 113.49 are amended; and new sections §§ 113.65–113.112 are added to read:

PART 113—STANDARD REQUIREMENTS

Applicability

Sec.

113.1 Compliance.
113.2 Reserved.
113.3 Sampling of biological products.
113.4 Exemptions to tests.
113.5 General testing.
113.6 Veterinary Services testing.
113.7 Multiple fractions.
113.8 Virus titrations in lieu of test for antigenicity.

Standard Procedures

113.25 Culture Media for detection of bacteria and fungi.
113.26 Detection of viable extraneous bacteria and fungi in all biological products except live vaccines.
113.27 Detection of viable extraneous bacteria and fungi in live vaccines.
113.28 Detection of mycoplasma contamination.
113.29 Determination of moisture content in desiccated biological products.
113.30 Detection of Salmonella contamination.
113.31 Detection of avian lymphoid leukosis.
113.32 Detection of Brucella contamination.
113.33 Mouse safety tests.
113.34 Detection of hemagglutinating viruses.
113.35 Detection of viricidal activity.
113.36 Detection of extraneous pathogens by the chicken inoculation test.
113.37 Detection of extraneous pathogens by the chick embryo inoculation test.
113.38 Guinea pig safety test.
113.39–113.49 Reserved.

Ingredient Requirements

113.50 Ingredients of biological products.
113.51 Requirements for primary cells used in biological products production.
113.52 Requirements for selection of cell lines.
113.53 Requirements for ingredients of animal origin.

Diagnostics and Reagents

113.65 Tuberculin, Intradermic.
113.66 Pullorum Antigen.
113.67 Avian Mycoplasma Antigen.
113.68–113.74 Reserved.

Blood Origin Products

113.75 General requirements for biological products of animal blood origin.
113.76 Tetanus Antitoxin.
113.77 Swine Erysipelas Antiserum.
113.78 Canine Distemper-Hepatitis-Leptospira Antiserum.
113.79 Clostridium Perfringens Type C Antitoxin.
113.80 Clostridium Perfringens Type D Antitoxin.
113.81–113.84 Reserved.

Inactivated Bacterial Products

113.85 Bacterins, toxoids, and bacterin toxoids.
113.86 Leptospira Pomona Bacterin.
113.87 Leptospira Icterohaemorrhagiae Bacterin.
113.88 Leptospira Canicola Bacterin.
113.89–113.90 Reserved.
113.91 Clostridium Chauvoei Bacterin.
113.92 Clostridium Hemolyticum Bacterin.
113.93 Clostridium Novyi Bacterin-Toxoid.
113.94 Clostridium Sordellii Bacterin-Toxoid.
113.95 Clostridium Botulinum Type C Bacterin-Toxoid.
113.96 Clostridium Perfringens Type C Toxoid and Bacterin-Toxoid.
113.97 Clostridium Perfringens Type D Toxoid and Bacterin-Toxoid.
113.98 Reserved.
113.99 Tetanus Toxoid.
113.100 Staphylococcus Aureus Bacterin-Toxoid.
113.101 General requirements for Pasteurella Multocida (avicida) Bacterins.
113.102 Pasteurella Multocida (avicida) Bacterin, Type 1.
113.103 Pasteurella Multocida (avicida) Bacterin, Type 3.
113.104 Erysipelas Bacterin.
113.105–113.109 Reserved.

Live Bacterial Vaccines

113.110 Brucella Abortus Vaccine.
113.111 Anthrax Spore Vaccine-Nonencapsulated.
113.112 Erysipelas Vaccine.

Authority: 37 Stat. 832–833 (21 U.S.C. 151–158).

* * * * *

§ 113.33 Mouse safety test.

* * * * *

(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.

* * * * *

§ 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 does 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.

§§ 113.39–113.49 [Reserved]

* * * * *

DIAGNOSTICS AND REAGENTS

§ 113.65 Tuberculin, Intradermic.

Tuberculin, Intradermic, is a filtrate produced from cultures of Pn, C, and Dt strains of *Mycobacterium tuberculosis* (supplied by Veterinary Services) which has been inactivated and is nontoxic. 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 Veterinary Services. 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 paragraph (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.

§ 113.66 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. Gross 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.

§ 113.67 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 an acceptable dye, but tube antigens shall be unstained. 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 Sorenson's buffer solution (use buffer solution at pH 6.0 for Mycoplasma Gallisepticum Plate Antigen and at pH 7.0 for Mycoplasma Gallisepticum Tube Antigen and Mycoplasma Synoviae Plate Antigen) in a modified Hopkins tube and 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 1.0 percent (± 0.2 percent), the serial is unsatisfactory.

(b) *Preservative requirements.* Only preservatives written in an approved Outline of Production shall be used. If phenol is used, a direct titration with a standardized bromide-bromate solution shall be made; *Provided,* That 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. If plate antigen is not homogeneous and free of visible particles and clumps, the serial is unsatisfactory.

(2) Tube antigen shall be checked for homogeneity and autoagglutination. If tube antigen is not homogeneous and free of large visible particles and clumps, or if it autoagglutinates, the serial is unsatisfactory.

(3) 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 Gallisepticum Tube Antigen and Mycoplasma Synoviae Plate 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.* (1) Mycoplasma Gallisepticum Antigen—Plate or Tube Test reactions of the serial shall be compared with a reference antigen using both known positive and negative chicken and turkey serums (the positive serums shall have varying degrees of reactivity from weakly positive to strongly positive). Five positive serums shall be tested against plate antigen in the 1:4 dilution or tube antigen in the 1:20 dilution; five negative serums shall be tested against plate antigen undiluted or tube antigen in the 1:20 dilution. 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 similar reactions with the positive serums, the serial is unsatisfactory; *Provided,* That a variation is permitted with one of the five serums in the set of positive serums used.

(2) Mycoplasma Synoviae Antigen, Plate—Test reactions of the serial shall be compared with a reference antigen using both known negative chicken serum and positive chicken serums. The five negative serums shall be tested against the antigen undiluted and the positive serums shall be tested against the antigen in the 1:4 dilution. 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 similar reactions with the positive serums, the serial is unsatisfactory; Provided, That a variation is permitted with one of the five serums in the set of positive serums used.

(g) *Specificity requirements.* Mycoplasma Synoviae Antigen shall be examined for cross-agglutination with Mycoplasma gallisepticum antiserum. If cross-agglutination occurs, the serial is unsatisfactory.

§§ 113.68–113.74 [Reserved]

Blood Origin Products

§ 113.75 General requirements for biological products of animal blood origin.

Each serial of biological product prepared from the blood of animals shall comply with the general requirements prescribed in this section unless otherwise provided for in a Standard Requirement or approved Outline of Production for such product. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Biological products of animal blood origin prepared in the United States shall be obtained from blood of animals maintained at licensed establishments. Such products offered for importation into the United States shall be prepared from the blood of animals maintained at the establishment entered on the permit as having prepared the product.

(b) Only healthy animals shall be used and the fitness shall be determined by physical examination by, or under the supervision of, a veterinarian and by tests for infectious diseases.

(1) No animal shall be used while showing clinical signs of disease. If the body temperature is normal, this restriction shall not apply to an animal having localized lesions, contusions, or slight lameness, but shall apply if the animal is feverish, in pain, or distress.

(2) New animals shall be subjected to applicable tests for the species. Such tests shall be listed in the filed Outline of Production and records maintained of results obtained. No animal shall be used for production of blood which has been

found positive when tested for an infectious disease. Retests shall be conducted as deemed necessary by the Deputy Administrator.

(c) Serum and antiserum of equine origin shall be heated at 58.5° C. for 60 minutes, with a tolerance of 0.5° above and below that temperature. Serum and antiserum of bovine and porcine origin shall be heated in like manner for 30 minutes. Neither serum nor antiserum shall contain preservative at the time of heating.

(d) Serum and antiserum heated as provided in paragraph (c) of this section, shall be cooled immediately thereafter to 15° C. or lower, and thus held until properly preserved. It shall be preserved, mixed, and tested by methods described in the licensee's outline.

(e) Licensees shall keep detailed records relative to each batch of antiserum or serum pasteurized and each serial prepared for marketing. Recording thermometer charts shall bear full information concerning the antiserum or serum heated and tests made of the equipment.

(f) *Purity requirements.* Biological products of animal blood origin shall contain only serum constituents, preservatives, and safe amounts of chemicals used in the manufacturing procedures. Final container samples of completed product shall be tested for viable microorganisms as provided in § 113.26.

(g) *Safety requirements.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(h) If no label claim for specific antibody content can be substantiated by the licensee, the product shall be considered a normal serum; *Provided,* That, if the serum does contain demonstrable antibodies which have been produced in response to specific antigens injected into the blood producing animals, the product shall be considered an antiserum, except that:

(1) An antiserum concentrated at least twofold by the removal of water may be considered an antibody concentrate; and

(2) An antiserum fractionated by suitable means to remove most of the nonglobulin material and concentrated to increase the amount of globulin at least twofold over the amount in the original antiserum may be considered a globulin concentrate.

(i) Normal serum, antiserum, and antiserum derivatives shall contain at least one preservative in the following table. The amount shall be within the range of concentration set forth and the type used shall be indicated on the label.

(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 approved Outline of Production for the product.

(j) If a potency test has been issued in a Standard Requirement or is written in an approved Outline of Production for an antiserum, an antiserum concentrate, or a globulin concentrate, the potency shall be sufficient for the product to satisfactorily meet the requirements of such test. Unless otherwise specified in the test procedure, the test sample of antiserum concentrate or globulin concentrate shall be diluted with 1 percent peptone solution to a volume equal to the contents of the sample times the concentration factor specified in such approved Outline of Production prior to conducting the test.

(k) If a potency test has not been issued in a Standard Requirement or is not written into an approved Outline of Production for an antiserum, an antiserum concentrate, or a globulin concentrate, such product shall meet the requirements in this paragraph.

(1) The globulin content of antiserum, normal serum, and globulin concentrate shall be determined by electrophoretic mobility or other equally acceptable tests.

(i) The globulin content of antiserum and normal serum shall equal or exceed the amounts per ml listed according to the species of origin as follows:

Species:	*Globulin (mg/ml)*
Bovine	45
Equine	50
Porcine	35
Canine	25
Feline	25

(ii) The globulin content of globulin concentrate shall exceed the value given for the antiserum from a particular species by the multiple of the concentration factor prescribed in the approved Outline of Production.

(iii) Globulin concentrates shall be fractionated in such a manner that the protein content shall contain greater than 90 percent globulin as determined by electrophoretic mobility or other acceptable tests.

(2) The level of protein content for antiserum and normal serum shall equal or exceed the amounts per ml listed according to the species of origin as follows:

Species:	*Protein (mg/ml)*
Bovine	60
Equine	60
Porcine	55
Canine	45
Feline	45

(3) The protein content of an antiserum concentrate shall exceed the value given in the table for the antiserum from a particular species in paragraph (f)(2) of this section by the multiple of the concentration factor prescribed in the approved Outline of Production.

§ 113.76 Tetanus Antitoxin.

Tetanus Antitoxin is a specific antibody obtained from the blood of healthy horses hyperimmunized with an antigen prepared with a toxin-producing strain of *Clostridium tetani.* Each serial shall meet the general requirements provided in paragraph (a) of this section and shall be tested for purity, safety, and potency as provided in paragraphs (b), (c), and (d) 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 Bureau of Standards requirements shall be used. The reading shall be made at the bottom of the meniscus and recorded to the nearest 0.1 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 demonstrating that the recoverable volume of the final container provides a 20 percent excess over the labeled unitage.

(b) *Purity test.* Final container samples of completed product from each serial shall be tested for viable bacteria and fungi as prescribed in § 113.26.

(c) *Safety test.* Final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(d) *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 with a 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 dose 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 350 gram guinea pig, results in death of that guinea pig within approximately 96 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 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 anti-toxin shall be based on an estimation of the expected potency. The final titration shall include a test at the expected unit value, one dilution above and one below.

(5) Normal guinea pigs weighing within a range of 340–380 grams shall be used. Pregnant guinea pigs must not be used.

(i) Each of two guinea pigs (controls) shall be injected subcutaneously with a 3.0 ml dose of the standard toxin-antitoxin mixture. Injections shall be made in the same order that antitoxins were added to the toxin-antitoxin mixtures. 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 of three dilutions of the unknown antitoxin. A 3.0 ml dose shall be injected subcutaneously into each animal.

(6) Controls shall be observed until all are dead, or for 6 days, the time of death being recorded in hours. For a satisfactory test, the controls must die with clinical signs of tetanus within 12 hours of each other and within an overall time of 60 to 120 hours with the mean time of death being approximately 96 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 flat 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 a new 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.

§ 113.77 Swine erysipelas antiserum.

Swine Erysipelas Antiserum shall be prepared from the blood of horses hyperimmunized with antigenic strains of *Erysipelothrix insidiosa*. Each serial shall be tested for purity, safety, and potency as provided 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 subserial shall be tested for viable bacteria and fungi as prescribed 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 using the following two-stage test:

(1) In the first stage, each of 40 Swiss albino mice, each weighing 16–20 grams, shall be injected subcutaneously with 0.1 ml of antiserum. Twenty-four hours postinjection, the injected mice and 10 additional mice designated as controls shall be challenged subcutaneously with the same culture of *Erysipelothrix insidiosa*.

(2) If less than eight of the 10 controls do not 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 the erysipelas organism.

(3) The antiserum injected mice shall be observed for 10 days post-challenge and all deaths recorded. The second stage shall be required when 7–10 of the antiserum injected mice 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 giving an unsatisfactory test
1	40	40	6 or less	11 or more.
2	40	80	12 or less	13 or more.

§ 113.78 Canine Distemper-Hepatitis-Leptospira Antiserum.

The antiserum shall be prepared from blood of dogs hyperimmunized with canine distemper virus, canine hepatitis virus, and one or more leptospira species until the final product shall provide passive immunity against the disease agents named on the label. Each serial and subserial shall be tested for purity, safety, and potency as provided 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 prescribed 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). Also, each of two healthy dogs shall be injected subcutaneously with the maximum dose recommended on the label per 1 pound body weight of a dog and each shall be injected intravenously with the maximum dose recommended on the label per 1 pound body weight of a dog. The injected dogs shall be observed for 14 days for unfavorable reactions. If unfavorable reactions attributable to the product occur in one or both of the dogs, 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 shall be declared unsatisfactory.

(c) *Potency tests.* Bulk or final container samples of completed product from each serial shall be tested for potency as provided in this paragraph. Concentrated products shall be diluted to the equivalence of regular serum before testing. Standard antiserums and standard viruses, available upon request to Veterinary Services, shall be used.

(1) *Distemper fraction.* A virus neutralization test using chick embryo inoculation technique or acceptable tissue culture system shall be conducted.

(i) Serial threefold dilutions shall be made of the serum under test and the standard antiserum. A volume of each serial dilution of serum shall be mixed with an equal volume of standard virus. For use in the test, the standard virus shall be diluted to contain 100–1,000 ID_{50} per 0.1 ml.

(ii) A 1:2 dilution of the standard virus, as prepared for use in the test, shall be titrated in tenfold dilutions to determine the actual amount of virus used in the neutralization test. These dilutions shall be incubated along with serum/virus mixtures.

(iii) After 1 hour incubation at room temperature, 0.2 ml of each serum/virus mixture shall be inoculated onto the chorioallantoic membrane of at least five chicken embryos 6–8 days old. The inoculated embryos shall be incubated at 35–37° C for 6–7 days and the chorioallantoic membranes examined. Calculate the 50 percent end point of neutralization (ND_{50}) by the Reed and Muench or other acceptable statistical method.

(iv) The reciprocal of the ND_{50} dilution of the test serum divided by the reciprocal of the ND_{50} dilution of the standard serum is the test serum to standard ratio. A serial is unsatisfactory unless the average ratio of two tests is 0.67 or more.

(2) *Hepatitis fraction.* A virus neutralization test using canine kidney cell culture as the indicator system shall be conducted.

(i) Serial threefold dilutions shall be made of the antiserum under test and the standard antiserum. A volume of each serial dilution of serum shall be mixed with an equal volume of standard virus. The standard virus shall contain 100–1,000 $TCID_{50}$ per 0.1 ml.

(ii) A 1:2 dilution of the standard virus, as prepared for use in the test, shall be titrated in tenfold dilutions to determine the actual amount of virus used in the neutralization test. These dilutions shall be incubated along with serum/virus mixtures before inoculation of culture tubes.

(iii) After 1 hour incubation at room temperature, 0.2 ml of each serum/virus mixture shall be inoculated into five culture tubes containing dog kidney cell monolayers. The inoculated tubes shall be incubated at 35–37° C.

(iv) The tubes shall be observed daily for specific cytophathogenic changes from the 4th to 10th day postinoculation. The test shall be terminated before the 10th day if additional cytopathogenic changes do not occur in virus titration tubes over a 48 hour period.

(v) Results shall be recorded and the 50 percent end point of neutralization (ND_{50}) calculated by the Reed and Muench or other acceptable statistical method.

(vi) The reciprocal of the ND_{50} dilution of the test serum divided by the reciprocal of the ND_{50} dilution of the standard serum is the test serum to standard ratio. A serial is unsatisfactory unless the average ratio of two tests is 0.67 or more.

(3) *Leptospira fraction.* Young adult hamsters, each weighing 40–50 grams, shall be used as test animals. For each species of leptospira antibody included

in the product, each of at least five hamsters shall be injected with 60 percent of the minimum dose recommended on the label for 1 pound body weight of a dog and at least five additional hamsters shall be used as controls.

(i) Within 24 hours following injection, the five injected hamsters and the five control hamsters for each species of leptospira shall be challenged intraperitoneally with a suspension of virulent leptospira organisms representative of the species. A dose of 10–1,000 hamster LD_{50} shall be used. Both groups shall be observed for 14 days post-challenge and the results noted.

(ii) The test shall be conclusive if at least 80 percent of the controls in each group die from leptospirosis as demonstrated by acceptable cultural methods.

(iii) If the test is considered conclusive as provided in paragraph (c) (3) (ii) of this section, and less than 80 percent of the injected hamsters in a group remain normal during the observation period, the serial is unsatisfactory.

§ 113.79 Clostridium Perfringens Type C Antitoxin.

Clostridium Perfringens Type C Antitoxin shall be prepared from the blood of animals hyperimmunized with Clostridium Perfringens Type C toxin. Each serial shall be tested for purity, safety, and potency as provided 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 using the toxin-neutralization test for Beta Antitoxin 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_o and L_+ doses of Standard Toxin according to their definitions.

(ii) *L_o 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 Veterinary Services. 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 Veterinary Services.

(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_o 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_o doses.

(iv) Combine 10 International Units of Standard Antitoxin with 10 L_o 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_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 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_o doses of Standard Toxin die, the antitoxin is considered to contain less than 500 International Unit per ml and the serial is unsatisfactory.

§ 113.80 Clostridium Perfringens Type D Antitoxin.

Clostridium Perfringens Type D Antitoxin shall be prepared from the blood of animals hyperimmunized with Clostridium Perfringens Type D toxin. Each serial shall be tested for purity, safety, and potency as provided 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 using the toxin-neutralization test for Epsilon Antitoxin 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_o and L_+ doses of Standard Toxin according to their definitions.

(ii) *L_o 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 Veterinary Services. 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 Veterinary Services.

(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_o 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_o doses.

(iv) Combine 1 International Unit of Standard Antitoxin with 10 L_o 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_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 Clostridium Perfringens Type D Antitixin diluted 1:34 and 10 L_0 doses of Standard Toxin die, the antitoxin is considered to contain less than 34 International Unit per ml and the serial is unsatisfactory.

§ 113.81–§ 113.84 [Reserved]

INACTIVATED BACTERIAL PRODUCTS

§ 113.85 Bacterins, toxoids, and bacterin-toxoids.

Basic requirements for bacterins, toxoids, and bacterin-toxoids are provided in this section.

(a) A *bacterin* shall be an antigenic suspension 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 writen into the filed Outline of Production for the product.

(b) A *toxoid* shall be a sterile, antigenic filtrate resulting from growth of bacterial organisms in a cultural medium which has had the bacterial cells removed, has been inactivated without appreciable loss of antigenic value as measured by suitable tests, and which is nontoxic as demonstrated by acceptable tests written into the filed Outline of Production.

(c) A *bacterin-toxoid* shall be:

(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 antigenic value 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 combining one or more toxoids or bacterin-toxoids with one or more bacterins or one or more bacterin-toxoids.

(d) Bacterins used as diluents. Bacterins authorized for use as diluents in combination packages include Leptospira Pomona Bacterin, Leptospira Canicola Bacterin, Leptospira Icterohaemorrhagiae Bacterin, and Pasteurella Bacterin. All serials and subserials of these bacterins shall be subject to the applicable requirements in paragraphs (e) and (f) of this section.

(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 shall be tested for residual free formaldehyde content using the Basic Fuchsin Test.

(1) The residual free formaldehyde content of biological products containing Clostridial antigens shall not exceed the equivalent of 0.5 percent formaldehyde solution (1,850 parts per million formaldehyde.)

(2) The residual free formaldehyde content of bacterins, bacterin-toxoids, and toxoids other than those containing Clostridial antigens, shall not exceed the equivalent of 0.2 percent formaldehyde solution (740 parts per million formaldehyde.)

§ 113.86 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 § 113.85 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 10 young adult hamsters, each weighing 50–90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly in accordance with the label recommendations for use.

(2) *Controls.* Retain 10 additional hamsters from the same group as unvaccinated controls.

(3) *Challenge.* From 14–18 days postvaccination, challenge each vaccinate and each control intraperitoneally with a suspension of virulent *Leptospira pomona* organisms, using a dose of 10–1,000 hamster LD_{50} as determined by titration.

(4) *Post-challenge period.* Observe the vaccinates and controls for 14 days postchallenge 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.

§ 113.87 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 § 113.85 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 10 young adult hamsters, each weighing 50–90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly in accordance with the label recommendations for use.

(2) *Controls.* Retain 10 additional hamsters from the same group as unvaccinated controls.

(3) *Challenge.* From 14-18 days postvaccination, challenge each vaccinate and each control intraperitoneally with a suspension of virulent *Leptospira icterohaemorrhagiae* organisms using a dose of 10–1,000 hamster LD_{50} as determined by titration.

(4) *Post-challenge period.* Observe the vaccinates and controls for 14 days postchallenge 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.

§ 113.88 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 § 113.85 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 10 young adult hamsters, each weighing 50–90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly in accordance with the label recommendations for use.

(2) Controls. Retain 10 additional hamsters from the same group as unvaccinated controls.

(3) Challenge. From 14–18 days postvaccination, challenge each vaccinate and each control intraperitoneally with a suspension of virulent *Leptospira canicola* organisms using a dose of 10–1,000 hamster LD_{50} as determined by titration.

(4) Post-challenge period. Observe the vaccinates and controls for 14 days postchallenge 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.

§ 113.89–§ 113.90 [Reserved]

§ 113.91 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 § 113.85 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 eight 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 9 to 10 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 Veterinary Services, shall be used for challenge 14 to 15 days following the last injection of the product. Each of the 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 post-challenge 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	Cumulatives total number of deaths giving 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.

§ 113.92 Clostridium Hemolyticum Bacterin.

Clostridium Hemolyticum Bacterin shall be produced from a culture of *Clostridium hemolyticum* which has been inactivated and is nontoxic. Each serial of biological product containing *Clostridium hemolyticum fraction* shall meet the applicable requirements in § 113.85 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 the eight 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 9 to 10 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 hemolyticum* challenge material, available upon request from Veterinary Services, shall be used for challenge 14 to 15 days following the last injection of the product. Each of the eight vaccinates and each of five additional nonvaccinated guinea pigs for controls shall be injcted 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 post-challenge 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 giving 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.

§ 113.93 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 § 113.85 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 following two-stage test provided in this paragraph.

(1) Each of eight 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 9 to 10 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 novyi* challenge material, available upon request from Veterinary Services, shall be used for challenge 14 to 15 days following the last injection of the product. Each of the 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 post-challenge 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 table:

Stage	Number of vaccinates	Cumulative number of animals	Cumulative total number of deaths for a satisfactory test	Cumulative total number of deaths giving 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.

§ 113.94 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 § 113.85 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.

§ 113.95 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 § 113.85 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.

(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 orally with botulinum Type C toxin which has been titrated in mice to provide for a $10^{4.0}$ mouse MLD_{50} 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.

§ 113.96 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 have been inactivated and is nontoxic. Each serial shall meet the applicable requirements in § 113.85 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 Veterinary Services. 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 Veterinary Services.

(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, each weighing 4–8 pounds, shall be injected subcutaneously with not more than half of the recommended cattle dose. The dose for a combination product having both Type C and Type D fractions shall be 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) When eight or more rabbits are bled, equal quantities of serum from each shall be combined and tested as a single pooled serum.

(ii) When only four to seven rabbits are bled, the serums shall be individually tested.

(iii) If less than four rabbits are bled, 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_o 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_o 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_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 10 International Units 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 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_o doses of Standard Toxin die, the serum is considered to contain less than 10 International Units per ml.

(iv) If less than 4 of 4 or less than 80 percent of individual serums from at least five to seven rabbits or if the single pooled serum from eight or more rabbits contain(s) less than 10 International Units per ml, the serial is unsatisfactory.

§ 113.97 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 have been inactivated and is nontoxic. Each serial shall meet the applicable requirements in § 113.85 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_o and L_+ doses of Standard Toxin according to their definitions.

(ii) *L_o 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 Veterinary Services. 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 Veterinary Services.

(vi) *Diluent.* The solution used to make proper dilutions prescribed in this test. Such solutions shall be made by dissolving 1 gram of peptone at 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, each weighing 4–8 pounds, shall be injected subcutaneously with not more than half of the recommended sheep dose. The dose for a combination product having both Type C and Type D fractions shall be 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) When eight or more rabbits are bled, equal quantities of serum from each shall be combined and tested as a single pooled serum.

(ii) When only four to seven rabbits are bled, the serums shall be individually tested.

(iii) If less than four rabbits are bled, 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 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 does of Standard Toxin die, the serum is considered to contain less than 2 International Units per ml.

(iv) If less than four of four or less than 80 percent of individual serums from at least five to seven rabbits or if the single pooled serum from eight or more rabbits contain(s) less than 2 International Units per ml, the serial is unsatisfactory.

§ 113.98 [Reserved]

§ 113.99 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 adsorbed, precipitated, or purified and concentrated. Each serial of biological product containing *tetanus toxoid* fraction shall meet the applicable requirements in § 113.85 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. Each of at least 10 guinea pigs, each weighing 500 grams±10 percent, shall be injected subcutaneously with 0.4 of the dose recommended on the label for a horse.

(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. Serum from not less than eight animals shall be used.

(2) The pooled serum shall contain at least two antitoxin units per ml as determined by titrating it in the manner prescribed for tetanus antitoxin in § 113.76. A 1:20 dilution of the pooled serum shall be made so that the final dilution contains 0.1 antitoxin unit per ml. The dilution shall be held at 20 to 25° C for 30 minutes prior to combining with a test dose of toxin. The test dose of standard toxin shall be mixed in proper proportion with the diluted pooled serum, incubated at 20 to 25° C for 1 hour and inoculated subcutaneously into two guinea pigs.

(3) The test dose of the standard toxin shall be verified against 0.1 of a unit of standard antitoxin in two guinea pigs which serve as the control animals.

(4) If the control animals do not die of tetanus within 60–120 hours, the test is not valid and must be repeated. In a valid test, if the vaccinated animals do not survive as long as the control animals, the serial is unsatisfactory.

§ 113.100 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 § 113.85 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 paragraph (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, usng 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.

§ 113.101 General Requirements for Pasteurella Multocida (avicida) Bacterins.

Pasteurella Multocida (avicida) Bacterin shall be prepared from cultures of *Pasteurella multocida*, Avian Strains, Type 1 or Type 3 or both (Little and Lyons Classification) which have been inactivated and is nontoxic; *Provided*, That, avian strains of types other than Types 1 and 3 may be added if written into the filed Outline of Production for the product.

(a) Each serial shall meet the applicable requirements in § 113.85. Each serial containing Type 1 strains shall be tested as provided in § 113.102 and each serial containing Type 3 strains shall be tested as provided in § 113.103. Serials containing Types 1 and 3 shall be tested as provided in § 113.102 and § 113.103.

(b) If avian strains of types other than Types 1 and 3 have been added, each serial shall also be tested for such strains as provided in the filed Outline of Production.

§ 113.102 Pasteurella Multocida (avicida) Bacterin, Type 1.

Each serial of Pasteurella Multocida (avicida) Bacterin containing Type 1 strains shall be tested 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 in the potency test provided in paragraph (c) of this section shall constitute a safety test. If unfavorable reactions attributable to the product occur in the chickens during the prechallenge 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 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) *Positive controls.* Each of not more than 21 chickens shall be injected with two doses of a reference bacterin available from Veterinary Services upon request.

(3) *Unvaccinated controls.* Each of not more than 21 chickens shall held as controls.

(4) *Challenge.* Not less than 14 days after the section injection, each of 20 vaccinates, each of 20 positive controls, and each of 20 unvaccinated controls shall be challenged intramuscularly with a minimum of 250 colony-forming units of virulent *Pasteurella multosida*. Strain X–73, Type 1 (Little and Lyons Classification) and observed daily for a 14 day post-challenge period. Only dead birds shall be considered in evaluating the product.

(5) If nine or more positive controls die, the test may be declared inconclusive and repeated.

(6) If 16 or more unvaccinated controls die, the test is valid and stage one potency test results shall be evaluated according to stage one of the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of dead vaccinates for satisfactory serial	Cumulative total number of dead vaccinates for unsatisfactory serial
1	20	20	6 or less	9 or more.
2	20	40	15 or less	16 or more.

(7) The serial shall pass or fail based on the stage one results of the potency test; *Provided*, That, if seven or eight

vaccinates die in the first stage, the second stage shall be required.

(8) 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, a serial shall either pass or fail the second stage.

§ 113.103 Pasteurella multocida (avicida) Bacterin, Type 3.

Each serial of *Pasteurella multocida* (avicida) Bacterin containing Type 3 strains shall be tested 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 in the potency test provided in paragraph (c) of this section shall constitute a safety test. If unfavorable reactions attributable to the product occur in the turkeys during the prechallenge 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 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) *Positive controls.* Each of not more than 21 turkeys shall be injected with two doses of a reference bacterin available from Veterinary Services upon request.

(3) *Unvaccinated controls.* Each of not more than 21 turkeys shall be held as controls.

(4) *Challenge.* Not less than 14 days after the second injection, each of 20 vaccinates, each of 20 positive controls, and each of 20 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 post-challenge period. Only dead birds shall be considered in evaluating the product.

(5) If nine or more positive controls die, the test may be declared inconclusive and repeated.

(6) If 16 or more unvaccinated controls die, the test is valid and stage one potency test results shall be evaluated according to stage one of the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of dead vaccinates for satisfactory serial	Cumulative total number of dead vaccinates for unsatisfactory serial
1	20	20	6 or less	9 or more.
2	20	40	15 or less	16 or more.

(7) The serial shall pass or fail based on the stage one results of the potency test; *Provided,* That, if seven or eight vaccinates die in the first stage, the second stage shall be required.

(8) 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, a serial shall either pass or fail the second stage.

§ 113.104 Erysipelas Bacterin.

Erysipelas Bacterin shall be produced from a culture of *Erysipelothrix insidiosa* which has been inactivated and is nontoxic. Each serial of biological product containing erysipelas bacterin shall meet the applicable requirements in § 113.85 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 tests.* Bulk or final container samples of completed product shall be tested by either the mouse protection test provided in paragraph (d) of this section or the swine protection test provided in paragraph (e) of this section.

(d) When the mouse protection test is conducted, the ability of the bacterin to be tested (Unknown) to protect mice shall be compared with a standard bacterin (Standard) which is either supplied by or is acceptable to Veterinary Services.

(1) Bacterins with a 2 ml recommended dose for swine shall be diluted 1:2.5 with physiological saline solution and tested the same as bacterin with a 5 ml recommended dose. At least three threefold dilutions shall be made with the Standard and comparable threefold dilutions for the Unknowns.

(2) For each dilution of the Standard and each dilution of the Unknown, a group of at least 16 mice, each weighing 16–20 grams, shall be used. Each mouse in a group shall be injected subcutaneously with 0.5 ml from the appropriate dilution.

(3) Each injected mouse shall be challenged subcutaneously 14–21 days after being injected with the diluted bacterin. A 0.5 ml dose containing at least 100 mouse LD_{50} of a suitable culture of *Erysipelothrix insidiosa* shall be used. All survivors in each group of mice shall be recorded 10 days post-challenge.

(4) *Test for valid assay.* The same consecutive dilutions of the Standard and Unknown need not to be used in the test for valid assay, but the following requirements shall be met:

(i) At least two dilutions of the Standard and two dilutions of the Unknown shall protect more than 0 percent and less than 100 percent of the mice inoculated. The lowest dilution of the Standard shall protect more than 50 percent and the highest dilution less than 50 percent.

(ii) Subtract the numbers of surviving mice injected with the lowest dilution, middle dilution, and highest dilution of the Unknown from the numbers of surviving mice injected with the corresponding dilutions of the Standard. Designate the differences as No. 1, No. 2, and No. 3.

(iii) Adjust difference No. 2 to zero by adding the proper figure using a plus or minus sign as needed. Add the same figure to Difference No. 1 and to Difference No. 3.

(iv) Consult this table to see if the results obtained are included.

Difference 1	*Range of Difference 3*
−9	−7 to −2 inclusive
−8	−8 to 0
−7	−9 to +2
−6	−9 to +3
−5	−9 to +4
−4	−9 to +5
−3	−9 to +6
−2	−9 to +7
−1	−8 to +7
0	−8 to +8
1	−7 to +8
2	−7 to +9
3	−6 to +9
4	−5 to +9
5	−4 to +9
6	−3 to +9
7	−2 to +9
8	0 to +8
9	2 to +7

(v) If the results do not appear, the test is invalid. If the results do appear, the test is valid and the potency of the Unknown can be compared with the Standard.

(5) Using the same dilutions used to determine validity, obtain the total survivors of the Unknown and the total survivors of the Standard. If the total number of survivors for the Standard exceeds the total number of survivors for the Unknown by a number greater than six, the Unknown is unsatisfactory.

(e) When the swine protection test is conducted, susceptible pigs shall be used as test animals. Each of four pigs (vaccinates) shall be injected with one pig dose as recommended on the label. Four additional pigs shall be held as unvaccinated controls. Fourteen to twenty-one days post-vaccination, the vaccinates and the controls shall be challenged with a virulent *Erysipelothrix insidiosa* culture by the percutaneous, or intradermal, or intramuscular route and observed for 7 days.

(1) A satisfactory challenge shall be evidenced in the controls by a bacteremia, a high body temperature, and clinical signs of sickness, including but not limited to acute illness with hyperemia

of the abdomen and ears possibly terminating in suddent death; moribund and anorexia, with or without metastatic skin lesions; slowness with variable anorexia, stiffness, and/or joint involvement; and any combination of these symptoms and lesions.

(2) If at least 75 percent of the controls do not show characteristic symptoms of swine erysipelas during the observation period, including but not limited to a body temperature of 105.6° F or higher on at least 2 days or 107° F or higher for at least 16 hours, 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 insidiosa* can be isolated from the blood, spleen, or other organs.

(3) To demonstrate immunity after challenge, the vaccinates shall remain free of lesions or symptoms of erysipelas and the body temperature shall not exceed 104.6° F for longer than 1 day. If at least 75 percent of the vaccinates do not demonstrate immunity to erysipelas throughout the observation period, the serial or subserial is unsatisfactory.

§ 113.105–§ 113.109 [Reserved]

LIVE BACTERIAL VACCINES

§ 113.110 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) To meet the requirements prescribed in § 113.27(b), at least four single-dose or two multiple-dose final container samples of completed product shall be tested by inoculating one potato agar slant, one tube of Dextrose Andrades broth with gas tube (or equivalent) and one tube of thioglycollate broth from each sample. If organisms not typical of *Brucella abortus* organisms are 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) *Potency test.* Each serial and each subserial shall be tested for potency.

(1) At least four single-dose or two multiple-dose final container samples of completed product shall be tested for the number of viable organisms per cubic centimeter of rehydrated vaccine. A bacterial count shall be made on tryptose agar plates from suitable dilutions using 1 percent peptons as a diluent.

(2) If freshly prepared *Brucella abortus* vaccine does not contain at least 10 billion viable *Brucella abortus* organisms per cubic centimeter of rehydrated vaccine, the serial or subserial is unsatisfactory.

(3) If tested at any time within the expiration period, each cubic centimeter of rehydrated vaccine does not contain 5 billion viable *Brucella abortus* organisms, the serial or subserial is unsatisfactory.

§ 113.111 Anthrax Spore Vaccine—Nonencapsulated.

Anthrax Spore Vaccine—Nonencapsulated shall be a live spore suspension prepared for nonencapsulated variants of *Bacillus anthracis* which has been proven to be immunogenic and relatively nonpathogenic. Each serial and subserial 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.* Each serial or first subserial shall be tested for purity as provided in this paragraph to meet the requirements prescribed in § 113.27(b).

(1) Bulk samples of spore suspension or completed product shall be tested for microorganisms other than *Bacillus anthracis.* One ml shall be inoculated onto each of five plates of nutrient agar, incubated at 35–37° C and examined macroscopically and microscopically daily for 7 days. If growth not characteristic of *Bacillus anthracis* is detected, the serial or subserial is unsatisfactory.

(2) Each of five final container samples of completed product shall be tested as follows:

(i) One ml shall be inoculated into one tube of either thioglycollate or nutrient broth, incubated at 37° C for 7 days and examined for microorganisms other than *Bacillus anthracis.* If microorganisms other than *Bacillus anthracis* are detected, the serial or subserial is unsatisfactory.

(ii) One ml shall be inoculated into one vessel of Soybean Casein Digest Medium, incubated at 20–25° C for 14 days, and examined for fungi. If fungi are detected, the serial is unsatisfactory.

(3) Bacterial dissociation test. Vaccine from each bulk container shall be streaked onto the surface of two serum agar plates (50 percent bovine serum with nutrient agar) in such a manner so to produce isolated colonies over a major portion of the plates. The plates shall be incubated under an atmosphere of 30 percent CO_2 at 37° C and examined under a dissection-type microscope using obliquely transmitted light daily for at least 3 days. If the colonies are not of the rough opaque type, the serial is unsatisfactory. Questionable colonies shall be subcultured and the organisms inoculated into guinea pigs to check for undesirable reactions. If undesirable reactions occur, the serial is unsatisfactory.

(b) *Safety test.* The prechallenge period of the potency test provided in subparagraph (c) (2) of this section constitutes a safety test. If 80 percent of the vaccinates survive the prechallenge period, the serial is satisfactory for safety; *Provided,* That

(1) If less than 80 percent of the vaccinates survive the prechallenge period, each of five young sheep or goats shall be inoculated subcutaneously with a total of two cattle doses which may be fractionally administered in two sites and the animals observed for 14 days; and

(2) If any sheep or goat shows evidence of persistent or progressive edema (does not subside in 4 or 5 days) or does not survive the observation period, the serial or subserial is unsatisfactory.

(c) *Potency tests.* The spore count provided in this paragraph shall be conducted on each serial and each subserial. The vaccination challenge test provided in this paragraph shall be conducted on each serial.

(1) Spore count. Final container samples of completed product shall be diluted in tenfold steps. Each dilution that may be significant shall be plated in triplicate on nutrient or tryptose agar, inverted, and incubated at 35–37° C for 24–48 hours. The plates showing proper dispersion of colonies shall be counted and averaged. If the spore count average is less than 2,000,000 spores per bovine dose, the serial or subserial is unsatisfactory.

(2) Bulk or final container samples of completed product shall be tested for potency in healthy guinea pigs weighing 400 to 500 grams each.

(i) Each of at least 10 guinea pigs shall be vaccinated subcutaneously on the abdominal area with one-half the dose recommended for cattle and observed for 14 days. The vaccinated guinea pigs and six unvaccinated control guinea pigs shall be challenged with a suspension of anthrax spores virulent to guinea pigs and observed an additional 10 days.

(ii) If five of the six unvaccinated controls do not die during the 10 day post-challenge observation period, the test is inconclusive and may be repeated.

(iii) If at least five of the six unvaccinated controls die and less than 80 percent of the vaccinates remain well during

the post-challenge observation period, the serial or subserial is unsatisfactory.

§ 113.112 Erysipelas Vaccine.

Erysipelas Vaccine shall be prepared as a desiccated live culture of an avirulent or modified strain of *Erysipelothrix insidiosa* which has been proven to be nonpathogenic and nontoxic. Each serial and subserial shall be tested for purity, safety, potency, and moisture content. A serial or subserial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Bulk or final container samples of completed product from each serial shall be tested by colonial observation and gram stain of organisms plated on a suitable solid media to meet the requirements prescribed in § 113.27(b).

(b) *Safety test.* Final container samples of completed product from each serial and subserial shall be tested as provided in § 113.33(b).

(c) *Potency tests.* Final container samples of completed product from each serial and subserial shall be tested for potency as provided in § 113.104(e).

6. Part 114 is amended to read:

PART 114—PRODUCTION REQUIREMENTS FOR BIOLOGICAL PRODUCTS

Sec.

AUTHORITY: 37 Stat. 832–833; 21 U.S.C. 151–158.

§ 114.1 Applicability.

Unless otherwise authorized by the Deputy Administrator, all biological products prepared for shipment or delivered for shipment from one State or territory or the District of Columbia to another State or territory or the District of Columbia 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 Deputy Administrator prescribes for carrying out such regulations.

§ 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) After an establishment license has been issued, biological products shall not be prepared in or brought onto the premises of the licensed establishment if such products are not prepared in compliance with the regulations.

§ 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 unless authorized in advance by the Deputy Administrator.

§ 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 Veterinary Services upon request of the Deputy Administrator.

§ 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 Veterinary Services 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 Veterinary Services 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.

§ 114.8 Outline of Production required.

An Outline of Production shall be on file with Veterinary Services 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 Veterinary Services 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 Veterinary Services for filing. When objectionable features, if any, are corrected and no further exceptions are taken by Veterinary Services 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 Veterinary Services 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 Veterinary Services 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 Veterinary Services.

(e) When a list of licensed products to be continued in production at a licensed establishment is requested by the Deputy 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 Veterinary Services 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 Deputy 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 Deputy 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.

§ 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 Veterinary Services 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 Veterinary Services.

(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 Veterinary Services.

(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.

§ 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 Deputy 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.

§ 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 [Reserved]

§ 114.13 Expiration date determination.

Unless otherwise provided for in a Standard Requirement or filed Outline of Production, the expiration date for each serial and subserial shall be computed in accordance with the conditions provided in this section.

(a) *Harvest date.* The earliest date of harvest for any component of a biological product and the date of the last satisfactory potency test shall be considered in determining the expiration date.

(1) The date of harvest for products prepared from animal blood or live animal tissues including chicken embryos shall be the date such blood or tissues were collected.

(2) The date of harvest for products prepared by inactivating (killing) micro-organisms shall be the date the chemical or physical inactivating method is applied.

(3) The date of harvest for products prepared with living micro-organisms grown in artificial media shall be the date the cultures are removed from the production incubators.

(4) If the harvest date cannot be established by the criteria provided in paragraph (a) (1), (2), or (3) of this section, the means of establishing the harvest date shall be written into the filed Outline of Production.

(b) *Live vaccines.* The expiration dates for live vaccines shall be determined in accordance with the conditions prescribed in this paragraph.

(1) Each serial of vaccine shall be tested for virus content at release and at its approximate expiration date until a statistically acceptable stability record has been established. All estimations of virus content shall be based on valid 50 percent end point titrations.

(2) The date of lyophilization shall not exceed 12 months from date of harvest. The storage conditions from date of harvest to date of lyophilization shall be shown to be suitable as established by adequate data.

(c) *Inactivated biological products.* The expiration dates for inactivated products, normal serums, and antiserums shall be determined in accordance with the conditions prescribed in a Standard Requirement or filed Outline of Production for the product. At no time shall the date be more than 3 years from the date of harvest.

(1) The expiration date for a product shall be determined by stability tests confirmed by potency tests on at least five

consecutive serials until 6 months beyond the date requested by the licensee.

(2) The expiration dates of antitoxins shall be calculated from the date of a satisfactory potency test as prescribed in a Standard Requirement or filed Outline of Production.

(3) Subsequent changes in the expiration date may be granted based upon stability data designed to show adequate potency of the product on or after the dating requested.

§ 114.14 Extension of the expiration date for a serial or subserial.

(a) The expiration date shall not be extended:

(1) If a U.S. Standard of Potency has not been developed for all fractions of the product; or

(2) For any serial or any portion of any serial which has left the licensed premises; or

(3) For a serial or portion of a serial if the expiration date has been extended previously.

(b) An extension of the expiration date may be granted if a request from the licensee is substantiated by valid test data obtained prior to the original expiration date and the following conditions are met:

(1) The new expiration date shall not exceed 6 months beyond the maximum time from harvest permitted by the currently filed Outline of Production; and

(2) Redated serials shall be retitrated or otherwise tested for potency by the licensee at or near the original expiration date and the results submitted to Veterinary Services. If found to be below the potency standards established by Veterinary Services, such serials shall be removed from the market.

§ 114.15 Disposal of unsatisfactory products and byproducts.

All biological products found to be unsatisfactory for marketing, all refuse or other materials deemed unsatisfactory for production purposes, all carcasses (part or whole) of production or test animals, and all undesirable byproducts of manufacture shall be disposed of as may be required by the Deputy Administrator.

§ 114.16 Producing subsidiaries.

(a) A production facility shall be used by only one producer (licensee or subsidiary) at one time except when cultures are incubated and when in-process, partially processed, or completed products are stored.

(b) For the purpose of maintaining separate production:

(1) Beginning of production shall be when the media is inoculated; or when tissue-producing animals or cell cultures are inoculated; or when the bleeding of serum animals occurs.

(2) Ending of production shall be when the product is a completed product in bulk containers with only bottling, labeling, or testing remaining.

(c) There shall be sufficient delay between operations of two producers to permit proper cleaning and disinfection of facilities when required.

(d) Storage of completed products may be in a common facility provided they are adequately identified. If not labeled, tags or markers shall be used.

§ 114.17 Rebottling of biological products.

The Deputy 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 Veterinary Services.

(e) The licensee shall not release the rebottled product unless notified by Veterinary Services that such product is eligible for release. Production records shall show the results of all tests conducted and shall accurately reflect the actions taken.

§ 114.18 Reprocessing of biological products.

The Deputy Administrator may authorize a licensee to reprocess a serial of completed product in liquid form 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) The serial shall be uniformly reprocessed and thoroughly mixed prior to bottling in final containers.

(c) All required 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.

(d) The reprocessed serial shall be identified by a new serial number and the records for the serial shall accurately reflect the action taken.

(e) Test samples of the reprocessed serial and test reports for all tests conducted shall be submitted to Veterinary Services. The licensee shall not release the serial until notified by Veterinary Services that the serial is eligible for release.

7. Part 116 is amended to read:

PART 116—RECORDS

Sec.

AUTHORITY: 37 Stat. 832–833; 21 U.S.C. 151–158.

§ 116.1 Applicability and general considerations.

Each licensee and each foreign manufacturer of biological products imported into the United States shall maintain detailed records of information necessary to give a complete accounting of the activities within each establishment. Such records shall include, but shall not be limited to the items enumerated in this part.

(a) Records shall be made concurrently with the performance of successive steps in the preparation of a biological product. 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 loss or gain from start to finish in such preparation.

(b) 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.

(c) 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.

§ 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 Deputy 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.

§ 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.

§ 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.

§ 116.5 Reports.

When required by the Deputy Administrator, reports containing accurate information of production activities in each establishment by the licensee or the foreign manufacturer whose products are either being offered for importation or being imported into the United States, as the case may be, shall be prepared and forwarded to Veterinary Services. Records necessary to make such reports shall be maintained in each establishment.

§ 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.

§ 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 Veterinary Services prior to release of the serial or subserial. Blank forms for such summaries shall be available from Veterinary Services upon request.

§ 116.8 Completion and retention of records.

All 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 may be marketed in the United States or exported. All records shall be retained for a period of 2 years after the expiration date of the product involved and for such longer period as may be required by the Deputy Administrator in specific cases.

NOTE.—The recordkeeping and reporting requirements contained herein have been approved by the Office of Management and Budget in accordance with the Federal Reports Act of 1942.

Effective Date: The amendments to Part 112 in § 112.2(a)(11), § 112.3(g), § 112.7(d)(4)(iv), § 112.7(d)(5), and § 112.7(h) shall become effective January 1, 1975. The amendments to Part 112 in § 112.2(a)(8), § 112.2(e), the lead paragraph in § 112.7, and § 112.7(g), and the amendments to Part 108, Part 113, Part 114, and Part 116 shall become effective June 10, 1974.

Done at Washington, D.C., this 6th day of MAY 1974.

J. M. HEJL,
Acting Deputy Administrator Veterinary Services, Animal and Plant Health Inspection Service.

[FR Doc.74-10880 Filed 5-9-74;8:45 am]

Title 10—Energy

CHAPTER II—FEDERAL ENERGY OFFICE

PART 211—MANDATORY PETROLEUM ALLOCATION REGULATIONS

Correction

In FR Doc. 74-10367, appearing at page 15960 in the issue for Monday, May 6, 1974, in § 211.2, between the undesignated first paragraph and paragraph (a), insert the following section heading:

§ 211.9 Supplier/purchaser relationships.

Title 12—Banks and Banking

CHAPTER II—FEDERAL RESERVE SYSTEM

SUBCHAPTER A—BOARD OF GOVERNORS OF THE FEDERAL RESERVE SYSTEM

PART 201—EXTENSIONS OF CREDIT BY FEDERAL RESERVE BANKS

Changes in Rates

Pursuant to section 14(d) of the Federal Reserve Act (12 U.S.C. 357), and for the purpose of adjusting discount rates with a view to accommodating commerce and business in accordance with other related rates and the general credit situation of the country, Part 201 is amended as set forth below:

1. Section 201.51 is amended to read as follows:

§ 201.51 Advances and discounts for member banks under sections 13 and 13a.

The rates for all advances and discounts under sections 13 and 13a of the Federal Reserve Act (except advances under the last paragraph of such section 13 to individuals, partnerships, or corporations other than member banks) are:

Federal Reserve Bank of—	Rate	Effective
Boston	8	Apr. 30, 1974
New York	8	Apr. 25, 1974
Philadelphia	8	Do.
Cleveland	8	Do.
Richmond	8	Do.
Atlanta	8	Apr. 29, 1974
Chicago	8	Apr. 26, 1974
St. Louis	8	Do.
Minneapolis	8	Do.
Kansas City	8	Apr. 25, 1974
Dallas	8	Do.
San Francisco	8	Do.

2. Section 201.52 is amended to read as follows:

§ 201.52 Advances to member banks under section 10(b).

The rates for advances to member banks under section 10(b) of the Federal Reserve Act are:

Federal Reserve Bank of—	Rate	Effective
Boston	8½	Apr. 30, 1974
New York	8½	Apr. 25, 1974
Philadelphia	8½	Do.
Cleveland	8½	Do.
Richmond	8½	Do.
Atlanta	8½	Apr. 29, 1974
Chicago	8½	Apr. 26, 1974
St. Louis	8½	Do.
Minneapolis	8½	Do.
Kansas City	8½	Apr. 25, 1974
Dallas	8½	Do.
San Francisco	8½	Do.

3. Section 201.53 is amended to read as follows:

§ 201.53 Advances to persons other than member banks.

The rates for advances under the last paragraph of section 13 of the Federal Reserve Act to individuals, partnerships, or corporations other than member banks secured by direct obligations of, or obligations fully guaranteed as to principal and interest by, the United States or any agency thereof are:

Federal Reserve Bank of—	Rate	Effective
Boston [1]	10	Apr. 30, 1974
New York	10	Apr. 25, 1974
Philadelphia	10	Do.
Cleveland	10	Do.
Richmond [1]	10	Do.
Atlanta [1]	10	Apr. 29, 1974
Chicago [1]	10	Apr. 26, 1974
St. Louis [1]	10	Do.
Minneapolis [1]	10	Do.
Kansas City [1]	10	Apr. 25, 1974
Dallas [1]	10	Do.
San Francisco	10	Do.

[1] A rate of 8 percent was approved, effective on the indicated dates on advances to nonmember banks, to be applicable in special circumstances resulting from implementation of changes in Regulation J (see 37 FR 12714).

(12 U.S.C. 248(1). Interprets or applies 12 U.S.C. 357.)

By order of the Board of Governors, May 1, 1974.

[SEAL] THEODORE E. ALLISON,
Assistant Secretary of the Board.

[FR Doc.74-10836 Filed 5-9-74;8:45 am]

Title 14—Aeronautics and Space

CHAPTER I—FEDERAL AVIATION ADMINISTRATION, DEPARTMENT OF TRANSPORTATION

[Airworthiness Docket No. 74-WE-18-AD; Amdt. 39-1842]

PART 39—AIRWORTHINESS DIRECTIVES

AiResearch Model TPE331-1-151B, -2-201C, -3U-307G, -5-251C -5-251K and -6-251M Engines

There have been failures of the fuel control system on Airesearch Model TPE 331-1-151B, -2-201C, -3U-307G, -5-251C, -5-251K and -6-251M engines that could result in the engine not properly responding to power and condition lever movement and setting. Since this condition is likely to exist or develop in other engines of the same type design, an airworthiness directive is being issued to require a modification and recurring inspection of the fuel control assembly

mounting and support bracket fasteners on AiResearch Model TPE331–1–151B, –2–201C, –3U–307G, –5–251C, –5–251K and –6–251M engines. The manufacturer has also issued operational instructions covering this subject. Although not mandatory, the agency concurs with the substance of these instructions, and urges observance herewith. The AD contains a note to reflect this determination.

Since a situation exists that requires immediate adoption of this regulation, it is found that notice and public procedure hereon are impracticable and good cause exists for making this amendment effective in less than 30 days.

In consideration of the foregoing, and pursuant to the authority delegated to me by the Administrator (31 FR 13697), § 39.13 of Part 39 of the Federal Aviation Regulations is amended by adding the following new airworthiness directive:

AiResearch Manufacturing Company of Arizona: Applies to Model TPE331–1–151B, –2–201C, –3U–307G, –5–251C, –5–251K, and –6–251M engines.

Compliance required as indicated.

To detect, correct and prevent loosening of the fuel control assembly mounting and support bracket fasteners, accomplish the following:

(a) Within the next 25 hours time in service after the effective date of this AD, unless previously accomplished, replace the fuel control assembly support bracket with a new bracket in accordance with instructions contained in paragraph 2.B. of AiResearch Service Bulletin TPE331–73–0028, dated April 29, 1974, or later FAA-approved revisions.

(b) Within 100 hours time in service after accomplishment of paragraph (a), above, or 25 hours additional time in service after the effective date of this AD, whichever occurs later, and intervals not to exceed 100 hours time in service thereafter, inspect and retorque the fuel control assembly and support bracket mounting fasteners in accordance with the procedures contained in paragraph 2.C. of AiResearch Service Bulletin TPE331–73–0028, dated April 29, 1974, or later FAA-approved revisions.

(c) Equivalent procedures may be approved by the Chief, Aircraft Engineering Division, FAA Western Region, upon submission of adequate substantiation data.

Note.—AiResearch Operating Information Letter OI–331–3, Revision A, dated April 19, 1974, provides pertinent operational information. Flight crews are urged to obtain a copy of this letter and acquaint themselves with the information presented therein.

This amendment becomes effective May 13, 1974.

This amendment is made under the authority of section 313(a), 601 and 603 of the Federal Aviation Act of 1958 (49 U.S.C. 1354(a), 1421 and 1423) and of section 6(c) of the Department of Transportation Act (49 U.S.C. 1655(c)).

Issued in Los Angeles, California, on May 2, 1974.

Arvin O. Basnight,
Director,
FAA Western Region.

[FR Doc.74–10778 Filed 5–9–74;8:45 am]

[Airworthiness Docket No. 74–WE–19–AD; Amdt. 39–1837]

PART 39—AIRWORTHINESS DIRECTIVES

AiResearch TSCP 700–4B Auxiliary Power Units

Pursuant to the authority delegated to me by the Administrator (31 FR 13697), an airworthiness directive was adopted April 17, 1974, and made effective immediately by telegram dated April 17, 1974, to all known United States operators or owners of aircraft in which the AiResearch Model TSCP 700–4B auxiliary power units (APU) are installed (McDonnell Douglas Model DC–10). The airworthiness directive requires the installation of a placard to prohibit all inflight operation of the APU. The APU may still be used in ground operations. The telegraphic AD constituted an interim action. An inspection program and modifications are under development for future AD action. Equivalent procedures and placards may be approved by the Chief, Aircraft Engineering Division, FAA Western Region.

Since it was found that immediate corrective action was required, notice and public procedure thereon was impracticable and contrary to the public interest and good cause existed for making the airworthiness directive effective immediately to all known operators or owners of aircraft incorporating AiResearch Model TSCP 700–4B Auxiliary Power Units (APU) (installed in McDonnell Douglas DC–10 Series airplanes). These conditions still exist and the airworthiness directive is hereby published in the Federal Register as an amendment to § 39.13 of Part 39 of the Federal Aviation Regulations to make it effective as to all persons:

AiResearch: Applies to Model TSCP 700–4B Auxiliary Power Units (APU) installed in McDonnell Douglas Model DC–10 aircraft.

Pursuant to the authority of the Federal Aviation Act of 1958, delegated to me by the Administrator the following airworthiness directive applicable to AiResearch Model TSCP 700–4B Auxiliary Power Units (APU) (installed in McDonnell-Douglas Model DC–10 Series Aircraft) is effective immediately upon receipt of this telegram because of numerous reports of fatigue cracks in the fuel control differential pressure regulator body. The presence of crack(s) can allow fuel to leak into the APU compartment. Compliance required as indicated, unless already accomplished. Within 25 hours additional time in service, install a placard in view of the flight crew to prohibit all in-flight operation of the APU. Thereafter the APU may not be used in flight but may be used in ground operations.

Note.—This is interim AD action. An inspection program and modifications are under development for future AD action. Equivalent procedures and placards may be approved by the Chief, Aircraft Engineering Division, FAA Western Region.

This amendment is effective May 13, 1974, for all persons except those to whom it was made effective immediately by telegram dated April 17, 1974.

This amendment is made under the authority of sections 313(a), 601 and 603 of the Federal Aviation Act of 1958 (49 U.S.C. 1354(a), 1421 and 1423) and of section 6(c) of the Department of Transportation Act (49 U.S.C. 1655(c)).

Issued in Los Angeles, California on May 1, 1974.

Arvin O. Basnight,
Director,
FAA Western Region.

[FR Doc.74–10779 Filed 5–9–74;8:45 am]

[Docket No. 74–NW–7–AD; Amdt. 39–1838]

PART 39—AIRWORTHINESS DIRECTIVES

Boeing Model 707–100, –100B and –200 Series Airplanes

There have been cracks in the upper wing splice at wing station 360 on Boeing Model 707 series airplanes. One crack was as long as 67 inches. These cracks impair the structural integrity of the wing and could lead to structural failure. Since this condition is likely to exist or develop in other airplanes of the same type design, an airworthiness directive is being issued to require inspection and repair, as necessary, of the upper wing splice at wing station 360.

Since a situation exists that requires immediate adoption of this regulation, it is found that notice and public procedure hereon are impracticable and good cause exists for making this amendment effective in less than 30 days.

In consideration of the foregoing, and pursuant to the authority delegated to me by the Administrator (31 FR 13697 § 39.13 of Part 39 of the Federal Aviation Administration is amended by adding the following new airworthiness directive:

Boeing: Applies to Boeing Model 707–100 series, –100B series and –200 series airplanes certificated in all categories.

Compliance required as indicated. To detect cracks in the upper wing splice plate and upper rib cap at wing station 360, accomplish the following:

Unless X-Ray inspected within the last 300 flights, X-Ray inspect the upper wing surface splice plate and rib cap at wing station 360 within the next 25 flights after the effective date of this AD, on airplanes with more than 24,000 flights, or within the next 50 flights after the effective date of this AD, on airplanes with more than 17,000 flights in accordance with instructions in Boeing Alert Service Bulletin No. 3160 dated April 19, 1974, or later FAA approved revisions, or in a manner approved by the Chief, Engineering and Manufacturing Branch, FAA, Northwest Region. If cracks are found, repair, as necessary, prior to further flight in accordance with instructions approved by the Chief, Engineering and Manufacturing Branch, FAA, Northwest Region.

The Manufacturer's specifications and procedures identified and described in this directive are incorporated herein and made a part hereof pursuant to 5 U.S.C. 552(a)(1). All persons affected by this directive who have not already received these documents may obtain copies upon request to The Boeing Company, P.O. Box 3707, Seattle, Washington 98124. These documents may also be examined at FAA Northwest Region, Boeing Field, Seattle, Washington.