

from each serial shall be tested for safety in young adult mice in accordance with the test provided in §113.33(b) unless:

(1) The product contains material which is inherently lethal for mice. In such instances, the guinea pig safety test provided in §113.38 shall be conducted in place of the mouse safety test.

(2) The product is recommended for poultry. In such instances, the product shall be safety tested in poultry as defined in the specific Standard Requirement or Outline of Production for the product.

(3) The product is recommended for fish, other aquatic species, or reptiles. In such instances, the product shall be safety tested in fish, other aquatic species, or reptiles as required by specific Standard Requirement or Outline of Production for the product.

(c) *Identity test.* Methods of identification of Master Seed Bacteria to the genus and species level by laboratory tests shall be sufficient to distinguish the bacteria from other similar bacteria according to criteria described in the most recent edition of "Bergey's Manual of Systematic Bacteriology" or the American Society for Microbiology "Manual of Clinical Microbiology". If Master Seed Bacteria are referred to by serotype, serovar, subtype, pilus type, strain or other taxonomic subdivision below the species level, adequate testing must be used to identify the bacteria to that level. Tests which may be used to identify Master Seed Bacteria include, but are not limited to:

- (1) Cultural characteristics,
- (2) Staining reaction,
- (3) Biochemical reactivity,
- (4) Fluorescent antibody tests,
- (5) Serologic tests,
- (6) Toxin typing,
- (7) Somatic or flagellar antigen characterization, and
- (8) Restriction endonuclease analysis.

(d) *Ingredient requirements.* Ingredients used for the growth and preparation of Master Seed Bacteria and of final product shall meet the requirements provided in §113.50. Ingredients of animal origin shall meet the applicable requirements provided in §113.53.

(e) Only serials tested for viricidal activity in accordance with the test

provided in §113.35 and found satisfactory by such test shall be packaged as diluent for desiccated fractions in combination packages.

(f) If formaldehyde is used as the inactivating agent and the serial has not been found satisfactory by the viricidal activity test, bulk or final container samples of completed product from each serial 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.)

[39 FR 16862, May 10, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 60 FR 14355, Mar. 17, 1995]

§ 113.101 *Leptospira Pomona* Bacterin.

Leptospira Pomona Bacterin shall be produced from a culture of *Leptospira pomona* which has been inactivated and is nontoxic. Each serial of biological product containing *Leptospira pomona* fraction shall meet the applicable requirements in §113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

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

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in §113.38.

(c) *Potency test.* Bulk or final container samples of completed product shall be diluted with physiological saline so that each 0.25 ml contains not more than 1/800th of the dose recommended on the label and shall be tested for potency, using the two-stage test provided in this paragraph.

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(1) *Vaccinates*. Inject each of at least 10 but not more than 12 young adult hamsters, each weighing 50 to 90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly, in accordance with the label recommendations for use.

(2) *Controls*. Retain at least 10 but not more than 12 additional hamsters from the same group as unvaccinated controls.

(3) *Challenge*. From 14 to 18 days postvaccination, challenge each of 10 vaccinates and each of 10 controls intraperitoneally with a suspension of virulent *Leptospira pomona* organisms, using a dose of 10-10,000 hamster LD₅₀ as determined by titration.

(4) *Post-challenge period*. Observe the vaccinates and controls for 14 days post-challenge and record all deaths. If eight or more controls die of leptospirosis, the test is valid and the results shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total dead hamsters for satisfactory serial	Cumulative total dead hamsters for unsatisfactory serial
1	10	10	2 or less	5 or more.
2	10	20	5 or less	6 or more.

(5) If three or four vaccinates die in the first stage, the second stage shall be conducted in a manner identical to the first stage.

(6) If the second stage is used, each serial shall be evaluated according to the second part of the table. On the basis of cumulative results, each serial shall either pass or fail.

[39 FR 16862, May 10, 1974, as amended at 40 FR 20067, May 8, 1975; 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.102 *Leptospira Icterohaemorrhagiae* Bacterin.

Leptospira Icterohaemorrhagiae Bacterin shall be produced from a culture of *Leptospira icterohaemorrhagiae* which has been inactivated and is nontoxic. Each serial of biological product containing *Leptospira icterohaemorrhagiae* fraction shall meet the applicable requirements in § 113.100 and be tested for purity, safety, and potency as prescribed in this section. A

serial found unsatisfactory by any prescribed test shall not be released.

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

(b) *Safety test*. Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.38.

(c) *Potency test*. Bulk or final container samples of completed product shall be diluted with physiological saline so that each 0.25 ml contains not more than 1/80th of the dose recommended on the label and shall be tested for potency, using the two-stage test provided in this paragraph.

(1) *Vaccinates*. Inject each of at least 10 but not more than 12 young adult hamsters, each weighing 50 to 90 grams, with 0.25 ml of the diluted bacterin either subcutaneously or intramuscularly, in accordance with the label recommendations for use.

(2) *Controls*. Retain at least 10 but not more than 12 additional hamsters from the same group as unvaccinated controls.

(3) *Challenge*. From 14 to 18 days postvaccination, challenge each of 10 vaccinates and each of 10 controls intraperitoneally with a suspension of virulent *Leptospira icterohaemorrhagiae* organisms, using a dose of 10-10,000 hamster LD₅₀ as determined by titration.

(4) *Post-challenge period*. Observe the vaccinates and controls for 14 days post-challenge and record all deaths. If eight or more controls die from leptospirosis, the test is valid and the results shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total dead hamsters for satisfactory serial	Cumulative total dead hamsters for unsatisfactory serial
1	10	10	2 or less	5 or more.
2	10	20	5 or less	6 or more.

(5) If three or four vaccinates die in the first stage, the second stage shall be used. The second stage shall be conducted in a manner identical to the first stage.

(6) If the second stage is used, each serial shall be evaluated according to