

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

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

§ 113.7 Multiple fractions.

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

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

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

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

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

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

§ 113.8 In vitro tests for serial release.

(a) Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seed for production as specified in the Standard Requirements or in the filed Outline of Production. The Administrator may exempt a product from a required animal potency test for

release when an evaluation can, with reasonable certainty, be made by:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(c) In the case of inactivated products, bulk or final container samples of completed product from each serial derived from an approved Master Seed, shall be evaluated for relative antigen content (potency) as compared with an unexpired reference by a parallel line immunoassay or other procedure ac-

ceptable to APHIS.¹ Firms currently using immunoassays which do not satisfy this requirement shall have 2 years from the effective date of the final rule to update their filed Outlines of Production to be in compliance with this requirement unless granted an extension by the Administrator based on a showing by the firm seeking the extension that they have made a good faith effort with due diligence to achieve compliance. On the basis of the results of such test procedures, each serial that meets the required minimum potency shall be released to the firm for marketing; each serial not meeting the required minimum potency shall be withheld from the market. The evaluation of such products shall be made in accordance with the following criteria:

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

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

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

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

(i) If: The first retest (test 2) following an initial equivocal test; the second retest (test 3) following two consecutive equivocal tests (tests 1 and

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

2); or the third retest (test 4) following three consecutive equivocal tests (tests 1, 2, and 3) shows that the potency equals or exceeds the required minimum potency, the serial is satisfactory.

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

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

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

(ii) If one of the retests (tests 2 or 3) shows that the potency equals or exceeds the required minimum potency and the other retest (test 2 or 3) is an equivocal test, a third retest (test 4) may be performed. If the third retest (test 4) shows that the potency of the

serial equals or exceeds the required minimum potency, the serial is deemed satisfactory. If both retests (tests 2 and 3) or if the third retest (test 4) is an equivocal test, the accumulated test results shall be considered indicative of a lack of potency and release of the serial withheld, in which case the licensee may submit data confirming the continued validity of the test system to APHIS for review and approval. If the data are acceptable to APHIS, the potency test may be repeated by the firm, subject to the provisions specified in paragraphs (c)(4) (i) and (ii) and (c)(5) (i) and (ii) of this section, and confirmatory testing by APHIS.

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

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established, it shall be allowed an initial dating period equal to the dating of the product or as supported by data acceptable to APHIS, except that frozen references may have an initial dating period of 5 years, or as supported by data acceptable to APHIS. Prior to the expiration date, such reference may be granted an extension of dating by confirming its immunogenicity using a Qualifying Serial of product.

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

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

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

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

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

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

(i) The Administrator shall require that at least two additional serials prepared with the same Master Seed be

subjected to similar animal potency tests by Animal and Plant Health Inspection Service or the licensee or both.

(ii) If another serial is found unsatisfactory for potency, the product shall be removed from the market while a reevaluation of the product is made and the problem is resolved.

[49 FR 22625, May 31, 1984, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 62 FR 19038, Apr. 18, 1997; 72 FR 72564, Dec. 21, 2007]

§ 113.9 New potency test.

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

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

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

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

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

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

[49 FR 45846, Nov. 21, 1984]