

specificity in accordance with the conditions prescribed for each test. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Density requirements.* A 2.5 ml sample of completed antigen shall be diluted with 2.5 ml of buffer solution formulated in the same manner as the vehicle of the antigen being tested in a modified Hopkins tube and then sedimented at 1,000 x g in a refrigerated centrifuge at 20 °C for 90 minutes. If the packed cell volume of the completed antigen is not 1.2 percent (± 0.4 percent), the serial is unsatisfactory.

(b) *Preservative requirements.* Preservatives shall be as specified in the Outline of Production filed with APHIS in accordance with 9 CFR 114.8. If phenol is used, a direct titration with a standardized bromide-bromate solution shall be made. If the final concentration of phenol is not 0.25 percent (± 0.05 percent), the serial is unsatisfactory.

(c) *Homogeneity requirements.* (1) Plate antigen shall be checked on a plate for homogeneity and autoagglutination. If plate antigen is not homogeneous and free of large visible particles (strands or clumps) or if it autoagglutinates, the serial is unsatisfactory.

(2) Stereo-microscopic examination shall be used when necessary to evaluate a granular appearing antigen.

(d) *Hydrogen ion concentration.* The hydrogen ion concentration shall be determined with a pH meter which has been standardized with a pH buffer just prior to use. The pH of *Mycoplasma Gallisepticum* Antigen shall be 6.0 ± 0.2 . The pH of *Mycoplasma Synoviae* Antigen and *Mycoplasma Meleagridis* Antigen shall be 7.0 ± 0.2 .

(e) *Purity requirements.* The antigen shall be tested for viable bacteria and fungi as prescribed in § 113.26.

(f) *Sensitivity requirements.* The reactivity of each antigen shall be tested by comparing the agglutination reactions of each serial of antigen with the agglutination reactions of a standard reference antigen which is supplied by or acceptable to APHIS. A set consisting of five known positive and five known negative serums shall be used. The negative serums shall be tested against the antigens undiluted and the positive serums shall be tested against the antigens diluted 1:4 in buffer solu-

tion formulated in the same manner as the vehicle of the antigen being tested. If negative serums do not have negative reactions in this test, the serial is unsatisfactory. If the test antigen and the reference antigen do not have the same agglutination reactions with at least four of the five positive serums used, the serial is unsatisfactory.

(1) The sensitivity of *Mycoplasma Gallisepticum* Antigen shall be tested using a set of chicken and a set of turkey serums (the positive serums shall have varying degrees of reactivity from weakly positive to strongly positive).

(2) The sensitivity of *Mycoplasma Synoviae* Antigen shall be tested using chicken serums.

(3) The sensitivity of *Mycoplasma Meleagridis* Antigen shall be tested using turkey serums.

(g) *Specificity requirements.* *Mycoplasma Synoviae* Antigen shall be examined for cross-agglutination with five *Mycoplasma gallisepticum* antiserums (chicken origin); *Mycoplasma Meleagridis* Antigen shall be examined for cross-agglutination with five *Mycoplasma gallisepticum* antiserums (turkey origin) and five *Mycoplasma synoviae* antiserums (turkey origin). Tests shall be conducted with undiluted antigen. If cross-agglutination occurs, the serial is unsatisfactory.

[48 FR 33474, July 22, 1983. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.409 Tuberculin—PPD Bovis, Intradermic.

Tuberculin—PPD Bovis, Intradermic is a purified protein derivative produced from cultures of *Mycobacterium bovis* Strain AN-5 (supplied by Animal and Plant Health Inspection Service), which has been inactivated and is nontoxic. Each serial shall be tested for purity, safety, potency, and special chemical characteristics in accordance with the conditions prescribed for each test. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Each serial shall be tested for viable bacteria and fungi as prescribed in § 113.26.

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

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be subjected to a comparison specificity test using a Reference PPD Tuberculin supplied by Animal and Plant Health Inspection Service.

(1) *Test animals.* White female guinea pigs from one source, which weigh 500 to 700 grams at the beginning of the test, and which have not been used in a previous test, shall be used in the specificity test. Twenty-three guinea pigs (10 sensitized with *M. bovis*, 10 sensitized with *M. avium* and three unsensitized) shall be required for each serial being tested, and 20 guinea pigs (10 sensitized with *M. bovis* and 10 sensitized with *M. avium*) shall be required for the Reference PPD Tuberculin. Allowance should be made for deaths during the sensitization period.

(2) *Sensitization of guinea pigs.*

(i) Sensitize one group of guinea pigs to *M. bovis*. Inject each animal intramuscularly with 0.5 ml of a sterile heat-killed suspension of *M. bovis* Strain AN-5 supplied by Animal and Plant Health Inspection Service.

(ii) Sensitize one group of guinea pigs to *M. avium*. Inject each animal intramuscularly with 0.5 ml of a sterile heat-killed suspension of *M. avium* Strain D-4 supplied by Animal and Plant Health Inspection Service.

(iii) Maintain an unsensitized group as control animals.

(3) Thirty-five days post-injection, the guinea pigs shall be used for tuberculin testing.

(4) The sensitized animals and controls shall be prepared at least 4 hours prior to injection of PPD tuberculin by clipping the hair from the entire abdominal and flank areas, applying a depilatory agent for 5 to 10 minutes, then rinsing with warm water and drying.

(i) Select four sites on each guinea pig for injection of PPD tuberculin. Two sites shall be on each side of the midline and spaced a sufficient distance from each other to avoid overlapping of skin reactions.

(ii) Prepare four dilutions of the Reference PPD Tuberculin and each serial

of PPD tuberculin being tested so as to contain 0.6, 1.2, 2.4, and 4.8 micrograms of protein per 0.1 ml dose. Each of the four dilutions of the same tuberculin shall be randomly assigned a site on a guinea pig.

(iii) Inject one dose of each dilution at the assigned site using a tuberculin syringe.

(5) *Measurement of skin reactions.* Measure the area of erythema produced at each site on each guinea pig 24 hours following injection of PPD tuberculin. Measurements in millimeters shall be made anterior to posterior across the greatest diameter and perpendicular to the first measurement. Calculate the area of erythema in square millimeters at each site by multiplying the two measurements.

(6) *Calculation of average response per guinea pig.* Obtain the total area of erythema for each guinea pig by adding the areas of the four test sites. Add these composite areas of erythema from all guinea pigs with the same sensitization and the same PPD tuberculin injection, then divide by the number of animals in the group. The number obtained is the average response per guinea pig to the PPD tuberculin for the given type of sensitization.

(7) *Determination of specificity index.* The specificity index of a PPD tuberculin is determined by subtracting the average response obtained on *M. avium* sensitized guinea pigs from the average response obtained on *M. bovis* sensitized guinea pigs.

(8) *Validity of bioassay.* The bioassay test results obtained on serials tested concurrently in a single test series are valid if the specificity index of the reference PPD tuberculin is at least 400 square millimeters. If the results are not valid, the bioassay test series must be repeated with a different set of sensitized guinea pigs.

(9) *Reactions in unsensitized guinea pigs.* If a positive reaction (erythema) is observed in one or more of the 3 unsensitized guinea pigs, the serial is unsatisfactory.

(10) *Interpretation of specificity index.* When a bioassay is valid and reactions are not observed in unsensitized guinea pigs, the following interpretation of the specificity index will be used for

classifying each serial of PPD tuberculin:

Specificity index	Classification
440 mm ² or greater	Satisfactory.
Between 360 mm ² and 440 mm ²	Inconclusive.
Less than 360 mm ²	Unsatisfactory.

(11) *Second stage test.* If a serial is classified as inconclusive, it can be declared unsatisfactory or undergo a second stage test. The second stage shall be conducted in a manner identical to the first stage, except that unsensitized guinea pig controls are not necessary. The results are evaluated by combining the results obtained on all guinea pigs tested in stages one and two. Calculate the average response on the 20 *M. bovis* sensitized animals and on the 20 *M. avium* sensitized animals and determine the specificity index. An inconclusive serial is satisfactory after the second stage test, if its specificity index is 400 square millimeters or more, and unsatisfactory if its specificity index is less than 400 square millimeters.

(d) *Special chemical tests and requirements.* Final container samples of completed product from each serial shall be tested as follows:

(1) *Protein concentration.* The final product shall contain a protein concentration of 1.0±0.1 mg/ml. The Microkjeldahl Test for Nitrogen shall be used.

(2) *Phenol content.* Phenol content of the final product shall be 0.50 percent plus or minus 0.04 percent. A direct titration with a standardized bromide-bromate solution shall be conducted.

[41 FR 8471, Feb. 27, 1976, as amended at 41 FR 21760, May 28, 1976; 41 FR 32883, Aug. 6, 1976. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991]

ANTIBODY PRODUCTS

§ 113.450 General requirements for antibody products.

Unless otherwise prescribed in a Standard Requirement or in a filed Outline of Production, all antibody products shall meet the applicable requirements of this section.

(a) *Terminology.* The following terms in the regulations and standards concerning antibody products shall mean:

Antibody. An immunoglobulin molecule, having a precise glycoprotein structure, produced by certain cells of the B lymphocyte lineage in response to antigenic stimulation, and functioning to specifically bind and influence the antigens that induced its synthesis.

IgG (Immunoglobulin G). One of the several recognized classes of structurally related glycoproteins whose representatives include all known antibodies.

Monoclonal. Produced by, or derived from, the offspring of a single common progenitor cell.

Failure of passive transfer. A condition of neonates characterized by an abnormally low concentration of circulating maternal IgG.

(b) *Nomenclature.* Antibody products shall be named as follows:

(1) *Virus-specific products.* The true name of a virus-specific product shall include the term "antibody," specify the disease for which the product is intended, and indicate the type of animal that supplied the component antibodies. If the antibodies are monoclonal, the term "monoclonal" shall be used. Example: "Duck Virus Hepatitis Antibody, Duck Origin."

(2) *Bacterium-specific products.* The true name of a bacterium-specific product shall include the term "antibody" if the component antibodies are directed against a nontoxin antigen or the term "antitoxin" if the component antibodies are directed against toxin, specify the organism against which the product is intended, and indicate the type of animal that supplied the component antibodies. If the antibodies are monoclonal, the term "monoclonal" shall be used. Example: "Escherichia Coli Monoclonal Antibody, Murine Origin."

(3) *Failure of passive transfer products.* The true name of a product for treatment of failure of passive transfer shall include the term "IgG" and indicate the type of animal that supplied the component IgG. Example: "Bovine IgG."

(4) *Combination products.* The true name of a product for treatment of failure of passive transfer as well as for the prevention and/or alleviation of a specific viral or bacterial disease shall