§ 113.408 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 a dye acceptable to Animal and Plant Health Inspection Service (APHIS). 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 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×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 4.0 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 solution 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 antiseraums (chicken origin); Mycoplasma Meleagridis Antigen shall be examined for cross-agglutination with five Mycoplasma gallisepticum antiseraums (turkey origin) and five Mycoplasma synoviae antiserums (turkey origin). Tests shall be conducted with undiluted antigen. If cross-agglutination occurs, the serial is unsatisfactory.


§ 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 §113.28.

(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.

(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.