

§ 113.407

(5) *Clarity.* The product shall be optically clear and free from any extraneous particles.

[39 FR 16857, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.407 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

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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. 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.

[39 FR 16857, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, and amended at 40 FR 760, Jan. 3, 1975. Redesignated at 55 FR 35561, Aug. 31, 1990]

§ 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 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.