

§ 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