§ 147.5 The microagglutination test for pullorum-typhoid.

Routinely, the microagglutination test is applied as a single-dilution test and only a single 18–24 hour reading is made.

(a) The procedure for the collection and delivery of blood samples in the microagglutination test is the same as that described in §147.1(a). A method that has proven advantageous is to transfer the serum samples from the blood clot to a microplate as described in “Applied Microbiology,” volume 24, No. 4, October 1972, pages 671–672. The dilutions are then performed according to paragraphs (d) or (e) of this section.

(b) Stained microtest antigen for pullorum-typhoid is supplied as concentrated stock suspension and must be approved by the Department. Directions for diluting will be provided with the antigen. The stock as well as the diluted antigen prepared each day should be kept sealed in the dark at 5° to 10 °C, when not in use.

(c) Available data indicate that a 1:40 dilution for the microagglutination test is most efficient for the detection of pullorum-typhoid agglutinins in both chickens and turkeys. In all official reports on the blood test, the serum dilutions shall be indicated.

(d) The recommended procedure for the 1:40 dilution in the microagglutination test is as follows:

1. Add 100 microliters (0.10 cc.) of 0.85 percent physiological saline to each well of the microplate.
2. Using a microdiluter or a multimicrodiluter handle fitted with twelve 10 microliter microdiluters, transfer 5 microliters (0.005 cc.) of the serum sample from the collected specimen to the corresponding well of the microplate. This is accomplished by touching the surface of the serum sample with the microdiluter and then transferring and mixing with the diluent in the microplate well. The microdiluter is removed, blotted, touched to the surface of the distilled water wash, and again blotted. Other acceptable methods of serum delivery are described in “Applied Microbiology,” volume 21, No. 3, March 1971, pages 394–399.
3. Dilute the microtest antigens with 0.50 percent phenolized saline and add 100 microliters (0.1 cc.) to each microplate well.


(4) Information as to criteria and procedures for approval of concentrated stock suspension of stained microtest antigens may be obtained from the National Poultry Improvement Plan, Veterinary Services, APHIS, USDA, 1506 Klondike Road, Suite 300, Conyers, GA 30012.
§ 147.6 Procedure for determining the status of flocks reacting to tests for Mycoplasma gallisepticum, Mycoplasma synoviae, and Mycoplasma meleagridis.


(a) The status of a flock for Mycoplasma shall be determined according to the following criteria:

(1) If the tube agglutination test, enzyme-labeled immunosorbent assay (ELISA), official molecular examination procedure, or serum plate test is negative, the flock qualifies for the classification for which it was tested.

(2) If the tube agglutination, ELISA, or serum plate test is positive, the hemaglutination inhibition (HI) test or a molecular examination procedure shall be conducted: Provided, for the HI test, that if more than 50 percent of the samples are positive for M. gallisepticum, M. meleagridis, or M. synoviae, the HI test shall be conducted on 10 percent of the positive samples or 25 positive samples, whichever is greater. HI titers of 1:40 or more may be interpreted as suspicious and appropriate antigen detection samples should be taken promptly (within 7 days of the original sampling) from 30 clinically affected birds and examined by an approved cultural technique individually, or pooled (up to 5 swabs per test) and used in a molecular examination procedure or in vivo bioassay.