§ 147.4 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 30094.

(a) The procedure for the collection and delivery of blood samples in the microagglutination test is applied as a single-dilution test and only a single 18–24 hour reading is made.

(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.

§ 147.5 The microagglutination test for pullorum-typhoid.

Routinely, the microagglutination test is applied as a single-dilution test 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.

(d) Various degrees of reaction are observed in this as in other agglutination tests. The greater the agglutinating ability of the blood, the more rapid the clumping and the larger the clumps. A positive reaction consists of a definite clumping of the antigen surrounded by clear spaces. Such reaction is easily distinguished against a white background. A somewhat weaker reaction consists of small but still clearly visible clumps of antigen surrounded by spaces only partially clear. Between this point and a negative or homogenous smear, there sometimes occurs a very fine granulation barely visible to the naked eye; this should be disregarded in making a diagnosis. The very fine marginal clumping which may occur just before drying up is also regarded as negative. In a nonreactor, the smear remains homogeneous. (Allowance should be made for differences in the sensitiveness of different antigens and different set-ups, and therefore, a certain amount of independent, intelligent judgment must be exercised at all times. Also, the histories of the flocks require consideration. In flocks where individuals show a suspicious agglutination, it is desirable to examine representative birds bacteriologically to determine the presence or absence of S. pullorum.)

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(4) Seal each plate with a plastic sealer or place unsealed in a tight incubation box as described in “Applied Microbiology,” volume 23, No. 5, May 1972, pages 931–937. Incubate at 37°C for 18–24 hours.

(5) Read the test results as described in paragraph (f) of this section.

(e) The recommended procedure for a microagglutination test titration is as follows:

(1) Add 50 microliters (0.05 cc.) of 0.85 percent physiological saline to each well of the microplate.

(2) To the wells representative of the lowest dilution in the titration, add an additional 50 microliters (0.05 cc.) of 0.85 percent physiological saline making a total of 100 microliters in these wells.

(3) Transfer each serum sample as described in § 147.5(d)(2) of this section to the first well containing 100 microliters (0.10 cc.) in the titration, which represents the lowest dilution.

(4) Make twofold serial dilutions of each serum by transferring 50 microliters (0.05 cc.) of diluted serum from one well to the next using twelve 50 microliter microdiluters fitted in a multitmicroliter handle. When transfers have been made to all of the wells of the desired series, the 50 microliters remaining in the microdiluters are removed by blotting, touching the microdiluters to the surface of the distilled water wash, and blotting again.

(5) Dilute the desired microtest antigen with 0.50 percent phenolized saline and add 50 microliters (0.05 cc.) to each microplate well.

(6) Seal each plate with a plastic sealer or place the unsealed microplates in a tight incubation box and incubate at 37°C for 18–24 hours.

(7) Read the test results as described in paragraph (f) of this section.

(f) Read the test results with the aid of a reading mirror. Results are interpreted as follows:

(1) N, or (negative) when the microplate well has a large, distinct button of stained cells; or

(2) P, or + (positive) when the microplate well reveals no antigen button; or

(3) S, or ? (suspicious) when the microplate well has a small button. Suspicious reactions may tend to be more positive than negative [+] or vice versa [−] and can be so noted if desired.

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§ 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 hemagglutination 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.