carefully withdrawn, the loop becomes properly filled. On looking down edgewise at the filled loop, one observes that the blood appears to bulge. The loopful of blood then should be stirred into the drop of antigen, and the mixture spread to a diameter of about 1 inch. The loop then should be rinsed in clean water and dried by touching it to a piece of clean blotting paper, if necessary. The test plate should be rocked from side to side a few times to mix the antigen and blood thoroughly, and to facilitate agglutination. The antigen should be used according to the directions of the producer.

(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 homogeneous 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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[36 FR 23121, Dec. 3, 1971. Redesignated at 44 FR 61586, Oct. 26, 1979, as amended at 59 FR 12799, Mar. 18, 1994]

§ 147.4 [Reserved]

§ 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.[4] 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 1:20 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 1:20 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 10 microliters (0.01 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

[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, 1498 Klondike Road, Suite 200, Conyers, GA 30094.

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) 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 (0.05cc.) 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 (0.10cc.) in the titration, which represents the lowest dilution.

(4) Make twofold serial dilutions of each serum by transferring 50 microliters (0.05cc.) of diluted serum from one well to the next using twelve 50 microliter microdiluters fitted in a multimicrodiluter 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.

The macroagglutination tests for Mycoplasma antibodies, as described in ''Standard Methods for Testing Avian Sera for the Presence of Mycoplasma Gallisepticum Antibodies'' published by the Agricultural Research Service, USDA, March 1966, and the microagglutination tests, as reported in the Proceedings, Sixteenth Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, 1973, shall be the official tests. Procedures for isolation and identification of Mycoplasma may be found in Isolation and Identification of Avian Pathogens, published by the American Association of Avian Pathologists and §§147.15 and 147.16.

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

(1) If the tube agglutination or the serum plate test is negative, the flock qualifies.

(2) If the tube agglutination or the serum plate test is positive, the hemaglutination inhibition (HI) test and/or the Serum Plate Dilution (SPD) test shall be conducted. *Provided*, that for egg-type and meat-type chicken and waterfowl, exhibition poultry, and