

strains should be combined in equal volume-density and stored in the refrigerator (5 °to 10 °C.) in tightly stoppered bottles.

(g) The tests should be conducted on a suitable, smooth plate. The serum-antigen dilution should be made so that the dilution will not exceed 1:50 when compared to the standard tube agglutination test. When testing turkey blood samples, it is desirable to use a serum-antigen dilution equivalent to the 1:25 in the tube method. The serum should be added to the antigen and mixed thoroughly by use of the tip of the serum pipette. Most strong positive reactions will be plainly evident within 15 to 20 seconds. The final reading should be made at the end of 2 or 3 minutes. Heating the plate at approximately 37 °C. will hasten agglutination. Before reading, the plate should be rotated several times.

(h) The results shall be recorded as described in § 147.1(h).

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§ 147.3 The stained-antigen, rapid, whole-blood test.³

(a) The description of the preparation of antigen is not herein included because the antigen is a proprietary product produced only under license from the Secretary of Agriculture.

(b) A loop for measuring the correct quantity of blood can usually be obtained from the manufacturer of the antigen. A satisfactory loop may be made from a piece of No. 20 gage nichrome wire, 2½ inches long, at the end of which is fashioned a loop three-sixteenths of an inch in diameter. Such a loop, when filled with blood so that the blood appears to bulge, delivers 0.02 cc. A medicine dropper whose tip is adjusted to deliver 0.05 cc. is used to measure the antigen. A glass plate about 15 inches square, providing space for 48 tests, has proved satisfactory for this work. The use of such a plate en-

ables the tester to have a number of successive test mixtures under observation without holding up the work to wait for results before proceeding to the next bird.

(c) A drop of antigen should be placed on the testing plate. A loopful of blood should be taken up from the wing vein. When submerged in the blood and then carefully withdrawn, the loop becomes properly filled. On looking down edge-wise 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

³The procedure described is a modification of the method reported by Schaffer, MacDonald, Hall, and Bunyea, Jour. Amer. Vet. Med. Assoc. 79 (N. S. 32): 236-240 (1931).

§ 147.4

to determine the presence or absence of *S. pullorum*.)

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§ 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 1:40 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,

⁴Information 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.

9 CFR Ch. I (1-1-07 Edition)

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) 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.