

§ 147.2 The rapid serum test.²

(a) The procedure for the collection and delivery of blood samples in the rapid serum test is the same as that described in §147.1(a).

(b) The selection and maintenance of suitable strains of *S. pullorum* and the composition of a satisfactory medium are described in §147.1 (b) and (c).

(c) Large 1-inch test tubes, Kolle flasks, or Blake bottles are streaked liberally from 48-hour slant-agar cultures prepared from stock cultures of the selected strains.

(d) The antigen-growing tubes or bottles should be incubated 48 hours at 37 °C., and the surface growth washed off with a very slight amount of 12 percent solution of sodium chloride containing 0.25 to 0.5 percent phenol, filtered through lightly packed sterile absorbent cotton placed in the apex of a sterile funnel.

(e) The washings should be adjusted (using 12 percent sodium chloride containing 0.25 to 0.5 percent phenol) so that the turbidity is 50 times greater than tube 0.75 of McFarland's nephelometer, or to a reading of 7 mm. by the Gates nephelometer.

(f) The individual strain antigens should be tested with negative sera for their insensitivity and with positive sera for high agglutinability in comparison with known satisfactory antigen. The antigens of the separate 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 read-

ing 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 enables 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

²The procedure described is a modification of the method reported by Runnels, Coon, Farley, and Thorpe, *Amer. Vet. Med. Assoc. Jour.* 70 (N. S. 23): 660-662 (1927).

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).

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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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§ 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, 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.

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, 1506 Klondike Road, Suite 300, Conyers, GA 30094.