

(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 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.<sup>3</sup>

(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 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

<sup>3</sup>The 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).

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 The tube agglutination test for *S. typhimurium*.**

(a) The procedure for the collection and delivery of blood samples in the tube agglutination test for *S. typhimurium* is the same as that described in § 147.1(a).

(b) The “O” antigen should be prepared as follows:

(1) The antigen shall consist of a representative nonmotile strain of *S. typhimurium* which is of known antigenic composition and high agglutinability but is not sensitive to negative and nonspecific sera. Strain P 10 meets these requirements.

(2) The stock culture is maintained on 1 percent nutrient agar deeps, which have been incubated for 18–24 hours at 37° C. They are stored at room temperature.

(3) A satisfactory medium used for growing the organism is veal infusion agar (Difco). It is dispensed in 50 ml.

amounts into 500 ml. medicine bottles, with screw caps, and sterilized at 15 pounds pressure for 20 minutes. The bottles are then laid flat upon an even surface until the medium has solidified.

(4) The inoculum used for preparation of “O” antigen is a nonmotile strain of *S. typhimurium*. The organism is grown in veal infusion broth (Difco) for 18–24 hours at 37° C.; then plated, for single colony isolation, on veal infusion agar plates. These plates are incubated for 18–24 hours at 37° C. After incubation, single colonies are picked and transferred to veal infusion agar slants, which are incubated for 18–24 hours at 37° C. After this, the cultures are tested for smoothness by using a 1:500 dilution of acriflavine.

(5) Smooth cultures are inoculated into flasks containing veal or beef infusion broth which is incubated for 18–24 hours at 37° C. The incubated broth suspension of organisms is dispensed into the antigen bottles containing veal infusion agar. The suspension is distributed evenly over the agar surface by gently tilting the bottles from side to side. The inoculated bottles are then laid flat, agar side down, for 10–20 minutes. They are subsequently incubated, agar side upward, for 24–48 hours at 37° C. before harvesting.

(6) The harvesting of the organism consists of washing the growth from each antigen bottle with 0.5 percent phenolized physiological saline. The bacterial suspension from each bottle is filtered through sterile milk pad filters into a large sterile container or through a thin layer of absorbent cotton in a Buchner funnel with the aid of suction. To each 100 ml. of the bacterial suspension is added additional phenol to make the final concentration 0.5 percent. The concentrated antigen is tested for sterility at intervals after 24 hours. After sterility is proved, the stock antigen is standardized to determine the density according to the McFarland nephelometer scale.

(7) The diluted antigen to be used in routine testing is prepared from stock antigen, by diluting with 0.25 percent phenolized saline, and is standardized to a turbidity corresponding to 0.75–1.00 of the McFarland nephelometer scale.