§ 147.2 The rapid serum test.

(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 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.

(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...
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§ 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. 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 °C 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.

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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. 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 °C 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.

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