

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 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 When submerged in the blood and then carefully withdrawn, the loop becomes properly filled.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

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

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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§ 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.*