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 When submerged in the blood and then carefully withdrawn, the loop becomes properly filled.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

(Approved by the Office of Management and Budget under control number 0579–0007)

§ 147.6 Procedure for determining the status of flocks reacting to tests for Mycoplasma gallisepticum, Mycoplasma synoviae, and Mycoplasma meleagridis.

The macroagglutination tests for Mycoplasma antibodies, as described in “Standard Methods for Testing Avian Sera for the Presence of Mycoplasma Gallisepticum Antibodies” published by the Agricultural Research Service, USDA, March 1966, and the microagglutination tests, as reported in the Proceedings, Sixteenth Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, 1973, shall be the official tests. Procedures for isolation and identification of Mycoplasma may be found in Isolation and Identification of Avian Pathogens, published by the American Association.