

(6) Seal each plate with a plastic sealer or place the unsealed microplates in a tight incubation box and incubate at 37 °C. for 18-24 hours.

(7) Read the test results as described in paragraph (f) of this section.

(f) Read the test results with the aid of a reading mirror. Results are interpreted as follows:

(1) N, or - (negative) when the microplate well has a large, distinct button of stained cells; or

(2) P, or + (positive) when the microplate well reveals no antigen button; or

(3) S, or ? (suspicious) when the microplate well has a small button. Suspicious reactions may tend to be more positive than negative [ $\pm$ ] or vice versa [ $\mp$ ] and can be so noted if desired.

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[41 FR 48726, Nov. 5, 1976. Redesignated at 44 FR 61586, Oct. 26, 1979, and amended at 57 FR 57342, Dec. 4, 1992; 59 FR 12799, Mar. 18, 1994; 59 FR 67617, Dec. 30, 1994; 61 FR 11521, Mar. 21, 1996; 63 FR 3, Jan. 2, 1998; 67 FR 8469, Feb. 25, 2002]

**§ 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 of Avian Pathologists and §§147.15 and 147.16.

(a) The status of a flock for *Mycoplasma* shall be determined according to the following criteria:

(1) If the tube agglutination or the serum plate test is negative, the flock qualifies.

(2) If the tube agglutination or the serum plate test is positive, the

hemagglutination inhibition (HI) test and/or the Serum Plate Dilution (SPD) test shall be conducted. *Provided*, that for egg-type and meat-type chicken and waterfowl, exhibition poultry, and game bird flocks, if more than 50 percent of the samples are positive for either *Mycoplasma gallisepticum*, *M. synoviae*, or both, the HI and/or the SPD test shall be conducted on 10 percent of the positive samples or 25 positive samples, whichever is greater. The results of the HI and/or SPD tests must be followed by the action prescribed in paragraphs (a)(3), (a)(4), and (a)(5) of this section.

(3) If the tube agglutination or serum plate tests are positive and HI and/or the SPD tests are negative, the flock shall be retested in accordance with paragraph (a)(6) of this section.

(4) If HI titers of 1:40 or SPD titers of 1:5 are found, the flock shall be considered suspicious and shall be retested in accordance with paragraph (a)(6) of this section.

(5) If HI titers of 1:80, positive enzyme-labeled immunosorbent assay (ELISA) titers, or SPD titers of 1:10 or higher are found, the Official State Agency shall presume the flock to be infected. If the indicated titers are found, tracheal swabs from 30 randomly selected birds shall be taken promptly and cultured individually or a PCR-based procedure conducted on these specimens for *Mycoplasma*, and additional tests conducted in accordance with paragraph (a)(6) of this section before final determination of the flock status is made.

(6) Fourteen days after the previous bleeding date, all birds or a random sample comprised of 75 birds shall be tested by the serum plate or tube agglutination test. Tested birds shall be identified by numbered bands.

(7) If the tube agglutination test or serum plate test is negative for the *Mycoplasma* for which the flock was tested, the flock qualifies.

(8) If the tube agglutination or serum plate test is positive on the retest, the HI and/or SPD test shall be conducted on the reacting samples.

(9) On the retest, if the tube agglutination or serum plate tests are positive at the same or higher rate and the HI or SPD tests are negative, the flock

shall be considered suspicious and shall be retested in accordance with paragraph (a)(6) of this section.

(10) On the retest if HI titers of 1:80 and/or SPD titers of 1:10 or higher are found, the flock shall be considered infected: *Provided*, That, at the discretion of the Official State Agency, additional tests may be conducted in accordance with paragraph (a)(6) of this section before final determination of the flock status is made.

(11) If HI titers of 1:80 and/or SPD titers of 1:10 or higher are found on the second retest, the flock shall be considered infected for the Mycoplasma for which it was tested.

(12) If the tube agglutination or serum plate tests are found on the second retest to be positive at the same or higher rate and the HI and/or SPD tests are negative, the flock should be considered infected: *Provided*, That if the status of the flock is considered to be equivocal, the Official State Agency may examine reactors by the in vivo bio-assay, PCR-based procedures, and/or culture procedures before final determination of the flock status is made.

(13) If the in vivo bio-assay, PCR-based procedures, and culture procedures are negative, the Official State Agency may qualify the flock for the classification for which it was tested.

(14) If the in vivo bio-assay, PCR-based procedures, or culture procedures are positive, the flock will be considered infected. However, the following considerations may apply:

(i) In PCR-positive flocks for which there are other negative mycoplasma test results, the flock's mycoplasma status should be confirmed through either seroconversion or culture isolation of the organism, or through both methods, before final determination of the flock's status is made.

(ii) In flocks for which only the bio-assay is positive, additional in vivo bio-assay, PCR-based procedures, or cultural examinations may be conducted by the Official State Agency before final determination of the flock's status is made.

(15) If the in vivo bio-assay, PCR-based procedures, or cultures are positive on retest, the flock shall be con-

sidered infected for the mycoplasma for which it was tested.

(b) [Reserved]

[40 FR 1504, Jan. 8, 1975, as amended at 41 FR 48726, Nov. 5, 1976. Redesignated at 44 FR 61586, Oct. 26, 1979, and amended at 47 FR 21993, May 20, 1982; 50 FR 19900, May 13, 1985; 54 FR 23957, June 5, 1989; 59 FR 12799, Mar. 18, 1994; 61 FR 11521, Mar. 21, 1996; 62 FR 44070, Aug. 19, 1997; 63 FR 3, Jan. 2, 1998; 65 FR 8019, Feb. 17, 2000]

#### § 147.7 Standard test procedures for mycoplasma.<sup>5</sup>

The serum plate agglutination test, the tube agglutination test, and the enzyme-linked immunosorbent assay (ELISA) test should be considered basic screening tests for mycoplasma antibodies. The test selected will depend on preference, laboratory facilities, and availability of antigen. These three tests, though quite accurate, determine flock status rather than individual bird status, since occasional reactions are nonspecific. Under normal circumstances, the rate of such nonspecific reactions is low. Nonspecific reactions may occasionally be high, particularly after the use of erysipelas bacterin in turkeys and where mycoplasma antibodies are present for closely related mycoplasma other than for the species being tested. The hemagglutination inhibition (HI) test is too cumbersome for routine screening use. Positive reactions are extremely accurate however, and are useful in evaluating serum samples that react with the ELISA, plate, and/or tube antigens. The test should be conducted with 4 HA units. Titers of 1:80 or greater for both chicken and turkey sera are considered positive, while a 1:40 or 1:20 titer would be strongly suspicious and additional tests should be required.

(a) *Serum plate agglutination test.* (1) The serum plate agglutination test for mycoplasma is conducted by contacting and mixing 0.02 ml of test

<sup>5</sup>For additional information on mycoplasma test procedures, refer to the following references: Proc. 77th Annual Meeting, U.S. Animal Health Association, 1973; Isolation and Identification of Avian Pathogens, 3rd Edition; Methods for Examining Poultry Biologics and for Identifying and Quantifying Avian Pathogens, 1991.