

(K) Cover the plate with sealing tape. Incubate at room temperature for 30 minutes or until control RBC's give a tight button.

(L) Read the reaction on a mirrored plate reader.

(iv) *Results.*

(A) The titer is reported as the reciprocal of the last dilution to give a tight button of RBC's. The final dilution scheme includes the antigen in the dilution calculation and is as follows: B=1:20, C=1:40, D=1:80, E=1:160, F=1:320, G=1:640, H=1:1,280.

(B) For the assay to be valid:

(1) The positive control sera must give a result within one dilution of the previously determined titer.

(2) The negative control sera must be negative.

(3) The backtitration of the antigen must be 1:4 or 1:8.

(4) The RBC control must give tight, non-hemolyzed buttons.

(5) Sera controls (well A of each test sera) must not have non-specific agglutination or hemolysis. If negative, report as "negative with non-specific agglutination or non-specific hemolysis" or "unable to evaluate due to non-specific agglutination or hemolysis" or treat the serum to remove the non-specific agglutination and repeat the test. (See paragraph (e)(2)(v) of this section.)

(v) *Treatment to remove non-specific agglutination.*

(A) *Purpose.* Treatment of serum to remove non-specific agglutination that is interfering with HI assays.

(B) *Specimen.* Serum.

(C) *Materials.* Homologous RBC's (chicken or turkey), 50 percent solution PBS, centrifuge, incubator, 4C (refrigerator).

(D) *Procedure.* (1) Prepare a 1:5 dilution of test serum by adding 50 μ L of serum to 200 μ L of PBS.

(2) Prepare a 50 percent solution of RBC's by adding equal volumes of packed RBC's to PBS. Mix well.

(3) Add 25 μ L of 50 percent RBC solution to the serum dilutions.

(4) Vortex gently to mix.

(5) Incubate at 4 °C for 1 hour.

(6) Centrifuge to pellet the RBC's.

(7) Use the supernatant to perform the HI assay. Modify the dilution scheme in the assay to consider the initial 1:5 dilution prepared in the treat-

ment. For the 1:5 dilution scheme, do not add PBS to row A. Add 50 μ L of the 1:5 treated supernatant to row A. Serially dilute 25 μ L from rows A through H. This prepares a serum dilution of 1:10 through 1:640 in rows B through H.

[49 FR 19803, May 10, 1984, as amended at 57 FR 57342, Dec. 4, 1992; 59 FR 12799, Mar. 18, 1994]

§ 147.8 Procedures for preparing egg yolk samples for diagnostic tests.

The following testing provisions may be used for retaining the classification U.S. M. Gallisepticum Clean under § 145.23(c)(1)(ii)(C) and § 145.33(c)(1)(ii)(C), and for retaining the classification U.S. M. Synoviae Clean under § 145.23(e)(1)(ii)(b) and § 145.33(e)(1)(ii)(b).

(a) Under the supervision of an Authorized Agent or State Inspector, the eggs which are used in egg yolk testing must be selected from the premises where the breeding flock is located, must include a representative sample of 30 eggs collected from a single day's production from the flock, must be identified as to flock of origin and pen, and must be delivered to an authorized laboratory for preparation for diagnostic testing.

(b) The authorized laboratory must identify each egg as to the breeding flock and pen from which it originated, and maintain this identity through each of the following:

(1) Crack the egg on the round end with a blunt instrument.

(2) Place the contents of the egg in an open dish (or a receptacle to expose the yolk) and prick the yolk with a needle.

(3) Using a 1 ml syringe without a needle, aspirate 0.5 ml of egg yolk from the opening in the yolk.

(4) Dispense the yolk material in a tube. Aspirate and dispense 0.5 ml of PBS (phosphate-buffered saline) into the same tube, and place in a rack.

(5) After all the eggs are sampled, place the rack of tubes on a vortex shaker for 30 seconds.

(6) Centrifuge the samples at 2500 RPM (1000 x g) for 30 minutes.

(7) Test the resultant supernatant for *M. gallisepticum* and *M. synoviae* by using test procedures specified for detecting IgG antibodies set forth for

testing serum in §147.7 (for these tests the resultant supernatant would be substituted for serum); except that a single 1:20 dilution hemagglutination inhibition (HI) test may be used as a screening test in accordance with the procedures set forth in §147.7.

NOTE.—For evaluating the test results of any egg yolk test, it should be remembered that a 1:2 dilution of the yolk in saline was made of the original specimen.

[50 FR 19900, May 13, 1985]

Subpart B—Bacteriological Examination Procedure

§147.10 Laboratory procedure recommended for the bacteriological examination of egg-type breeding flocks with salmonella enteritidis positive environments.

Birds selected for bacteriological examination from egg-type breeding flocks positive for *Salmonella enteritidis* after environmental monitoring should be examined as described in §147.11(a) of this subpart, with the following exceptions and modifications allowed due to the high number of birds required for examination:

(a) Except when visibly pathological tissues are present, direct culture, §147.11(a)(1) of this subpart, may be omitted; and

(b) Enrichment culture of organ (non-intestinal) tissues using a non-selective broth, §147.11(a)(2) of this subpart, may be omitted.

[59 FR 12801, Mar. 18, 1994]

§147.11 Laboratory procedure recommended for the bacteriological examination of salmonella.

(a) For egg- and meat-type chickens, waterfowl, exhibition poultry, and game birds. All reactors to the Pullorum-Typhoid tests, up to at least four birds, should be cultured in accordance with both *direct* (paragraph (a)(1)) and *selective enrichment* (paragraph (a)(2)) procedures described in this section. Careful aseptic technique should be used when collecting all tissue samples.

(1) Direct culture (refer to illustration 1). Grossly normal or diseased liver, heart, pericardial sac, spleen, lung, kidney, peritoneum, gallbladder, oviduct, misshapen ova or testes, in-

flamed or unabsorbed yolk sac, and other visibly pathological tissues where purulent, necrotic, or proliferative lesions are seen (including cysts, abscesses, hypopyon, and inflamed serosal surfaces), should be sampled for direct culture using either flamed wire loops or sterile swabs. Since some strains may not dependably survive and grow in certain selective media, inoculate *non-selective plates* in addition to two selective plating media. Refer to illustration 1 for recommended bacteriological recovery and identification procedures.⁶ Proceed immediately with collection of organs and tissues for selective enrichment culture.

(2) Selective enrichment culture (refer to illustration 2). Collect and culture organ samples separately from intestinal samples, with intestinal tissues collected last to prevent cross-contamination. Samples from the following organs or sites should be collected for culture in selective enrichment broth. A non-selective broth culture (illustration 1) of pooled organs and sites should also be included as described in paragraph (a)(3) of this section.

(i) Heart (apex, pericardial sac, and contents if present);

(ii) Liver (portions exhibiting lesions or, in grossly normal organs, the drained gallbladder and adjacent liver tissues);

(iii) Ovary-Testes (entire inactive ovary or testes, but if ovary is active, include any atypical ova);

(iv) Oviduct (if active, include any debris and dehydrated ova);

(v) Kidneys and spleen; and

(vi) Other visible pathological sites where purulent, necrotic, or proliferative lesions are seen.

(3) From each reactor, aseptically collect 10 to 15 g, or the nearest lesser amount available, from each organ or site listed in paragraph (a)(2) of this section and mince, grind, and blend

⁶Biochemical identification charts may be obtained from "A Laboratory Manual for the Isolation and Identification of Avian Pathogens," chapter 1, Salmonellosis. Third edition, 1989, American Association of Avian Pathologists, Inc., Kendall/Hunt Publishing Co., Dubuque, IA 52004-0539.