(*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 µL of serum to 200 µL 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 µL 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 treatment. For the 1:5 dilution scheme, do not add PBS to row A. Add 50 µL of the 1:5 treated supernatant to row A. Serially dilute 25 µL 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; 63 FR 3, Jan. 2, 1998]

§ 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*) of this chapter.

(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; 63 FR 3, Jan. 2, 1998]

§ 147.9 Standard test procedures for avian influenza.

(a) The agar gel immunodiffusion (AGID) test should be considered the basic screening test for antibodies to Type A influenza viruses. The AGID test is used to detect circulating antibodies to Type A influenza group-specific antigens, namely the ribonucleoprotein (RNP) and matrix