§ 147.8  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.

§ 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 and U.S. M. Synoviae Clean classifications, 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.

(ii) For egg yolk samples being tested to retain the U.S. H5/H7 Avian Influenza Monitored classification, test the resultant supernatant in accordance with the requirements in §146.13(b).

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.

§ 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