United States Department of Agriculture

National Veterinary Services Laboratories

Standard Operating Procedure

Preparation of Erythrocytes for Hemagglutination (HA) and Hemagglutination-Inhibition (HI) Tests

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Contact Person: Dennis Senne, (515) 663-7551

Approvals:

/s/ Brundaban Panigrahy Date: 6-1-05
Brundaban Panigrahy, Head
Avian Viruses Section

/s/ Beverly Schmitt Date: 6-1-05
Beverly J. Schmitt, Chief
Diagnostic Virology Laboratory

United States Department of Agriculture
Animal and Plant Health Inspection Service
P. O. Box 844
Ames, IA  50010

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Preparation of Erythrocytes for Hemagglutination (HA) and Hemagglutination-Inhibition (HI) Tests

Purpose:

This standard operating procedures (SOP) describes the washing and storage of chicken erythrocytes used in hemagglutination and hemagglutination-inhibition tests and for treatment of serum to remove natural serum agglutinins.

1. Materials and Equipment:

   1.1 Chicken blood, preserved in Alsever’s solution. Note: Male chicken (rooster) blood is preferred because erythrocytes from females may, at times, be less sensitive to hemagglutinins (due to hormonal changes).

   1.2 Phosphate buffered saline (PBS). See appendix 7.1

   1.3 Alsever’s solution. See appendix 7.2

   1.4 Common laboratory supplies and chemicals

   1.5 50 ml conical centrifuge tube(s)

   1.6 250 ml Erlenmeyer flask

   1.7 Serologic pipettes (1 ml and 10 ml)

   1.8 100 ml or 250 ml graduated cylinder

   1.9 Refrigerated centrifuge (4 °C ± 2 °C)

   1.10 Refrigerator (4 °C ± 2 °C)

   1.11 Vacuum for aspirating liquids (vacuum pump with side-arm flask or Chapman-type filter pump attached to a water line)

   1.12 Rack for 50 ml centrifuge tube(s)

2. Collection of chicken blood in Alsever’s solution:

Dispense 10 ml of sterile Alsever’s solution into a clean flask. Anesthetize donor rooster with 1 ml of a Ketamine/Xylazine mixture (10 ml Katamine [100 mg/ml], plus 0.1 ml Xylazine [100 mg/ml]). After bird is sufficiently anesthetized, collect 10 ml blood via cardiac puncture with a 20 ml syringe fitted with a 1 ½ inch, 20-gauge needle (Note: before bleeding, aspirate 2–3 ml of
Preparation of Erythrocytes for Hemagglutination (HA) and Hemagglutination-Inhibition (HI) Tests

Alsever’s solution into syringe barrel to prevent clotting of blood while bleeding). Immediately remove needle and gently expel blood into the flask containing Alsever’s solution. Mix gently but thoroughly. Chicken erythrocytes in Alsever’s solution may be stored for 1 to 2 weeks at 4 C.

3. Washing erythrocytes:

3.1 Place 20-30 ml chicken blood preserved in Alsever’s solution (equal volumes blood and Alsever’s solution) in a 50 ml centrifuge tube.

3.2 Fill centrifuge tube with PBS.

3.3 Gently invert tube several times to wash erythrocytes.

3.4 Centrifuge erythrocytes at 800 x g (1,800 rpm in a Beckman J6-B centrifuge with JS 4.2 rotor) for 10 min at 4 C.

3.5 Remove supernatant and the buffy coat (white blood cell layer) by aspiration.

3.6 Repeat steps 3.2 through 3.4 two additional times.

4. Storage of Washed, Packed Erythrocytes:

4.1 Washed, packed erythrocytes can be stored for up to 1 week at 4 C.

5. Preparation of 0.5% Erythrocyte Suspension:

5.1 Dispense 199 ml PBS into a 250 ml Erlenmeyer flask or other appropriate vessel.

5.2 Add 1 ml washed, packed erythrocytes to the PBS, rinsing the pipette thoroughly to remove all erythrocytes from the pipette.

5.3 Swirl flask gently but thoroughly to suspend erythrocytes.

5.4 Store erythrocytes at 4 C until needed. Note: Discard erythrocytes suspension if hemolysis is observed.
Preparation of Erythrocytes for Hemagglutination (HA) and Hemagglutination-Inhibition (HI) Tests

6. Preparation of 10% Erythrocyte Suspension (for treatment of serum to remove natural serum agglutinins):

6.1 Dispense 9 ml PBS into an appropriate vessel.

6.2 Add 1 ml washed, packed erythrocytes to the PBS, rinsing the pipette thoroughly to remove all erythrocytes from the pipette.

6.3 Mix to thoroughly suspend erythrocytes.

6.4 Store erythrocytes at 4°C until needed.

7. Appendix:

7.1 Phosphate buffered saline (PBS), 0.1 M, pH 7.2.

Combine the following reagents: Sodium chloride 8.5 g, sodium phosphate dibasic 1.33 g, sodium phosphate monobasic 0.22 g, distilled water q.s. to 1 liter. Mix thoroughly and check pH. The pH should be 7.2 ± 0.1. Store at 4°C or at room temperature.

7.2 Alsever’s solution (dextrose 0.8 gm, citric acid 0.055 gm, sodium chloride 0.42 gm, distilled water 100 ml). Autoclave or filter sterilize (0.22 µm filter). Store at 4°C.