

United States Department of Agriculture  
National Veterinary Services Laboratories  
Standard Operating Procedure

Removal of Natural Agglutinins from Avian Serums

Date: June 1, 2005  
Number: AVSOP2222.02  
Supersedes: AVSOP2222.01, September 3, 1998  
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Removal of Natural Agglutinins from Avian Serums

1. Purpose:

This Standard Operating Procedure (SOP) describes the method to remove natural serum agglutinins from avian serums prior to use in the hemagglutination-inhibition (HI) test.

2. General:

Natural serum agglutinins are present in some avian serums. These natural agglutinins may cause nonspecific agglutination (autoagglutination) of erythrocytes. If the natural agglutinins are not removed, false negative test result may be obtained (an antibody-positive serum interpreted as negative) when serums are tested by the HI procedure. As described in this SOP, natural serum agglutinins can be removed by adsorbing the serum with washed erythrocytes of the same specie as those used in the HI test.

3. Materials:

- 3.1 96-well U-bottom microtiter plates
- 3.2 Microtiter plate shaker
- 3.3 Washed chicken erythrocytes (see current version of AVSOP0801)
- 3.4 Phosphate buffered saline (PBS), 0.01 M, pH 7.2 (see appendix)
- 3.5 Pipettes or micro pipettes and tips

4. Procedure:

- 4.1 Prepare a 1:4 dilution of serum/plasma in a microtiter plate(s) well, e.g., 0.05 ml serum, 0.1 ml PBS, and 0.05 ml of a 1:10 dilution of washed erythrocytes. **Note: Keep erythrocytes thoroughly suspended while dispensing. Care should be taken when designing plate setup so that a multichannel micro pipette can be used to transfer treated serum to HI test plates.**
- 4.2 Place the microtiter plate(s) on a microtiter plate shaker and mix to suspend the erythrocytes.
- 4.3 Incubate at room temperature for 30 min. **Note: Shake plates every 10 min or so to keep erythrocytes suspended.**

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**4.4** Allow erythrocytes to settle by gravity or centrifuge at 200 x g (1,000 rpm in Beckman J-6B centrifuge with JS 4.2 rotor) for 2-3 min.

**4.5** Dispense the treated (1:4 dilution) serum to HI test plates as per protocol.

**5. Appendix:**

**5.1 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. The pH should be  $7.2 \pm 0.1$ . Store at room temperature.

**6. Summary of revisions.**

**6.1** Section 5.1, added: The pH should be  $7.2 \pm 0.1$ . Store at room temperature.