Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Standard Operating Procedure

Filter Paper Method of Blood Collection
for Serologic Test(s)

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Filter Paper Method of Blood Collection for Serologic Test(s)

1. **Purpose:**

Avian blood samples can be collected and preserved in filter paper. Blood collected in this manner can be used in place of serum and/or plasma for serologic tests such as hemagglutination-inhibition (HI) test. The technique is especially useful in field situations where aseptic collection and adequate preservation of serum and/or plasma may be a problem.

2. **Materials:**

   2.1 Filter paper (903, Schlicher and Schaell Inc., Keene, New Hampshire 03431)
   
   2.2 Flat-bottom microtiter plates (96-well) with covers
   
   2.3 Microtiter plate shaker
   
   2.4 Micropipettes (single and multichannel)
   
   2.5 Micropipette tips (200 µl)
   
   2.6 Refrigerator (4 C ± 2 C)
   
   2.7 Paper punch (5 mm diameter hole)
   
   2.8 Ink pen (indelible)
   
   2.9 Paper stapler
   
   2.10 Forceps (fine tip)
   
   2.11 Phosphate buffered saline (PBS). See appendix.

3. **Preparation and assembly of filter paper strip clusters:**

   3.1 Cut filter paper into 1.2 x 10 cm strips.
   
   3.2 Arrange 3 strips, each overlapping the other in the middle, to form a 6-spoke wheel configuration. Staple strips together at the center of the wheel. Each spoke is used to collect blood from one bird.

4. **Collection of blood on filter paper strips:**

   4.1 Puncture/nick the brachial wing vein or cut a toe nail of the bird to be sampled.
Filter Paper Method of Blood Collection for Serologic Test(s)

4.2 Briefly allow blood to pool on the skin at the puncture site. The blood should not be allowed to clot before soaking the filter paper strip.

4.3 Saturate the distal 1.2 to 2.5 cm of a filter paper strip with blood by soaking the flat side (not the edge) in the blood that has pooled at the puncture site on the wing or from a toe. Collect sufficient blood so that both surfaces of the strip become saturated.

4.4 Write the animal identification on the unused portion of the strip with an ink pen.

4.5 Bend the tip of each strip slightly upward so the cluster of 6 "spokes" will have a concave shape and can be placed on any flat surface so that the blood-containing portion of the strip will not be in contact with the storage surface.

4.6 Allow the strips to air-dry.

4.7 Place the dry strips in a plastic bag and seal the bag.

4.8 Ship/deliver samples to the laboratory without refrigeration.

4. Processing dried blood in filter paper:

4.1 Store the dried blood filter paper strips at 4°C until processed.

4.2 Label a flat-bottom microtiter plate to match the identification of samples on filter paper strips. One well per sample will be needed.

4.3 Punch 3, 5 mm discs from the saturated portion of a filter paper strip. Transfer the discs with a pair of forceps to the appropriate well of the microtiter plate.

4.4 Dispense 200 µl PBS to each well containing dried blood discs.

4.5 Place microtiter plate on a Micro plate shaker and mix for 1 hr (± 10 min) at a setting that will adequately mix the samples but not displace the liquid from the wells.
Filter Paper Method of Blood Collection for Serologic Test(s)

4.6 Incubate plates at 4 C overnight to allow for maximum elution of blood. The eluted sample is equivalent to a 1:10 dilution of serum.

4.7 Perform HI test according to the standard protocol (current version of AVPRO800, Hemagglutination-Inhibition Test to Detect Serum Antibodies to Avian Paramyxoviruses). Note: The initial serum dilution will be 1:20 when tested by the standard protocol.

5. References:


6. Appendix:

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.