AGID – Serum, Yolk
Antibody Extraction
Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Standard Operating Procedure

Procedure for Extracting Egg Yolk Antibodies

Date: July 28, 1998

Supersedes: New

Number: AVSOP2220.01

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Purpose:

This standard operating procedure (SOP) describes the extraction of antibodies from egg yolk samples for use in serologic procedures.

General:

The passive transfer of hen’s antibody to egg yolk is well documented. Egg yolk can be extracted to separate the antibody containing aqueous phase from the lipid phase. The resulting sample can be used in the agar gel immunodiffusion (AGID), hemagglutination-inhibition (HI), and neuraminidase-inhibition (NI), tests to determine the immune status of a flock. Egg yolk extract can be used in place of serum or plasma, and is especially useful for serosurveillance when spread of disease by on-farm visits are a concern. Eggs can be collected at processing plants which reduces the need for on-farm visits.

1. Materials

1.1 Paper dinner plates (6 inch) or petri dishes
1.2 Tuberculin syringes (1 ml)
1.3 12 x 75 mm snap-cap tubes or equivalent
1.4 Phosphate buffered saline (PBS), 0.01 M, pH 7.2. See appendix 3.1.
1.5 5 or 10 ml serologic pipettes or automated pipetting device
1.6 Vortex mixer
1.7 Low-speed centrifuge

2. Procedure:

2.1 Crack egg and empty contents with intact yolk sac onto a paper plate or petri dish.

2.2 Insert the blunt end of a 1 ml tuberculin syringe, without needle, into the yolk sac and withdraw 1 ml yolk material.
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2.3 Expel yolk into a 12 x 75 mm snap-cap tube containing 1 ml PBS. **Note**: The same syringe may be used to collect yolk from additional eggs from the same flock by rinsing the syringe with clean PBS after each use. To rinse the syringe, aspirate and expel the PBS several times to remove residual yolk material from the syringe barrel. If precise antibody titers for each egg are needed, a new syringe should be used for each egg.

2.4 Vortex yolk-PBS mixture vigorously at maximum speed setting for 10 to 15 sec.

2.5 Allow yolk-PBS mixture to stand at room temperature for 1 hr then repeat step 1.4.

2.6 Centrifuge yolk-PBS at 1,500 x g (2,500 rpm in Beckman J-6B centrifuge with JS 4.2 rotor) for 30 min at 4°C.

2.7 Harvest the supernatant and save for use in agar gel immunodiffusion (AGID), hemagglutination-inhibition (HI), and neuraminidase-inhibition (NI) tests. This represents a 1:2 dilution of yolk.

3. **Appendix**:

3.1 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. Store at room temperature.