Sample Submission Guidance for Detection of Highly Pathogenic Avian Influenza Virus (HPAIV)

NCVDLS recommends submission of the following avian samples to the Rollins Laboratory in Raleigh for the rapid detection of HPAIV using the polymerase chain reaction assay (PCR):

<table>
<thead>
<tr>
<th>Avian Source</th>
<th>Recommended Specimen*</th>
<th>Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallinaceous birds (turkeys, chickens, quail, pheasants)</td>
<td>Tracheal/oropharyngeal swab</td>
<td>1 swab/bird from single flock and species; up to 5 swabs may be pooled in 3ml of brain heart infusion broth (BHIB) or 11 swabs may be pooled in 5.5 ml of BHIB</td>
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<tr>
<td>Domestic waterfowl</td>
<td>Cloacal swab</td>
<td>1 swab/bird from single flock and species; maximum of 5 swabs may be pooled in 3 ml BHIB</td>
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<tr>
<td>Wild birds</td>
<td>Tracheal/oropharyngeal and cloacal swab</td>
<td>1 swab from each site from a single bird pooled in 3ml BHIB</td>
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</tbody>
</table>

*Appropriate swabs have a synthetic tip (e.g., rayon or dacron) and plastic shaft. Swabs with cotton tips and wooden shafts or those made of calcium alginate are not suitable because these have been shown to inactivate virus and inhibit the PCR assay thus invalidating test results.

Sample collection:

Tracheal swab: Hold the bird’s mouth and carefully introduce the swab into the trachea. Using gentle pressure and in a circular motion, swab the internal circumference of the trachea several times. The swab can also be rubbed along the choanal slit as it is being removed.

Oropharyngeal swab: Hold the bird’s mouth open and introduce the swab into the mouth. Gently rub the swab around the tracheal opening and, up along the choana, ensuring that it is rubbed through the choanal slit as it is being removed.

Cloacal swab: Hold the bird securely and introduce the swab into the cloaca through the vent to the appropriate depth while ensuring contact with the mucous membranes.

After collection, swabs are placed into tubes of BHIB which are available at any of our NCVDLS facilities or at staging areas. With the swab in the medium, swirl the stem end of the swab between fingers vigorously and remove it while gently expressing any adsorbed liquid on the sides of the tube. Samples should be placed inside sealed plastic bags and external surfaces of the bags sprayed with a disinfectant. When dried, place the bag inside another plastic bag along with a completed submission form. Samples should be kept cold immediately after collection and during transit to the laboratory.

Laboratory submission:

All sections of the avian/poultry laboratory submission form should be completed. This form can be downloaded on this website by clicking on the link for “NCVDL forms”. Required information includes: owner and collector, premises ID and physical address of flock, collection date, production type (e.g. commercial poultry, backyard, etc.), number of birds in flock, species, age, and type of samples submitted. Additional information on laboratory submission is found within our “Lab User Guide”.

For further questions or to discuss on-farm mortality issues, please contact either the Rollins Laboratory at 919.733.3986 or the Veterinary Division of the North Carolina Department of Agriculture & Consumer Services at 919.733.7601.

July, 15, 2015