Recommended Procedures for Sample Collection, Preservation and Shipping

Dennis A. Senne
dennis.a.senne@aphis.usda.gov
(515) 663-7551
USDA,APHIS,VS,
NVSL,Ames, Iowa 50010
Blood Collection
Dilute yolk 1:2 (1ml PBS & 1ml Yolk)
## AIV/ND Serology

### Types of Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>AGID</th>
<th>ELISA</th>
<th>HI/NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Plasma</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Egg Yolk</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Sample Collection

• **Please** submit 0.5ml – 1.0ml serum/plasma/yolk for serodiagnosis
  ✓ Need 0.15ml for HI
  ✓ Need 0.2ml for NI
Isolation of AIV/ND

Sample Collection:

• Tissues (do not pool tissues from different birds)
  ✓ Lung
  ✓ Spleen
  ✓ Brain if nervous signs

• Swabs (pool up to 5/tube)
  ✓ Tracheal (Tr) or oropharyngeal (Op)
  ✓ Cloacal (Cl)

Note: 1) Keep tissue and swabs cold (on ice),
2) Do not pool Tr/Op with Cl swabs
Cotton/dacron with plastic shafts

Can interfere with PCR
Tracheal Swab
Oropharyngeal Swab
Isolation of AIV/ND

Sample Collection:

- **Swab diluent (2-4ml/tube)**
  - ✓ Brain-heart infusion broth (BHI)
  - ✓ Viral transport media
  - ✓ Tris-buffered tryptose broth (TBTB)
  - ✓ Nutrient broth (NB)
  - ✓ Peptone broth (PB)
  - ✓ Cell culture medium w/1% BSA
Sample Collection

Number of Samples:

- Serology
  - 20 – 30 samples/flock
  - 95% confidence at 10% infection level
- Tissue
  - 3 – 5 birds
  - Do not pool tissue from different birds
- Swabs
  - 20 – 30 (pooled 5/tube)
### Sample Size as a Function of Population Size and Minimum Probability of Detection

<table>
<thead>
<tr>
<th>95% CI&lt;sup&gt;A&lt;/sup&gt;</th>
<th>#50&lt;sup&gt;B&lt;/sup&gt;</th>
<th>#100</th>
<th>#500</th>
<th>#1000</th>
<th>#10,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%&lt;sup&gt;C&lt;/sup&gt;</td>
<td>48&lt;sup&gt;D&lt;/sup&gt;</td>
<td>96</td>
<td>225</td>
<td>258</td>
<td>294</td>
</tr>
<tr>
<td>5%</td>
<td>31</td>
<td>45</td>
<td>56</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>10%</td>
<td>22</td>
<td>25</td>
<td>28</td>
<td>29</td>
<td>29</td>
</tr>
</tbody>
</table>

**99% CI**

<table>
<thead>
<tr>
<th>1%</th>
<th>50</th>
<th>99</th>
<th>300</th>
<th>368</th>
<th>448</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>39</td>
<td>59</td>
<td>83</td>
<td>86</td>
<td>90</td>
</tr>
<tr>
<td>10%</td>
<td>29</td>
<td>36</td>
<td>42</td>
<td>43</td>
<td>44</td>
</tr>
</tbody>
</table>

<sup>A</sup> Confidence Interval  
<sup>B</sup> Number of Birds in Flock  
<sup>C</sup> Prevalence Within the Flock  
<sup>D</sup> Number of Samples to Collect
Sampling for Disease Detection

- Rule of thumb for large populations for 95% confidence of detecting disease
- \( n = 3 \times \text{interval} \)

<table>
<thead>
<tr>
<th>Minimum Prevalence</th>
<th>Interval</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>1 in 100</td>
<td>3 x 100 = 300</td>
</tr>
<tr>
<td>10%</td>
<td>1 in 10</td>
<td>3 x 10 = 30</td>
</tr>
<tr>
<td>25%</td>
<td>1 in 4</td>
<td>3 x 4 = 12</td>
</tr>
</tbody>
</table>
Targeted Sampling Scenario – AI/ND in Vaccinated Populations

- Design a routine monitoring scheme (weekly, monthly etc.) – commercial and backyard flocks, especially ducks

- Within flock prevalence in vaccinated commercial flocks infected with HPAI could be as low as 0.1%.
  - Rule of 3 X interval (1 in 1000), n = 3000 samples
  - Not practical
Targeted Sampling Scenario – AI/ND in Vaccinated Populations

- If focus on daily mortality
- Assuming a normal daily mortality rate of 0.1%
- If the observed mortality rate in a suspect HPAI infected flock = 0.2 – 1% (2 – 10 fold increase in mortality)

  ✓ If HPAI was the cause of increased mortality, expect at least half the deaths are due to HPAI
  ✓ To detect 50% prevalence with 95% confidence
  \[ n = 6 \times (3 \times 2) \]
Targeted Surveillance “Barrel” Surveillance

- Very effective
- Focus on daily mortalities

- All flocks monitored weekly (10 birds)
- Compensates for under reporting of clinical disease
Waterfowl Sampling

• May differ from domestic gallinaceous bird surveillance
  ✓ Poultry – respiratory infections (primarily) – Tr/Op swabs
  ✓ Waterfowl – intestinal infections – cloacal swabs

• Wild birds – define goals – HPAI only, all AIV
  ✓ HPAI (H5N1) – tracheal/Op swabs from mortalities
  ✓ AIV – cloacal swabs from live birds and mortalities

• Diagnostic tests
  ✓ Virus/RNA detection – best
    ➢ Limitations of PCR (RNA extraction, PCR inhibitors)
  ✓ Antibody detection – limited value
Sample Preservation

- **Serum:**
  - 4C
  - -20C for storage

- **Tissue/swabs/virus:**
  - 4C (up to 72-96 hours)
  - -70C – long term storage
  - -20 not recommended
Stability of Hong Kong H5N1 Virus in Feces at Environmental Temperatures

Source: Shortridge **et al.** 1998
Shipping Specimens to the National Veterinary Services Laboratories (or other Institutions)
Definitions

Hazardous material: “…capable of posing an unreasonable risk to health, safety and property when transported in commerce.”

(Department of Transportation [DOT])

Dangerous Goods: “…capable of posing a risk to health, safety, property or the environment…” (International Air Transport Association [IATA])
Classes of Dangerous Goods

1. Explosives
2. Gas
3. Flammable liquids
4. Flammable solids
5. Oxidizing substances
6. Toxic or infectious
7. Radioactive
8. Corrosive
9. Miscellaneous
Applicable Regulations

• Department of Transportation (DOT)
  - 49 CFR Parts 171-175 (Hazardous Materials)
    - Applies to all shipments (ground, air, water)
    - www.dot.gov/regulations.html

• International Air Transport Assoc (IATA)
  - Dangerous Goods Regulations
    - Applies to air shipments
    - www.iata.org/whatwedo/dangerous_goods.html
Purpose of Regulations

“To ensure an acceptable level of safety for the transportation of hazardous & dangerous materials, including infectious and toxic substances, and to facilitate domestic and foreign transportation.”
Transport Regulations Apply To:

“Anyone who handles, offers for transport, transports or causes Dangerous Goods to be transported.”

These regulations apply to anyone who ships any infectious substance, diagnostic specimen or dry ice (to the NVSL or any other location).
Training Requirements

• **All shippers of DG must be trained**
  ✓ Training is valid for two years
  ✓ Training must include an exam

• **Records of training must be kept**

• **Training materials are available from IATA or from various private shipping companies**
Shippers’ Responsibilities

1. Classify
2. Identify
3. Package
4. Mark
5. Label
6. Document
7. Contact consignee
Classes of Dangerous Goods

1. Explosives
2. Gasses
3. Flammable liquids
4. Flammable solids
5. Oxidizing substances
6. Toxic or infectious
   6.1 Toxins
   6.2 Infectious substance
7. Radioactive
8. Corrosive
9. Miscellaneous (includes dry ice)
Proper Shipping Name and UN Number

UN 2814  Infectious substance, affecting humans

UN 2900  Infectious substance, affecting animals only

UN 3172  Toxic substance

UN 3373  Most diagnostic specimens

UN 1845  Dry ice
Infectious, Category A: UN 2814
(Infectious, affects humans)

Hendra virus
Nipah virus
Rift Valley fever virus
EEE and VEE virus
West Nile virus (cultures only)
Highly Pathogenic Avian Influenza (cultures only)

And many others – always check updated lists before shipping!
Infectious, Category A UN 2814
(Infectious, affects humans)

*Bacillus anthracis* cultures
*Brucella abortus* cultures
*Brucella melitensis* cultures
*Brucella suis* cultures
*Burkholderia mallei* cultures
*Chlamydia psittici* (avian cultures)
*Clostridium botulinum* cultures
*Escherichia coli* (verotoxigenic cultures)
*Francisella tularensis* cultures
*Mycobacterium tuberculosis* cultures
Infectious, Category A: UN 2900
(Infectious, affects animals only)

African horse sickness virus
African swine fever virus
Newcastle disease virus
Bluetongue virus
Classical swine fever virus
FMD virus

And many others – always check updated lists before shipping!
An infectious substance that does not meet the criteria for inclusion in Category A. Assign to:

- UN 3373 (diagnostic specimen)
- UN 2814 (infectious for humans), or
- UN 2900 (infectious for animals) as appropriate
Packing Instructions

Packing instruction 602
- Category A infectious substances

Packing instruction 650
- Category B infectious substances
- Diagnostic specimens
Category A shipments

Contact the NVSL (or any laboratory) for instructions before shipping any cultures or specimens suspected to contain a FAD or other Category A agent!!

These shipments require special permits and may require special chain of custody procedures.
Infectious Substance, Category B (UN 3373 – diagnostic specimens)

• Important requirements
  ✓ Leak proof primary container
  ✓ Leak proof secondary container
  ✓ Sufficient absorbent material to absorb entire volume of specimen
Infectious substance, Category B (dry ice)
Infectious Substance, Category B (dry ice)
Diagnostic Sample (ice pack)
Forms and Permits

NVSL Submission Form

Import Permit
Contact NVSL at this address for answers to shipping questions or to order supplies that are provided by the NVSL

NVSL Submission Forms:
http://www.aphis.usda.gov/vs/nvsl/JobAid/docs/VS10-4revised_v1.doc
Shipping Guidelines

• USDA permit required
  ✓ Can obtain from NVSL (515 663-7551)

• Shipping address:
  ✓ National Veterinary Services Laboratories
    1800 Dayton Ave
    Ames, Iowa  50010
Thank You For Your Attention!

NVSL, Ames, Iowa
OIE Reference Laboratory:
Avian Influenza
Newcastle disease