Post C&D Environmental Sampling Guidance - Poultry
May 5, 2022

Please note: These procedures may be revised as the situation continues to change.

SCOPE & INTENDED USE
This document applies to the sampling of poultry premises after a reportable disease event; the protocol provides general guidance to State Animal Health Officials (SAHOs), APHIS officials, and Incident Management Teams (IMTs) for environmental sample collection and testing. Updated guidance is pending current studies; more detailed instructions based upon this guidance are recommended as needed to address facility or situational differences. Contact NVSL at NVSL.AI.ND@usda.gov for examples of previous state sampling plans.

Biosecurity practices (on-site), including the use of personal protective equipment (PPE), will be followed for activities required for quarantine release, as directed by State and APHIS officials, and/or the IMTs.

PROTOCOL FOR ENVIRONMENTAL SAMPLE COLLECTION
Important Considerations
♦ Virus load can impact the likelihood of detecting virus on a particular surface.
♦ Environmental samples are highly diverse and have the potential to contain substances that pose challenges to diagnostic testing; this document is intended to provide guidance on sample collection from areas that have been shown to provide repeatable data. Be sure to notify the laboratory if sample type not described in this document is collected.
♦ There are many sample collection devices and sampling approaches that are useful; however, as above, this document is intended to provide guidance specifically for post virus elimination testing.
♦ There is no official requirement or option to release compost based upon environmental sampling and diagnostic testing of compost piles.
♦ Submissions other than official cleaning & disinfection (C&D) testing may be subject to user fees.
♦ Facilities vary widely within and across production sectors, therefore it is crucial to develop a sampling plan specific to the facility being tested (example in Appendix 1). It is the sampler’s responsibility to assess areas and make adjustments to the sampling as needed based on observations of cleanliness and layout of the facility.

General Collection Guidance
1. Refer to Optimizing sample collection methods for detection of respiratory viruses in poultry housing environments - Transboundary and Emerging Diseases - Wiley Online Library
2. For NAHLN labs, schedule sample collection for Monday, Tuesday, or Wednesday, and notify lab when to expect samples to ensure samples are processed without delay. Note: If samples arrive on Friday, they may sit at the lab over the weekend resulting in poor sample quality (low volume, bacterial contamination, etc.), causing prolonged test turnaround, and compromising analytical results. Contact NAHLN lab for specific schedules and around holidays.
3. **Recommended sampling devices**: 1,2,3
   - Pre-moistened 4x4 gauze cotton pads are ideal for a variety of surfaces.
   - **25-1607 1PF SC PurCollect** Environmental Sampling Collection Swabs can be used.
   - Dacron swabs with plastic handles may be used but lack of surface area means smaller areas are being sampled, requiring more samples in total.
   - Gloves and/or boot covers worn during sample collection can be collected separately Ziploc bags and sampled as well.

4. **Media: BHI with antibiotics is recommended (NVSL media #50067):**
   - Contact NVSL at NVSL.AI.ND@usda.gov for options to ship BHI media in 1-liter bottles.
   - A larger tube than is used for swab samples is recommended, such as 50ml conical tubes.
   - Label tubes with Date, House Number, AI Barcode, and number in series (e.g., 1-10).
   - Because this document is focused on sampling a facility after the virus elimination steps – the facility should be dry. Extra media is needed to moisten sampling device. Include at least 2x more media than number of samples planned and/or include several tubes containing extra media specifically to moisten sampling devices.

5. **Where to sample**: Target at least 10 selected locations in each house.
   - Sampling **areas** (general area) or specific items (e.g. feeders/waterers) should be identified; the locations can be further divided into specific **locations** to sample within the location/item; then select the specific **surfaces** to swab if needed.
   - Consider facility type and what virus elimination was performed when identifying locations to sample.
   - **Good areas to sample**:  
     1) Areas which are heavily contacted by birds, manure, and oral secretions should be targeted.
     2) Slat floors, walls (targeting corners where birds rest at the height of the birds’ faces), feeder and waterer (rims).
     3) Other surfaces to consider are those frequently touched by workers (switches, electric panels, handles, doors), other non-porous surfaces such as circulating and exhaust fans.
     4) If feathers with pulp are found, cut-off the plume and place the feather shaft in a media tube. Five feather shafts can be pooled into 3-5ml BHI media.
     5) Examples by facility type:  
       - For **layer facilities**, consider cages, surfaces associated with egg processing, pits, top of the drinker lines, and surfaces associated with manure handling.
       - For **turkey facilities**, consider bell drinkers, sills, curtains, and frames.

6. **How to sample**:  
   - Pre-moisten the collection device using the designated clean media tube (do not use Swiffer pads are not recommended for this testing as they may interfere with recovery of viable virus.  

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1. Identification of optimal sample collection devices and sampling locations for the detection of environmental viral contamination in wire poultry cages - Mo - 2021 - Transboundary and Emerging Diseases - Wiley Online Library
2. Optimizing sample collection methods for detection of respiratory viruses in poultry housing environments - Mo - Transboundary and Emerging Diseases - Wiley Online Library
the tube intended for sample collection, avoid saturating, media should not drip from the swab).

- Using the sampling device, swipe a ~2-3 square inch (~5x5 cm) surface from each area of the selected location (covering larger areas may affect sensitivity). If using a swab, sample smaller areas for example, no more than 1 square inch (~2x2 cm). If the area is smaller than 2 square inches, sample the entire surface.

- After sampling the surface, collect the sample in the labeled **SAMPLE** tube as follows:
  - **For GAUZE:** place the gauze pad in a sealable Ziploc plastic bag. Add the media from one collection tube, seal and using fingers to squish, squeeze, and mix the gauze pad for 10-15 seconds. Squeeze the media from the gauze pad and let it pool in one corner of the bag. Cut off the opposite corner of the bag and decant the media back into the collection tube.
  - **For SWABS:** immerse in the media and vigorously swirl, squeezing the excess liquid from the swab against the inside of the tube maintaining the volume of media provided (e.g., 50ml conical tube prefilled with 10mls of media). **The entire swab suspension is submitted for diagnostic testing.**

  **Note:** Swabs left inside the sample tube may result in media being drawn into the swab, leaving limited material for diagnostic testing.

- **Order:** Generally, sample cleaner areas first (e.g., egg storage areas). When collecting within houses – target areas at each end and the center; larger houses may require sample collection at more locations in the central areas. If the house is divided (e.g., brood chamber, pens) collect samples from each section that was occupied by birds while virus was present on the premises. If planning to sample the collector’s gloves and/or boot covers, prepare large Ziploc bags and label by area to collect them, collect gloves separately from boot covers.

**General Reminders – these samples are time sensitive!**

1. **Store unused media** at -20°C standard freezer or 4°C standard refrigerator
2. **Maintain the cold chain** (4°C) of samples using pre-frozen gel packs. **Avoid freezing BHI after sample has been collected; note that unused media may be frozen.**
3. **Bag the sample tubes and place in a pre-chilled cooler with correct lab accession form.**
4. **Return cooler to the NAHLN lab** as soon as possible for sample processing.
   a. Provide submission form and tracking number to lab as soon as possible.
5. **PCR is conducted per the NVSL Testing Guidance for Post C&D Environmental Samples NVSL-WI-0048.** Samples can be tested by PCR at the NAHLN laboratory. Virus isolation is usually performed at NVSL.
6. **Report results to the State Veterinarian.**
7. **When forwarding to NVSL, select FedEx First Overnight for arrival by 8 AM and notify NVSL by email that samples will be arriving; include FedEx tracking # and copy of VS Form 10-4 to NVSL.AI.ND@usda.gov**

Interested in sample plans from other states? Contact DVL at NVSL.AI.ND@usda.gov.