

## Post C&D Environmental Sampling Guidance - Poultry

November 25, 2022

**Please note: These procedures may be revised as the situation continues to change. Significant updates are present throughout the document.**

### 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. This updated and more specific guidance is based upon current data and recent publication; further updates are pending current studies and analysis.

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, e.g. the time that animals shedding virus have been present
- ◆ 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.
- ◆ 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.
- ◆ 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. **Schedule sample collection** for Sunday, Monday, Tuesday or Wednesday, **Note:** These samples are time-sensitive and lack of notification leading to delays in testing may require resampling of the facility.
3. **Sample collection supplies:**
  - 8 to 12 ply cotton gauze 4x4 pads (available at NVSL).
  - NOTE: AVOID synthetic products including Swiffer products.
  - Refer to [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](#)
4. **BHI with antibiotics when available (NVSL media #50067):**
  - Contact the NVSL Shipping department at [ncah.shipping@usda.gov](mailto:ncah.shipping@usda.gov) for BHI 1-liter



bottles, use order form [here](#) for 5 ml tubes.

- Use 5 ml of BHI per gauze pad for sampling.
- 50 ml conical tubes to collect the pooled samples.
  - Label tubes with Date, House Number, AI Barcode, and number in series (e.g., 1-10).
- AI Barcodes (available at NVSL).

5. **Where to sample:** A minimum of 5 pooled samples (each pooled sample is composed of 5 surface samples). The sampler may submit no more than 2 additional pooled samples per barn if they determine an additional area or material, is high risk for a maximum of 7 pooled samples from each barn/house: **target only high-risk areas.**

Collect one pooled sample (each pooled sample represents 5 surfaces) from each of these 5 targeted areas/items:

- 1) Bird contact areas: Surfaces heavily contacted by birds, eggs, manure, and oral secretions.

Examples: slat floors and cage walls (targeting corners where birds rest at the height of the birds' faces), egg handling equipment (belts, racks, flats), pits, surfaces associated with manure handling, for turkey facilities consider sills, curtains, and frames.

- 2) High-touch surfaces: areas frequently touched by workers.

Examples: switches, hand on wall height, railings, handles, doors

- 3) Feeders: sample feeders along the rims where the birds would have contacted.

- 4) Waterers: sample waterers based on type: if there are drinker lines present, sample the top of the waterline. If there are bell drinkers, sample underneath where the water pools (below the waterline) and under the rims.

- 5) Boot covers and gloves: Use gauze pad as directed in part E.1 to sample bottom of each boot, collect media into 50ml tube; collect gloves in a quart size sealable bag, add 5mls media mix as directed in part E.3 and collect the media in the same 50ul tube.

6. **How to sample:**

- The facility is ready to sample when it is DRY and after the virus elimination steps. When collecting within houses ensure the specific high-risk surfaces targeted for samples are evenly distributed throughout the high contact areas, for example, sampling feeder surfaces at the beginning, middle and end of the occupied space/barn.

**Items needed:**

- Disposable gloves and boot covers are needed. Changing of gloves between samples is not necessary, remove gloves once finished sampling in a barn/house.
- Additional items needed include scissors, sanitizing wipes for the scissors and to wipe down samples prior to placing in the cooler, and a cooler with gel ice packs.
- For each pooled sample you will need:
  - **five** gauze pads
  - **five** aliquots of 5mls BHI
  - **five** quart-size sealable plastic bags
  - **one** 50ml conical tube

**Collecting a pooled sample:**

1. Pre-moisten the gauze with 5 ml brain heart infusion (BHI) media immediately before sampling (the gauze should be damp, but not dripping).
2. Using the gauze, firmly wipe across the length and breadth of the target area a ~2-3 square inch (~5x5 cm) surface. Estimate a 25 cm<sup>2</sup> sample area for non-flat surfaces. If the area is smaller than 2 square inches, sample the entire surface.
3. After collecting the sample, place the gauze in a quart size sealable bag (pour in the remainder of the 5 ml BHI if any). Seal the bag and using fingers to mix the media with the gauze pad for 5 seconds. Wring/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 into the 50 ml collection tube.
4. Repeat steps 1-3 to collect 4 additional surfaces to create a pooled sample.

**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.
  - Provide submission form and tracking number to lab as soon as possible.
5. Enter environmental laboratory submission information into EMRS.
  - Refer to the EMRS2 Environmental Sampling Training document.
6. 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.
7. **Report results to the State Veterinarian.**
8. Laboratories (NAHLN and/or NVSL) should be notified **in advance of sampling**. When forwarding to NVSL, **select FedEx First Overnight for arrival by 8 AM as directed by NVSL 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](mailto:NVSL.AI.ND@usda.gov)**