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Please note: These procedures may be revised as needed. 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. 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.DVL.AVIAN@aphis.usda.gov](mailto:NVSL.DVL.AVIAN@aphis.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. **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.

2. **Recommended sampling devices:**<sup>1</sup>

- [25-1607 1PF SC PurCollect](#) Environmental Sampling Collection Swabs – ideal for multiple surfaces, and very rough surfaces.
- 4x4 gauze cotton pads are also good for a variety of surfaces.
- Dacron swabs with plastic handles for smooth surfaces or hard to reach areas.
- Gloves and/or boot covers worn during sample collection can be collected separately ziplock bags and sampled as well.

3. **Media: BHI with antibiotics is recommended (NVSL media #50067):**

- Contact NVSL at NVSL.DVL.AVIAN@aphis.usda.gov for options to ship BHI with antibiotics 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.

4. **Where to sample:** Target at least 10 selected locations in each house.

- Sampling **locations** (general area) or specific items (e.g. feeders/waterers) should be identified; the locations can be further divided into specific **areas** 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: 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 1 tube.
  - 5) Examples by facility type:
    - *For layer facilities* consider: cages, surfaces associated with egg processing, pits, and surfaces associated with manure handling.
    - *For turkey facilities* consider: drinkers, sills, curtains, and frames.

5. **How to sample:**

- Pre-moisten the swab or gauze using the designated clean media tube (do not use the tube intended for sample collection, avoid saturating, media should not drip from the swab).

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<sup>1</sup> Swiffer pads are not recommended for this testing as they may interfere with recovery of viable virus.

- 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 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 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.
  - For GAUZE: place the gauze pad in a sealable plastic sandwich bag. Add the media from one collection tube seal and using fingers to “stomach” 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.
- *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 ziplock bags and label by area to collect them, collect gloves separately from bootcovers.

### 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.***
6. **Bag the sample tubes and place in a pre-chilled cooler with correct lab accession form.**
7. **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.
8. PCR is conducted per the *NVSL Testing Guidance for Post C&D Environmental Samples* WI-AV-0045. Samples can be tested by PCR at the NAHLN laboratory. Virus isolation should be performed at NVSL.
9. **Report results to the State Veterinarian.**
10. 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.DVL.AVIAN@aphis.usda.gov](mailto:NVSL.DVL.AVIAN@aphis.usda.gov)**

## Appendix 1. Example Sampling Plan for a broiler breeder premises

Note: General Personal Protective Equipment (PPE) should be worn prior to entering any poultry premises. As directed by SAHOs, APHIS officials, and/or IMT, appropriate PPE will be used (e.g., **Tyvek coveralls, shoe covers, hairnet, gloves, respirator, and eye protection**).

### Supplies Required for Sampling:

1. In addition to PPE above, prepare sufficient disposable gloves to allow changing between each area in a house, and boot covers to allow a fresh pair in each house, and each egg room)
  - a. Bundle gloves in one-gallon Ziploc or large whirlpak bag (sufficient for 1 bag per house and egg storage)
2. Laboratory Submission Form (Appropriate form based on laboratory where samples will be submitted - one prefilled form per premise, include premise ID)
3. BHI antibiotic media (NVSL media #50067)
4. 50ml conical tubes (10 per house, 2 per egg storage, 3 for premoisting swabs, and at least 2 extra for sampling; **for 2 houses and 2 rooms = 27 tubes**)
  - a. Apply **barcode labels** on lab submission form and conical tubes
  - b. Serially number tubes (e.g. 1-10) and label with **Date & House Number**



5. Pre-fill the tubes with media listed in #3
  - a. 10 tubes with 10mls of media **per barn/house** placed in a one-gallon Ziploc or large whirlpak bag (e.g. two houses require 2 bags)
  - b. 2 tubes with 10mls of media **per distinct egg storage** placed in a one-gallon Ziploc or large whirlpak bag (e.g. two areas require 2 bags)
  - c. At least 3 tubes with 15-20mls media dedicated for pre-moistening the swabs prior to collecting samples, and include a few extra sample tubes filled with 10mls media – place these in a separate one-gallon Ziploc or large whirlpak bag
6. Sample collection devices: Large Puritan and standard Dacron swabs.
  - a. Prepare one Ziploc/whirlpak bag containing 12-13 Puritan and 20-25 Dacron swabs for each house and each collector (e.g. each collector will have 2 bags)
  - b. Have extra swabs on hand
7. Labeled one-gallon Ziploc bags for samples after they are collected
  - a. Label one bag per barn/house with **House Number and Premises ID**
  - b. Label one bag per egg storage area with **House Number and Premises ID**
8. An appropriate disinfectant for bag and conical tube surface decontamination (e.g. Lysol spray or spray bottle with VirkonS)
9. Cooler with pre-frozen gel ice packs (leave this in clean zone); designate assistant on clean side to receive samples
10. General supplies: Permanent markers, pens, trash bags (to dispose of boot covers, used whirlpak bags, and used swabs), paper towels, clipboards, tote, etc.