

# AI PCR Sample Collection

In this document we will review in details personnel, materials, and techniques necessary to collect samples from chickens and turkeys in the event of an AI outbreak.

## I. Personnel:

Ideally, two individuals are required for collecting AI PCR samples. One individual should be on the dirty side (inside the barns collecting the samples) and another individual on the clean side with clean insulated containers with clean ice packs and appropriate laboratory forms.

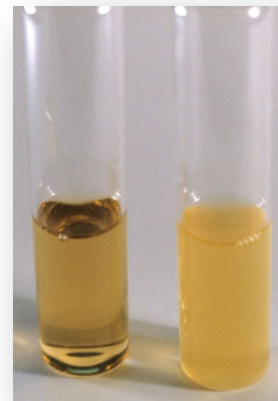
**The individual on the dirty side will collect the samples from the barns according to the instructions below.**

**The individual on the clean side will:**

- Receive collected samples in disinfected plastic bags
- Place the forms with the samples on the ice packs inside the insulated containers
- Disinfect the insulated containers.

## II. Materials Needed for Sample Collection:

- **Brain Heart Infusion (BHI) Broth:**
  - BHI is used as viral transport media and comes in a tube. It is available with or without antibiotics (either way is acceptable for HPAI testing). One tube can be used for 5 – 6 swabs (known as a “pooled sample”).
  - BHI broth should be stored in the refrigerator. Protect the tubes from light and freezing. Keep a log of your stock, and their expiration date. It is recommended that each farm unit has a stock of tubes, and that the store of tubes would be enough to support sample collection needs for 2-4 weeks in the event of an AI outbreak.
  - Do not use if the broth in the tube is turbid (cloudy) or not clear
  - It is acceptable to use tubes of 3 mL up to 5.5 mL size.
- **Swabs:**
  - For sources of swabs, please see the HPAI Toolkit on [iowapoultry.com](http://iowapoultry.com)
  - Use synthetic or semi-synthetic swabs (e.g. Dacron®/polyester, rayon, and nylon) with a plastic handle (flocked or spun head).
  - Avoid cotton or calcium alginate swabs or swabs with wooden handles
  - The swabs must be sterile and individually packaged.



Left: clear BHI-use  
Right: turbid BHI-don't use



Left: synthetic swabs with plastic handles (use)  
Right: cotton swabs with wooden handles (do not use)

- **Other Materials to take with you into the house:**
  - Gloves, disposable latex or nitrile
  - Personal protective equipment (coveralls, shoe covers, hairnets or bouffant, masks)
  - Ziplock bags
  - Markers
  - Sprayer bottles filled with a proper disinfectant (ie one approved to kill the influenza virus). Approved disinfectants include Virkon-S (1% solution), 10% bleach, Virocid, and Lysol.

**Helpful hint:** Look at the list, plan ahead, take what you need with you to the barn so that you do not have to make more than one trip back to your clean storage. Take with you slightly extra material (10%) to accommodate for any miscalculations or broken or malfunctioning material. Spray bottles should be left in the house; any other supplies that enter a poultry house should be discarded as they are considered to be contaminated.

- **Materials to have ready outside of the house in the clean area with the person on the clean side:**
  - Ice packs inside an insulated container(s).
  - Sprayer bottles filled with one of the listed disinfectants.
  - Laboratory forms.

### III. Sampling Procedure:

The choanal (palatine) cleft is the preferred site for collecting samples (respiratory) for gallinaceous poultry (chickens, turkeys, pheasants, quail). DO NOT USE this method for testing waterfowl.



1. Open the beak with one hand, using the fingers between the upper and lower beaks to prevent the bird from closing its mouth.
2. Insert the swab into the bird's mouth. Bring the swab up through the choanal cleft where to capture material from the upper respiratory tract. In other words, swab the middle of the roof of the mouth of the bird.

3. After swabbing the bird, place the swab in a tube with BHI broth. Swirl the swab in the liquid to dispel the contents of the swab into the media. Lift the swab out of the media, pressing the swab firmly against the side of the tube to remove any remaining liquid from the swab. You can collect up to 5 swabs and pool them together in one BHI tube. For pooled samples, all swabs in that tube must be from the same species as well as from the same premises/house. Collect used swabs in an appropriate container for proper disposal.



Place the swab with the sample into the BHI media and stir

4. Label the tubes with the barn number, farm name and type of bird (layer, broiler, turkey). I
5. Complete all required information on the appropriate laboratory submission form. The clean person outside the barn should do this.
6. Place the sample submission form in its own zip lock bag. Place each set of tubes in a zip lock bag and label the bags appropriately for the lab to which they are to go. Spray each bag with a disinfectant from the list above. Then perform the following steps.
  - Place the forms with the samples on the ice packs inside the insulated containers
  - Disinfect the insulated containers.