

Instructions for collecting samples for *M. ovipneumoniae* and pink eye agent testing

1. **Prior to collecting Samples:**
 - a. Owner must sign Consent Form
 - b. Fill out Herd Health Survey Questions
2. Issue a **Farm Code**: 5 characters, 3 letters, 2 numbers (ex, ABC12)
3. **Animal ID numbers**: use farm code followed by a number: ADC12-1, ABC12-2, ABC12-3....)
4. Gloves should be worn and changed or sprayed with ethanol or sanitized in between animals. If you do not use gloves sanitize your hands between animals.

NOTE: Duplicate Blood Samples and Nasal Swab samples will be sent to WADDL.

Keep the samples for the 2 labs separated use separate submission forms that are filled out completely

Samples to the USDA ARS Lab will be as follows:

All animals on the farm tested

First collection: 2 ocular swabs, 1 Nasal Swab, 1 Blood Tube

Second collection: 1 Nasal Swab only

Third collection: 1 Nasal Swab only

Samples to WADDL will be as follows:

Duplicate samples of blood and nasal swabs.

First collection: 1 Nasal Swab, 1 Blood Tube

Second collection: 1 Nasal Swab only

Third collection: 1 Nasal Swab only

Herds less than 10 animals collect duplicate samples for each sheep or goat.

Each animal should have collected:

- 2 red-top tubes of blood (half full minimum) from all animals older than 6 months. Each tube labeled with the animal ID# **ABC12-1**.
Duplicate samples - one for USDA-ARS, one for WADDL
- 2 duplicate nasal swabs on all animals: each swab is inserted into both nostrils in order to obtain duplicate swabs (One labelled "N1" **N1-ABC12-1** and duplicate labelled "N2" - **N2-ABC12-1**).
Duplicate samples - one for USDA-ARS, one for WADDL
- 2 ocular swab samples collected on all animals: 1 per eye (labelled "OD" right eye - **OD-ABC12-1**; and "OS" left eye - **OS-ABC12-1**). **Send to USDA-ARS lab only.**

At the 2nd and 3rd collection times, collect nasal swabs only.

Duplicate samples may be collected. We want to track individual animals on repeated sampling so use the same sample number for each animal at each collection time.

Herds larger than 10 animals collect duplicate samples on a minimum of 10 animals and up to a maximum of 20% of the flock or herd.

Selecting young stock as much as possible.

Duplicate Samples for at least 10 animals or up to maximum of 20% of the flock or herd described above.

Animals with no duplicate samples should have collected:

- 1 red-top tube of blood (half full minimum) tube labeled with the animal ID# **ABC12-11**.
- 2 ocular swab samples collected: 1 swab for each eye (labelled "OD" **OD-ABC12-11** and "OS" **OS-ABC12-10**)
- 1 nasal swabs: each swab is inserted into both nostrils in order to obtain duplicate swabs (labelled "N1" followed by the animal number)

At the 2nd and 3rd collection times, collect nasal swabs only.

Duplicate samples may be collected. We want to track individual animals on repeated sampling so use the same sample number for each animal at each collection time.

5. **Blood Collection:**

- a. Spray or wipe down the jugular groove with ethanol prior to performing venipuncture

6. **Swab Collection:**

Twist open the cylinder containing the swab by firmly gripping the colored cap with the "swabbing hand" and holding the tube/cylinder with the other hand (do not touch the end of swab). Wipe any debris or feed from the nares. Try to avoid touching outside of the location being sampled (i.e., the skin around the nose/eyes or anywhere else in the environment). After collecting sample, snugly place the swab back down into the plastic cylinder, avoiding touching the swab to anything outside of the cylinder.

Nasal swab:

- Gently insert the swab ("cotton end") deep into the nasal cavity and twist/move around for approximately 5 seconds. Insert up to within 1 cm of the cap that the swabs are attached to (only go until there is resistance on young kids, typically around 3-6 cm into the nasal passage; lambs seem to have wider nasal passages than goat kids, but only go as far as possible without resistance).
- Swab inserts easiest if you keep it along the ventromedial aspect of the nasal cavity. Gentle twisting or slight in and out movements to collect the sample and AVOID CAUSING excessive irritation or trauma and getting BLOOD ON SWAB.
- Both nasal cavities should be swabbed with the same swab to ensure a good collection of mucus and collect duplicate samples as needed (one is tested in my lab and the second is sent to an independent lab for confirmation).

Ocular swab:

- Swabs are labeled for right ("OD") and left ("OS") eye.
- Pull ventral eyelid out and gently insert swab into the conjunctival recess, roll 360 degrees and return swab to the cylinder

Shipping Samples:

USDA Samples: Place the **2 ocular swabs**, the **1 nasal swab** and the **1 tube of blood** into a plastic whirl-pak bag labeled with the animal ID. One bag per animal.

Place all the samples for USDA –ARS in one large plastic bag then put the sample forms in a separate plastic bag to keep it from getting wet or contaminated if a sample breaks. Remember to put sample submission forms in a plastic bag to protect the paperwork

WADDL Samples: Place the duplicate samples: **1 nasal swab**, **1 tube of blood** into a plastic whirl-pak bag labeled with the animal ID. The samples from each animal in a separate whirl-pak bag. Then all the individual whirl-pak bags then into one large plastic bag. This will allow the lab to process the samples more efficiently.

Keep these duplicate samples in a separate plastic bag with the WADDL submission forms. Put sample submission forms in a plastic bag to protect the paperwork.

WADDL submission forms are included with the sample kit they are partially filled out for your convenience. On the Front Page you must fill out the FARM CODE and the species of animal. If both sheep and goats are collected on one farm note which samples are from sheep and which are goat samples. On the back page print the Farm Code and the sample number for each sample. You can note the species next to the tube if both sheep and goats are collected on one farm.

Place the samples at refrigerator temperature or on ice pack as soon as possible and ship with icepack via FedEx Priority or overnight delivery. If samples are collected too late in the day, please place in the refrigerator (do not freeze) until the next day. Samples should be shipped within 24 hours of collection so they are received for processing within 48 hours post-collection.

Samples must be shipped Monday through Thursday morning to:

Dr. Maggie Highland
USDA-ARS-ADRU
3003 ADBF
Washington State University
Pullman, WA 99164-6630

Office phone: 509-335-6327

Cell phone: 608-213-3025

FedEx Account:

Note: Contact FedEx prior to shipping from your location on a Thursday. Shipping from some areas of Alaska FedEx will not guarantee overnight delivery.

Samples must not be shipped on FRIDAY. This results in substantially increased cost and requires someone to work over the weekend.

Plan ahead; if there is no alternative for a Thursday or Friday shipment, you must make special arranges this with Dr Highland prior to sending samples.

Additional information from Dr Highland:

Scenario A (best option): *****

Samples are collected and shipped out the day of collection for first overnight delivery arriving, the day after collection. Collection would need to be done on Mon, Tue, Wed, or Thurs. This is required for samples to be received Tue, Wed, Thurs, or Friday here in Pullman, WA.

Check with Fed Ex to determine if next day delivery is possible from your location. If no do not ship on Thursday.

Scenario B (2nd best option): ***

Samples are collected on Sun, Mon, Tue, Wed, then placed on ice packs or in a cooler at 4C then are shipped out the day after collection, arriving 2 days/48 hours post-collection.

With either scenario A or B – the blood doesn't need to be spun and serum pulled off/separated.

Scenario C (3rd option, less favorable): **

Samples are collected on Sat, Sun, Mon, or Tue, then shipped out 2 days after collection (shipped on Mon, Tue, Wed, and Thurs). Keeping the swabs on ice packs/at 4C is OK. Serum should be spun and separate though, prior to shipping, if at all possible, and can be kept at 4C. This will put the samples in our hands in Pullman, WA 3 days post collection. This is the maximum time for ocular and nasal swabs to stay at 4C prior to either being frozen or having DNA isolated; this is also the max time for leaving serum at refrigerator temperatures (or whole clotted blood, if there is no option for separating the serum from the RBC clot prior to shipping).

Note: If samples cannot be shipped within 48 hours of collection:

The serum should be separated and transferred into new tubes (ie. flip top or o-ring freezer tubes) and frozen. All swabs should also be placed in the freezer. Samples can be stored like this for a few weeks (we don't like to leave the dry nasal swabs in freezer longer than necessary) and then shipped on dry ice. If dry ice isn't available, holding off on shipping the serum until available may be best, otherwise pack well with abundant ice packs and freeze at -80 for a few days prior to shipping on ice packs. The nasal swabs can be sent after freezing on ice packs if dry ice isn't available, however, please let us know if this is the case so we can isolate the DNA the same day the samples arrive.