



Bovine Viral Diarrhea (BVD) Virus PCR Sampling Guidelines *For ear notch & nasal swab samples*

1. Materials and Equipment Needed for Ear Notch Collecting:
 - a. Ear notching tool that yields a 1cm a side notch: Recommend purchasing two or more tools.
 - b. Red Top blood tube: The preferred tube is a **VACUETTE® No Additive Tube**: 9ml.
 - c. 24-deep well plate: Can be obtained from WVDL. (Submission form available via email.)
 - d. Shipping container rack that holds individual tubes in slots.
 - e. Submission form: Use the “Serologic and Multiple Test Submission Form”. This form can be found on our web site: www.wvdl.wisc.edu (use with tubes)
 - f. Disinfectant for rinsing notching tool: 10% bleach (eg.100ml (3oz) bleach in 900ml, (27oz) water).
 - g. Clean rinse water: 3-5 gallon bucket. Change bucket water every 20-30 notches.
 - h. Use disposable gloves and wear clean coveralls.
 - i. Do not vaccinate or tattoo at the same time samples are taken.

2. Collection Procedure:
 - a. Label Red Top collection tubes with the animal ID and sequence number (#1 through number in submission). Alternatively, label side of 24-well plate with pool number.
 - b. Dip notching tool in disinfectant then ALWAYS rinse away disinfectant with copious quantities of clean water. **Caution:** Residual disinfectant on the notching tool will yield false negative results, therefore thorough rinsing with clean water is required!!
 - c. Take a proper size ear notch from a clean portion of the ear (figure 5). For comparison, a triangle notch size is superimposed on a nickel (figure 6). Place notch into either appropriate well in 24-well plate or a labeled, dry, red top collection tube (no formalin, other liquid or separator gel; do not use snap-cap milk tubes or whirl-pac bags). **Caution:** Collected ear notch **MUST** be free of contaminating dirt, feces, tattoo ink or BVD vaccine. Be sure to seal 24 well plates with rubber plate lids shipped with deep well plates prior to shipping.
 - d. Store collected ear notches for a maximum of 72 hours at refrigerator temperatures and ship over night to the WVDL on cold packs.



Figure 5: Best location for taking ear notch

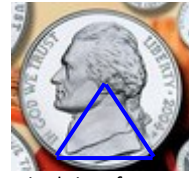


Figure 6: Required size of ear notch, ~1cm per side

3. Materials Needed for Nasal Swab Collecting:

- a. The following form and supplies can be found by visiting our website:

<http://www.wvdl.wisc.edu/index.php/forms/>

- i. BVD PCR NASAL SWAB SUBMISSION FORM
- ii. BVD NASAL SWAB KIT
 1. Polyester tip swab with Dacron shaft
 2. 96-array deep well plate
 3. Aluminum seal film
 4. Re-sealable plastic bag

4. Collection Procedure

- a. Using a single Dacron or polyester-tip nasal swab with a plastic applicator, (non-wood applicator) swab 2-3 inches inside one nostril with enough pressure to capture epithelial cells.
- b. Put the swab in the 96 deep-well plate.
- c. Snap off applicator stick so that the tip is below the top of the 96 deep-well plate and not protruding above the top of the array. If necessary, use scissors to cut applicator stick being careful not to touch the collection tip.
- d. Wipe down the scissors between cuts with dry, clean cloth.
- e. Do not add liquid to 96 deep-well plate.
- f. Cover with aluminum foil.
- g. Record the sample ID number on WVDL submission form in the corresponding well (an electronic spreadsheet can be used in addition to the WVDL submission form for ease of entry).
- h. Mark the 96 deep-well plate with the corresponding submission form page number.
- i. Place the 96 deep-well plate in a re-sealable bag provided and enclose the WVDL submission form in a separate bag.
- j. Ship the plate and form with cold packs overnight, not on Fridays.
- k. Refrigerate samples until packaging for up to one week. **Do not freeze.**
- l. If shipping from within Wisconsin, we recommend UPS Ground as a fast and inexpensive submission handler.
- m. A video of this collection procedure is located on our website at:

<http://www.wvdl.wisc.edu/index.php/forms/>