Wisconsin Veterinary Diagnostic Laboratory
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Use of Deep Nasopharyngeal Swabs for Bovine Respiratory Disease Testing

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Introduction

Deep nasopharyngeal swabs have been validated as a viable alternative to trans-tracheal wash or bronchial-alveolar lavage in cases of bovine respiratory disease and are superior to nasal swabs especially for *Mycoplasma bovis*.[1,2] The technique is simple and safe to perform and is very reliable.

Materials required for sample collection and submission:

1. **Double Guarded Culture Swab (33 inch length):** For bacteriological culture or viral and bacterial real time PCR, one swab is required for each animal sampled. If the submitting veterinarian wants bacteriology and virology real time PCR and bacterial culture done then two swabs are needed for each animal sampled.
2. **Bacterial Transport Media**
3. **BHI media (3ml) or sterile saline**
4. **WVDL Bovine Respiratory Disease Panels Form:** An electronic copy is available at www.wvdl.wisc.edu. Click on the submission guidelines link and select the forms option to download the WVDL Bovine Respiratory Disease Panels Form. The forms can be filled out either manually or electronically.
5. The double guarded culture swabs, real time PCR transport media (BHI) and the bacteriological culture media (Amies with charcoal) can be purchased from the Wisconsin Veterinary Diagnostic Laboratory Madison (WVDL), WI. An electronic copy is available at www.wvdl.wisc.edu. Click on the submission guidelines link and select the forms option to download the pharyngeal swab order form. The pharyngeal swab order form can be found in the supplies order section of the document. The pharyngeal swab order form can be filled out either manually or electronically.

Allow sufficient time (3-5 working days) for delivery of the kit. The cost of the bacterial culture kit which includes six double guarded culture swabs and bacterial transport media (Amies with charcoal) is $40.00. The cost of the viral and bacterial real time sampling kit which includes six double guarded culture swabs and BHI transport media is $45.00. The cost of the bacteriology/virology real time PCR and bacteriological culture sampling kit which includes 12 double guarded culture swabs, six bacterial transport media and six BHI transport media and a next-day air return shipping label is $65.00. In addition to the cost of the kit, the WDVL will also charge for the shipping costs to the clients as well.

Livestock producers can purchase a kit with a valid credit card. The cost of the kit does not include the testing costs. Pharyngeal swab samples must be submitted to the laboratory by a licensed veterinarian. Testing will not be done unless the WVDL receives a completed Bovine Respiratory Disease Panels Form that is signed by a veterinarian. Livestock producers should coordinate the collection of samples with their herd veterinarian.

Collection procedure: Real Time PCR for Bacteria and Viruses.

Veterinarians should plan on sampling 4-6 animals during an acute outbreak of respiratory disease. If at all possible, samples should be collected **before** the onset of antimicrobial treatment. Samples **must be chilled within 1-2 hours of collection**.

1. Restrain the animal's head. The animals head **cannot** move. Movement of the head can cause the swab to **break off** in the pharynx.
2. Clean the nostrils with a clean, disposable cloth.
3. Measure the distance from the nostril to the medial canthus of the eye.
4. Remove the twist tie from the culture swab.

5. Insert the 33 inch double guarded culture swab into the **ventral** meatus of the nose and advance it the pre-measured distance from the nostril to the medial canthus of the eye. Swabs placed in the dorsal meatus of the nose cannot advance far enough to obtain a deep pharyngeal sample.
6. Retract the culture swab approximately 1-2 inches.
7. Push the inner blue PVC swab sheath through the end of the outer clear PVC tube.
8. Push the polyester-tipped polystyrene swab through the blue PVC swab sheath for a distance of roughly 1-2 inches. Vigorously rotate the swab against the pharyngeal mucosa for **30-45 seconds.**
9. Retract the polyester tipped swab into the blue PVC swab sheath.
10. Remove the entire double guarded swab from the animal's nose.
12. Using a clean pair of scissors cut the polyester tipped swab roughly **5-6** inches from the tip. **Do not cut the swab too short;** short swabs are difficult to remove from the transport media. Place the swab in the liquid transport media (BHI).
13. Label the liquid transport media legibly with the animal's identification number or name. Please make sure the animal's I.D. matches exactly the I.D. on the WVDL Bovine Respiratory Disease Panels Form.
14. If the samples cannot be shipped immediately, they should be temporarily stored at 4 °C.

Collection procedure: Real Time PCR for Bacteria and Viruses plus Conventional Bacteriological Culture.

1. Restrain the animal's head. The animals head **cannot** move. Movement of the head can cause the swab to **break off** in the pharynx.
2. Clean the nostrils with a clean, disposable cloth.
3. Measure the distance from the nostril to the medial canthus of the eye.
4. Remove the twist tie from the culture swab.
5. Insert the 33 inch double guarded culture swab into the **ventral** meatus of the nose and advance it the pre-measured distance from the nostril to the medial canthus of the eye. Swabs placed in the dorsal meatus of the nose cannot advance far enough to obtain a deep pharyngeal sample.
6. Retract the culture swab approximately 1-2 inches.
7. Push the inner blue PVC swab sheath through the end of the outer clear PVC tube.
8. Push the polyester-tipped polystyrene swab through the blue PVC swab sheath for a distance of roughly 1-2 inches. Vigorously rotate the swab against the pharyngeal mucosa for **30-45 seconds**.
9. Retract the polyester tipped swab into the blue PVC swab sheath.
10. Remove the entire double guarded swab from the animal's nose.
11. Using a clean pair of scissors cut the polyester tipped swab roughly **5-6** inches from the tip. **Do not cut the swab too short;** short swabs are difficult to remove from the transport media. Place the swab in the bacterial transport media. **Make sure the polyester-tipped swab is fully immersed in the transport media**.
12. Repeat the procedure in the other nostril. Place the polyester tipped swab in the liquid (BHI) transport media.
13. Label all the transport media legibly with the animal's identification number or name. Please make sure the animal's I.D. matches exactly the I.D. on the WVDL Bovine Respiratory Disease Panels Form.
15. If the samples cannot be shipped immediately, they should be temporarily stored at 4 °C. Maintaining swabs at 4 °C instead of at room temperature increases the recovery rate of bacterial pathogens from diagnostic samples.[3,4]

Shipping Requirements

- **Completely fill out** the WVDL Bovine Respiratory Disease Panels Form. The form can be filled out either manually or electronically.
- Send the samples **overnight** with a sufficient number of ice packs to ensure they remain cold during shipment to the laboratory. The laboratory should receive the samples no later than 24-36 hours after collection.
- If possible, clients should schedule shipments to avoid weekend and holiday delivery of samples to the laboratory.

References

1. Godinho KS et al. 2007. Use of deep nasopharyngeal swabs as a predictive diagnostic method for natural respiratory infections in calves. Vet Rec 160: 22-25.
2. Thomas A. et al. 2002. Comparison of sampling procedures for isolating pulmonary mycoplasmas in cattle. Vet Res Comm 26: 333-339.
3. Rishmawi N et al. 2007. Survival of fastidious and nonfastidious aerobic bacteria in three bacterial transport swab systems. J Clin Microbiol 45: 1278-1283.
4. Perry JL 2001. Effects of temperature on fastidious organism viability during swab transport, abstr. C-55, p.51. Abstr 101st Gen Meet Am Soc Microbiol, Orlando, Fl.