



**Wisconsin Veterinary
Diagnostic Laboratory**
UNIVERSITY OF WISCONSIN-MADISON

Diagnostic Submission Guidelines: Bovine *Campylobacter* and *Trichomonas*

The diagnosis of *Campylobacter* and *Trichomonas* venereal infections in cattle is contingent upon proper sample collection and submission of the samples in the appropriate transport medium. Each organism and testing modality has specific transport medium requirements. PLEASE READ THE FOLLOWING CAREFULLY.

Materials required for sample collection and submission:

1. Sampling device—one for each animal to be sampled.

- A. Bovine Trichomoniasis Kit: Each kit comes with a 28 inch (71.12 cm) long collection catheter. The outside dimension of the catheter is 0.215 inches (0.546 cm) and it is assembled with a 19 inch (48.26 cm) tube protector.
- B. Rubber Bulb or 20ml syringe

The Bovine Trichomoniasis Kit and Rubber Bulb are available from Continental Plastic Corp., Delavan, WI 53115, telephone 262-728-4800; FAX 262-728-4810; www.continentalplastic.com. The catalog numbers are B8-6675 and B8-6677 for the Bovine Trichomoniasis Kit and Rubber Bulb, respectively. The kit also can be purchased from the WVDL website with the [WVDL Microbiology Media Order Form](#). Please allow sufficient time (3-5 working days) for delivery.

2. Transport media

- A. *Campylobacter fetus* subspecies *venerealis*—samples for culture must be submitted in Weybridge transport medium. Shelf life of Weybridge transport medium is six (6) months; medium must be **refrigerated** until used. Culture is the **ONLY** available testing for this organism at the WVDL.
 - Weybridge transport medium can be purchased from the WVDL website with the [WVDL Microbiology Media Order Form](#). Please allow sufficient time (3-5 working days) for delivery.

B. *Trichomonas foetus*—samples for either direct examination, or culture must be submitted in InPouch™ TF pouches. Please see the “Inoculation of transport media” section below for more information on InPouch™ TF and other acceptable submission types. Samples submitted for PCR testing can be submitted in 1.5 ml of saline (PBS) or Lactated Ringer’s solution. If culture and PCR are both needed, 2 samples must be submitted; one for culture (InPouch™ TF required) and one for PCR testing. The Biomed Diagnostic TF Transit Tube™ media is not recommended for culture or PCR.

Pouches in packs of ten (10) or one hundred (100) are available from Biomed Diagnostics, White City OR 97503, telephone 800-964-6466, FAX 541-830-3001; www.biomeddiagnostics.com. The catalog numbers are 12-011-001 and 12-011-002 for the 10 and 100 test kits, respectively. If fewer than ten (10) pouches are needed they can be purchased from the WVDL website with the [WVDL Microbiology Media Order Form](#). Please allow sufficient time (3-5 working days) for delivery.

3. Disposable gloves—one pair for each animal sampled.

4. Sterile Falcon tubes—5 ml size (BD Ref # 352054)

5. Sterile saline (PBS) or Lactated Ringer’s solution— used to dispense collected sample, 1.5 ml (for single test) or 3 ml (for two tests), into the sterile Falcon tubes.

6. Laboratory submission form— [Venereal Agents Submission Form](#). An electronic copy is available at www.wvdl.wisc.edu. Click on the form’s link to download the submission form.

Collection procedures: Male animals

The organisms inhabit the preputial cavity and epithelial crypts of the glans penis. The collection procedure is the same for both *Campylobacter* and *Tritrichomonas*; however, the transport media are different.

1. Restrain animal adequately.
2. Use a separate pair of sterile gloves and a separate collection device for each animal. *C. fetus* can be transmitted between bulls by contaminated hands or equipment.
3. Clean debris from the preputial orifice and clip preputial hairs to about one-half inch length.
4. Insert the sampling device (pipette inside tube) into the preputial opening to about the distal third of the preputial cavity.
5. Advance the collecting pipette through and beyond the protecting tube to the preputial fornix.
6. Collect the sample by rapidly scraping the pipette back and forth in short strokes on the mucosa of the distal penis and fornix area while applying suction with a rubber bulb or syringe and massaging the glans penis through the sheath to move smegma into the pipette. Fifteen to thirty (15-30) strokes of the pipette are required to obtain an adequate sample.
7. Retract the pipette back into the protecting tube and remove the entire device from the preputial cavity.
8. Collect at least one inch (2.54 cm) of smegma in the end of the collecting pipette.

Collection procedures: Female animals

The organisms are found in the cervical mucus. If cervical mucus or vaginal discharge cannot be obtained, the anterior vagina may be sampled. The sample collection procedure for both *Campylobacter* and *Tritrichomonas* is the same; however, the transport media are different.

1. Restrain animal adequately.
2. Use a separate pair of sterile gloves and a separate collection device for each animal.
3. Clean debris from the vulva.
4. Immobilize the cervix per rectum and insert the sampling device into the anterior third of the vagina.
5. Pass the collection pipette through the protecting tube and advance it to the cervical os.
6. Apply suction with a rubber bulb or syringe to aspirate cervical mucus into the pipette. Some persistence may be required to aspirate the thick mucus from this area. If postcoital pyometra due to *T. foetus* is suspected, the medium should be inoculated with a specimen of the uterine exudate.
7. Retract the pipette into the protecting tube and remove the entire collection device from the vagina.

Inoculation of transport media

Note: If the ambient temperature in the barn is **less than 40 °F (4 °C)**, the Weybridge transport medium must be kept on a heating pad or in an insulated container on top of hot water bottles.

Remove the collecting pipette from the protecting tube just prior to flushing the genital mucus (preputial smegma or cervical mucus) into 1.5 ml (for 1 test) or 3 ml (for 2 tests) of sterile saline or lactated Ringer's solution (without antibiotic preservatives) contained in a small sterile Falcon test tube (5 ml tube). Flush the sample back and forth several times until it is thoroughly mixed.

A. *Campylobacter* culture only—inoculate 1.5 ml of the sample suspension into the **bottom** of a tube of Weybridge medium, being careful to **avoid inoculation of air**.

B. *Tritrichomonas* culture only—inoculate 0.5-1.0 ml of the sample suspension in the InPouch™ TF pouches following the manufacturer's instructions.

- If Direct Exam is needed, DO NOT roll down the InPouch™ and clearly indicate on the submission for the need for Direct Exam.
- If a Direct Exam is NOT needed, please roll down each InPouch™ per the manufacturer's instructions.

C. *Tritrichomonas* PCR only—submit 1.5 ml of sample suspension, as stated above, directly for PCR.

D. *Campylobacter* and *Tritrichomonas* culture—inoculate one-half (1.5 ml) of the sample suspension into the **bottom** of a tube of Weybridge transport medium, being careful to **avoid inoculation of air** and the remaining one-half (1.5 ml) of the sample suspension in the InPouch™ TF pouches following the manufacturer's instructions.

Shipping Requirements

- Complete the [Venereal Agents Submission Form](#) and send samples to the WVDL-Madison by the most expeditious means. The laboratory should receive the samples within 24-48 hours after collection.
- Package the samples so they are protected from heat and cold. Samples should be **maintained at room temperature** (65-80 °F, 18-27 °C) while in transit. Weybridge samples, when shipped without InPouch™ TF pouches, can be shipped at 4-8 °C. Monke et al. (2002) provides evidence for shipment of samples at approximately 25 °C. Use extra insulation and you can use a room temp ice pack in cold weather and refrigerated ice packs in hot weather.
- Clients should schedule shipments to avoid weekend and holiday delivery of samples to the laboratory.

Test Sensitivity

Three consecutive negative cultures or a single negative real time PCR test for *Tritrichomonas* are required to provide evidence that a bull is free of infection with *Trichomonas*. Three consecutive negative cultures are required to provide evidence the bull is free of infection with *Campylobacter fetus* subspecies *venerealis*. Testing females for *Campylobacter* and *Tritrichomonas*, the only approved methods include the direct exam for *Trichomonas*, and culture for *Campylobacter* and *Tritrichomonas*.

Reference

- Monke HJ, Love BC, Wittum TE, Monke DR, Byrum BA. Effect of transport enrichment medium, transport time, and growth medium on the detection of *Campylobacter fetus* subsp. *venerealis*. *Journal of veterinary diagnostic investigation*. 2002 Jan;14(1):35-9.