

**BOVINE CAMPYLOBACTER
AND TRICHOMONAS**

Wisconsin Veterinary Diagnostic Laboratory
445 Easterday Lane
Madison, Wisconsin 53706-1253

Procedures for

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SAMPLE COLLECTION AND SUBMISSION

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 has specific transport medium requirements.

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

The Bovine Trichomoniasis Kit and Rubber Bulb are available from Continental Plastic Corp., Delavan, WI 53115, telephone 262-728-4800; FAX 262-728-4810. The URL for Continental Plastic Corporation is www.continentalplastic.com. The catalog numbers are B8-6675 and B8-6677 for the Bovine Trichomoniasis Kit and Rubber Bulb, respectively.

2. Transport media

- A. *Campylobacter fetus* var. *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.

Weybridge transport medium can be purchased from the Wisconsin Veterinary Diagnostic Laboratory Madison, WI. Telephone 608-262-5432. Ask for the Media Specialist; 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. Pouches in packs of twenty (20) or one hundred (100) are available

from Biomed Diagnostics, White City OR 97503, telephone 800-964-6466, FAX 541-830-3001. The catalog numbers are 11-1101 and 11-1003 for the 20 and 100 test kits, respectively. The URL for Biomed Diagnostics is www.biomeddiagnostics.com.

If fewer than ten (10) pouches are needed they can be purchased from the WVDL-Madison. Telephone 608-266-2465. Ask for the Media Specialist; 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**—dispense 3 ml into the Falcon tubes.**6. Laboratory submission form**—AH-HL-49 (Venereal Agents Test Report). An electronic copy is available at www.wvdl.wisc.edu. Click on the forms 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 *Trichomonas*; however, the transport media are different.

1. Restrain animal adequately.**2. Use a separate pair of 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 *Trichomonas* is the same; however, the transport media are different.

1. Restrain animal adequately.
2. Use a separate pair of 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.

- A. **Campylobacter only**—remove the collecting pipette from the protecting tube just prior to flushing the sample into 1.5 ml of sterile saline or lactated Ringer's solution (without antibiotic preservatives)

contained in a small sterile test tube (5 ml Falcon tube). Flush the sample back and forth several times until it is thoroughly mixed. 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. **Trichomonas only**—inoculate 1.5 ml of the sample suspension in the InPouch™ TF pouches following the manufacturer's instructions.
- C. **Campylobacter and Trichomonas**—Add the smegma to 3.0 ml of sterile saline or lactated Ringer's solution contained in a small sterile test tube (5 ml Falcon tube). Flush the sample back and forth several times until it is thoroughly mixed. 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 form AH-HL-49 and send samples to the WVDL-Madison by the most expeditious means. The laboratory should receive the samples within 24-48 hours after collection.
- When planning to collect more than twenty (20) samples, please schedule the submission with the laboratory prior to collecting the samples. Telephone 608-266-2465 and ask for the Bacteriology Section.
- Package the samples so they are protected from heat and cold. Samples must be **maintained at room temperature** (65-80 °F, 18-27 °C) while in transit.
- Clients should schedule shipments to avoid weekend and holiday delivery of samples to the laboratory.

Test Sensitivity

A single negative test of an individual animal is not sufficient to establish freedom from infection with *Campylobacter* or *Trichomonas*. At least three negative preputial cultures are necessary to provide adequate evidence that a bull is free of infection with these agents. Sampling of all bulls \geq 2 years of age, as well as an adequate number of recently exposed, non-pregnant cows, is the best means of establishing a herd diagnosis.