

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

The diagnosis of *Campylobacter* and *Tritrichomonas* 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.

* 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.
* Rubber Bulb or 20ml syringe

**The Bovine Trichomoniasis Kit and Rubber Bulb** are available from Continental Plastic Corp., Delavan, Wis. 53115, telephone 262-728-4800; FAX 262-728-4810;[www.continentalplastic.com](http://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](https://www.wvdl.wisc.edu/index.php/forms/). Please allow sufficient time (3-5 working days) for delivery.

**2. Transport media**

* *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.

**Weybridge transport medium** can be purchased from the WVDL website with the [WVDL Microbiology Media Order Form](https://www.wvdl.wisc.edu/index.php/forms/). Please allow sufficient time (3-5 working days) for delivery.

* *Tritrichomonas foetus*–samples for either direct examination, culture or PCR must be submitted in

InPouchTM TF pouches. The Biomed Diagnostic TF Transit Tube™ media is not recommended for culture or PCR.

**InPouchTM TF pouches** in packs of ten (10) or one hundred (100) are available from Biomed Diagnostics, White City Ore. 97503, telephone 800-964-6466, FAX 541-830-3001; [www.biomeddiagnostics.com](http://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](https://www.wvdl.wisc.edu/index.php/forms/). 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**–dispense 3 ml into the Falcon tubes.

**6**. **Laboratory submission form**– [Venereal Agents Submission Form](https://www.wvdl.wisc.edu/index.php/forms/). An electronic copy is available at [www.wvdl.wisc.edu](http://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.

**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). 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. *Tritrichomonas* only–**inoculate 0.5-1.0 ml of the sample suspension in the InPouchTM 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. *Campylobacter* and *Tritrichomonas***–Add the smegma to 3.0 ml of sterile saline or lactated Ringer’s solution contained in a small sterile test tube (5 ml). 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 InPouchTM TF pouches following the manufacturer’s instructions.

**Shipping Requirements**

* Complete the [Venereal Agents Submission Form](https://www.wvdl.wisc.edu/index.php/forms/) 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. 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*. For females, the direct exam and culture are the only approved methods.