Sample Collection Guide

Collection of samples from bulls and cows for culture of *Campylobacter fetus* and *Tritrichomonas foetus*

This procedure outlines sample collection for **culture** of *Campylobacter fetus* subsp. *venerealis* ('Vibriosis') in both bulls and cows. If you are sampling for antibody testing to look for evidence of exposure to *Campylobacter fetus* subsp. *venerealis* in cows, please refer to the procedure "Diagnosis of Bovine Venereal Campylobacterosis by ELISA".

Description

In bulls, *Campylobacter fetus* subsp. *venerealis* and *Tritrichomonas foetus* inhabit the mucosa of the glans penis, prepuce and the distal portion of the urethra. Bulls commonly become persistent carriers of these organisms and are the main reservoir of infection in the herd. Preputial scrapings (smegma) and preputial washings are suitable for culture.

In cows, *C. fetus* subsp. *venerealis* may colonise the vagina for a few months after infection, and *T. foetus* may colonise the vagina for about a month after infection. A persistent carrier stage in cows is uncommon. Vaginal washings are suitable for culture when active infection is present. Infertility is more common than abortion, but when abortion does occur, organisms may be cultured from abomasal contents of aborted foetuses, placental tissue and uterine discharges.

Storage of Media (both bulls and cows)

- Store both *Campylobacter* Enrichment Transport Media (CETM) and *Tritrichomonas foetus* enrichment media-(TFEM) at 5 ± 3°C once received.
- The CETM and TFEM may be kept at 5 ± 3°C until **the expiry date on the label.** After this time, the media should be not used and be discarded.

Inoculation of Media (both bulls and cows)

- Wear safety glasses, protective gloves and clothing when handling the media. Avoid contact with the media.
- Because *C. fetus* subsp. *venerealis* and *T. foetus* survive best at a temperature of 18-37°C, the CETM and TFEM vials must be allowed to warm prior to inoculating them.

Submission of Media (both bulls and cows)

- It is important to maintain the samples at 18-37°C during transport to the laboratory – **send the samples in an esky WITHOUT ice bricks.**
- Samples must be received by the laboratory within 72 h of collection (within 24 h is preferred). Samples submitted for *T. foetus* culture from cattle being exported for breeding must be delivered to the laboratory within 24 h of collection. **Include the date of collection on the submission form.**
- Label the inoculated containers clearly with the Animal IDs. If you are sampling large numbers of animals, it is best to label the specimens sequentially starting at 1 and use a key list to identify the animals.
- If it is suspected that samples have been exposed to temperatures outside of the range of 5-37°C during transit, target pathogen viability could have been affected. The laboratory reserves the right not to test these samples. Please refer to our terms and conditions on the NSW DPI Laboratory Services website:

https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/customer-service/nsw-dpi-laboratories-services-terms-and-conditions

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Sample collection from bulls

Bull sampling method A – Using a Tricamper™ tool

For an excellent video demonstrating safe collection of preputial samples from bulls visit https://www.youtube.com/watch?v=LUhUkg4jB-8. Note that this video demonstrates two different collection techniques; we recommend the technique which utilises a Tricamper™ sampling tool. The wet collection technique is also described below.

Equipment Required (available from the laboratory upon request)

1. One Tricamper™ sampling tool per bull.
2. Sterile, screw-capped, 10 mL containers, each containing **5 mL** phosphate buffered saline (PBS). Only one required per bull.
3. Sterile pipettes or syringes with 0.5 and 1 mL graduations (to transfer PBS into the transport media – not supplied by the laboratory).
4. *Campylobacter* enrichment transport medium (CETM).
5. *Tritrichomonas foetus* enrichment medium (TFEM).

Method

1. Minimise contamination of samples by clipping or removing hair and other material around the preputial orifice or vulva. Do not clean with disinfectants as this may reduce diagnostic sensitivity.
2. Whilst holding the anterior aspect of the sheath with one hand, insert the Tricamper™ tool (60 cm long polyethylene tube with a corrugated scraper head) into the prepuce, with the scraper head adjacent to the penis. Move the Tricamper™ back and forth, so that it scrapes across the preputial mucosa and surface of the penis. This action will cause some material from the prepuce and penis to adhere to the corrugated surface of the Tricamper™ head. Some preputial fluid will also be sucked into the hollow head of the Tricamper™ by capillary action. It is therefore important, when withdrawing the Tricamper™ from the prepuce, to block the hole in the end of the Tricamper™ handle with a finger, otherwise some of the sample may dribble out of the Tricamper™ head. Be careful also to keep the Tricamper™ as steady as possible when withdrawing it, as it is easy to accidentally flick the material off the Tricamper™ head.
3. Hold the Tricamper™ just off horizontal, insert the tip into the container of PBS and remove your finger from the end of the Tricamper™. Using side-cutters cut off the black head of the Tricamper™ into the container of PBS. Replace the lid securely on the container of PBS and shake vigorously to rinse the smegma off the Tricamper™.
4. Allow the smegma suspension to settle, then using a sterile pipette or a syringe; **inoculate the CETM with 1 mL of supernatant** and the **TFEM with between 0.5 to 1 mL (about 0.75 mL) of the sediment**.

It is important to remember that the rate of successful culture from *C. fetus* subsp. *venerealis*-infected bulls is estimated to be 35-40%, despite the use of *Campylobacter* enrichment transport media (CETM)[1]. Therefore, submitters should anticipate a high rate of false-negative results for *C. fetus* subsp. *venerealis* culture. In cases when *C. fetus* subsp. *venerealis* infection is highly suspected, multiple samplings taken at different time points may be required in order to confirm infection in a bull by a positive culture.

1. Chaban, B., García Guerra, A., Hendrick, S. H., Waldner, C. L., & Hill, J. E. (2013). Isolation rates of Campylobacter fetus subsp venerealis from bovine preputial samples via passive filtration on nonselective medium versus selective medium, with and without transport medium. American Journal of Veterinary Research, 74(8), 1066-1069.

Sample collection from bulls

Bull sampling method B – Wet method

We recommend using a Tricamper™ sampling tool for collection of preputial fluid, if possible (method A).

Equipment Required (available from the laboratory upon request)

1. Sterile insemination pipette connected by plastic or rubber tube to a 20 mL sterile disposable syringe per bull (NOTE – these items will need to be supplied yourself).
2. Sterile, screw-capped, 30 mL containers, each containing **20 mL** phosphate buffered saline (PBS).
3. Sterile pipettes or syringes with 0.5 and 1 mL graduations (to transfer PBS into the transport media – not supplied by the laboratory).
4. *Campylobacter* enrichment transport medium (CETM).
5. *Tritrichomonas foetus* enrichment medium (TFEM).

Method

1. Fill the 20 mL syringe with the 20 mL of PBS and connect the syringe to the pipette (plastic pipettes used for equine AI work are ideal).
2. Introduce the pipette to the full length of the preputial cavity and hold the preputial orifice firmly closed with one hand around the pipette to prevent PBS from escaping.
3. Inject 20 mL PBS into the prepuce and wash/massage thoroughly with one hand, forcing the saline up and down along the penis several times.
4. Withdraw the tip of the pipette close to the orifice, where the washing fluid has accumulated, and suck the fluid into the syringe.
5. Transfer the washings to the PBS container and stand to allow impurities to settle, then, using a sterile pipette or a syringe, **inoculate the CETM with 1 mL supernatant** and the **TFEM with between 0.5 to 1 mL (about 0.75 mL) of sediment**.

Sample collection from cows and heifers

The diagnosis rate of *Campylobacter* and *Tritrichomonas* infections is lower in cows than bulls, since vaginal infection is usually eliminated a few months post infection. The *Campylobacter* ELISA test, which detects antibodies in vaginal mucus, is usually a more reliable test to check for evidence of *Campylobacter* infection in cows and, therefore, recommend pursuing this before submitting samples for *Campylobacter* culture. It is a herd-based test – it is recommended to sample a representative number (10%) of infertile cows (at least 10 non-pregnant cows) around the time of pregnancy testing (refer to the procedure for "Diagnosis of Bovine Venereal Campylobacteriosis by ELISA").

Cow sampling method A – Using a Tricamper™ tool

We recommend using a Tricamper™ sampling tool for collection of vaginal fluid. For written instructions on the wet collection method which uses a sterile insemination pipette connected by plastic or rubber tube to a 20 mL sterile disposable syringe, please see Cow sampling method B.

Equipment Required (available from the laboratory upon request)

1. One Tricamper™ sampling tool per cow.
2. Sterile screw-capped, 10 mL containers, each containing **5 mL** phosphate buffered saline (PBS).
3. Sterile pipettes or syringes with 0.5 and 1 mL graduations (to transfer PBS into the transport media) – not supplied by the laboratory.
4. *Campylobacter* enrichment transport medium (CETM).
5. *Tritrichomonas foetus* enrichment medium (TFEM).

Method

1. Open the vulva with one hand and insert the Tricamper™ tool in a dorsocranial direction with the leading edge of the instrument in contact with the dorsal vagina. Once there is no risk of the instrument entering the urethra, entry progresses to a cranial movement so that the anterior end reaches the cervix. Move the Tricamper™ gently backwards and forwards, angling the instrument to collect scrapings from all mucosal surfaces. Block the end of the Tricamper™ (e.g. with a finger) to prevent any of the collected mucus being suctioned out. Remove the Tricamper™ from the vagina.
2. Hold the Tricamper™ just off horizontal, insert the tip into the container of PBS and remove your finger from the end of the Tricamper™. Using side-cutters, cut off the black head of the Tricamper™ into the container of PBS. Replace the lid securely on the container of PBS and shake vigorously to rinse the mucus off the Tricamper™.
3. Allow the suspension to settle, then using a sterile pipette or syringe, **inoculate the CETM with 1 mL of supernatant** and the **TFEM with between 0.5 to 1 mL (about 0.75 mL) of the sediment**.

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Sample collection from cows and heifers

Cow sampling method B – Wet method

We recommend using a Tricamper™ sampling tool for collection of vaginal fluid, if possible (method A).

Equipment Required (available from the laboratory upon request)

1. Sterile insemination pipette connected by plastic or rubber tube to a 20 mL sterile disposable syringe per cow (NOTE – these items will need to be supplied yourself).
2. Sterile, screw-capped, 30 mL containers, each containing 20 mL phosphate buffered saline (PBS).
3. Sterile pipettes or syringes with 0.5 and 1 mL graduations (to transfer PBS into the transport media) – not supplied by the laboratory.
4. *Campylobacter* enrichment transport medium (CETM).
5. *Tritrichomonas foetus* enrichment medium (TFEM).

Method

1. Fill the 20 mL syringe with 5 to 8 mL of PBS (discard the rest of the PBS so that the PBS container is empty) and connect the syringe to the pipette (plastic pipettes used for equine AI work are ideal).
2. Introduce the pipette into the vagina as far as the cervix and alternatively expel and suck the PBS back into the syringe several times to flush the vagina. It may be necessary to move the tube backwards and forwards along the floor of the vagina while applying suction with the syringe in order to locate the fluid.
3. Transfer the washings to the empty PBS container, allow them to settle, then using the pipette provided or a syringe, **inoculate the CETM with 1 mL of supernatant and the TFEM with between 0.5 to 1 mL (about 0.75 mL) of the sediment.**

Contact Us

For assistance or further information contact Customer Service.

Business hours:	8:30am – 5:00pm Monday – Friday (excluding public holidays)
Phone:	1800 675 623
Email:	laboratory.services@dpi.nsw.gov.au
Delivery address:	NSW DPI Laboratory Services Woodbridge Road Menangle, NSW 2568