Department of Primary Industries
and Regional Development

Sample Collection Guide

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

14th January 2026

ELISA testing (screening) of females for *C. fetus subsp. venerealis* is recommended prior to sample collection and testing via culture or PCR to look for antibody evidence of exposure. Refer to the procedure 'Diagnosis of Bovine Venereal Campylobacterosis by ELISA' for relevant sampling advice.

Description

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

In cows/heifers, 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 state in females with either infection is uncommon. Vaginal washings are suitable for culture when active infection is present. Infertility is more common than abortion, however, when abortion does occur, organisms may be cultured or detected via PCR from abomasal contents of aborted foetuses, placental tissue and uterine discharges.

Sample preparation

- Your preference of target pathogen, sampling method and test selection will determine the supplies you require. Sampling supplies can be ordered via this online form.

Storage of Media

- Store both *Campylobacter* enrichment transport media (CETM) and *Tritrichomonas foetus* enrichment media (TFEM) at 5 ± 3°C once received. Do not use these medias beyond the expiry date on the label.

Inoculation of Media

- Wear safety glasses, protective gloves and clothing when handling the media. Avoid skin 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 within this range prior to inoculation.

Submission of Media

- It is important to maintain the CETM and TFEM samples at 18-37°C during transport to the laboratory – send the samples in an esky WITHOUT ice bricks.
- Samples for culture must be received by the laboratory as soon as possible (within 24 h is preferred; 72 h maximum). Samples submitted for T. foetus culture for export purposes 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, 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 the range of 18-37°C during transit, target pathogen viability could be affected. The laboratory reserves the right to not test these samples. Please refer to our Terms and Conditions on the NSW DPIRD Laboratory Services website.

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Sample collection for bulls and cows/heifers

There are two sample collection methods for bulls and cows/heifers – dry and wet. There is an excellent video demonstrating safe collection of preputial samples from bulls.

When considering whether to sample the bulls or cows/heifers, the bulls are considered the main reservoir of infection for both C. fetus subsp. venerealis and T. foetus, whereas, the cows/heifers usually eliminate a vaginal infection within a few months of onset. Observation of pyometras and/or purulent vaginal discharges in females could be a good indication for sampling and testing females for T. foetus.

Sampling method A – Dry method

This sampling technique involves the use of a Tricamper™ tool.

Equipment Required (available from the laboratory upon request)

1. One Tricamper™ sampling tool per animal - 60 cm long polyethylene tube with a corrugated scraper head.
2. One sterile 10 mL container per animal, containing 5mL phosphate buffered saline (PBS).
3. Sterile pipettes or syringes with 0.5 and 1mL graduations (to transfer PBS into the transport media) – not supplied by the laboratory.
4. Campylobacter enrichment transport medium (CETM) and/or Tritrichomonas foetus enrichment medium (TFEM).

Method

1. Minimise contamination of samples by clipping hair and removing other foreign material around the preputial orifice or vulva with paper towel. Do not clean the prepuce or vulva with disinfectants as this may reduce diagnostic sensitivity. Clean, disinfect and dry any instruments between animal samplings.

   B. Bulls – whilst holding the cranial aspect of the sheath with one hand, insert the Tricamper™ tool 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 to withhold some of the sample within the Tricamper™ head. Keep the Tricamper™ as steady as possible when withdrawing it, as it is easy to accidentally flick the material off the Tricamper™ head.

   C. Cows/heifers – 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, advance the Tricamper™ in a cranial direction so that the leading 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™ with a finger to prevent loss of any of the collected mucus. Remove the Tricamper™ from the vagina.

   D. Hold the Tricamper™ just off horizontal, insert the tip into the container of PBS and remove your finger from the end of the Tricamper™. Gently snap the black Tricamper™ head off in the PBS. If this cannot be done easily, use side-cutters and clean, disinfect and dry these between samplings. Replace the lid securely on the container of PBS and shake vigorously to rinse the smegma off the Tricamper™.

Allow the smegma suspension in PBS to settle, then using a sterile pipette or a syringe; inoculate the CETM with 1 mL of supernatant (on top) and the TFEM with between 0.5 to 1 mL (about 0.75 mL) of the sediment (on the bottom).

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Sampling method B – Wet method

This sampling technique involves a wash of the prepuce or vagina using PBS.

Equipment Required (available from the laboratory upon request)

1. Sterile infusion pipette connected by a plastic or rubber end to a 20 mL sterile disposable syringe per animal (NOTE – these items are not supplied by the laboratory).
2. One sterile, screw-capped, 30 mL container per animal, 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) and/or Tritrichomonas foetus enrichment medium (TFEM).

Method

5. Fill the 20 mL syringe with the 20 mL of PBS and connect the syringe to the pipette (plastic equine infusion pipettes are ideal).
6. a. Bulls - introduce the pipette to the preputial fornix and hold the preputial orifice firmly closed with one hand around the pipette to prevent PBS from escaping. 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. Withdraw the tip of the pipette close to the orifice, where the wash fluid has accumulated, and suck the fluid back into the syringe.
7. b. Cows/heifers – introduce the pipette along the dorsal wall of the vagina to the cervix and expel the 20 mL PBS. Suck the fluid back and forth into the syringe several times to flush the vagina. To locate the fluid, it may be necessary to move the pipette backwards and forwards along the floor of the vagina while applying suction with the syringe.
8. Transfer the wash fluid to the PBS container and let it stand to allow impurities to settle, then, using a sterile pipette or a syringe, inoculate the CETM with 1 mL supernatant (on top) and the TFEM with between 0.5 to 1 mL (about 0.75 mL) of sediment (on the bottom).

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Test Selection

It is important to be aware of the differences between culture and PCR testing for your specific application. There are many variables such as infection load, sampling method, transport medias, dilution, transport temperatures and transit time to the laboratory that can impact the diagnostic sensitivity of culture. Note, culture detects live organisms only.

It is important to remember that the reported rate of successful culture from C. fetus subsp. venerealis-infected bulls is estimated to be 35-40%, despite the use of CETM1. 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 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. AJVR 74(8), 1066-1069.

Due to the diagnostic challenges presented with a low sensitivity for culture of C. fetus subsp. venerealis in bulls, PCR is preferred for diagnostic cases. Test optimisation of the PCR is ongoing at EMAI to improve diagnostic sensitivity and specificity and we therefore ask you to collect and submit both the PBS (with the head of the Tricamper™ tool) and CETM samples for PCR testing. Note, organism detection via PCR does not distinguish between live and dead organisms.

While bulls are considered the main reservoir of infection, levels of C. fetus subsp. venerealis are usually very low in the prepuce due to a chronic, asymptomatic carrier status. While PCR testing of PBS can detect low levels of the organism, levels can be so low that dilution can play a role and PCR will be unable to detect the infection, yielding false-negative PCR results. For this reason, to increase diagnostic sensitivity, our PCR protocol includes 3-4 days of incubation of the CETM samples in the laboratory prior to PCR for C. fetus subsp. venerealis. Note, this test approach results in an increased test turnaround time.

It's possible levels of C. fetus subsp. venerealis are transiently higher in heifers/cows when clinically affected (presenting as recent infertility or recent abortion), however, the challenge is predicting when suspect females might be transiently infected (typically within a few months of infection). If suspected females are sampled during this window of infection, recent in-house research at EMAI suggests PCR results will be highly specific and sensitive (approaching 100% for both), due to replication of live organisms in the CETM. Consequently, from a herd with suspected C. fetus subsp. venerealis infection, up to 10 CETM samples prepared from heifers/cows can be pooled following 3-4 days of incubation in the laboratory prior to PCR, with a single pooled test result reported per herd. A positive pooled PCR result confirms the presence of the organism in the herd and supports a diagnosis of reproductive failure due to vibriosis. Follow up testing of in-contact bulls or semen (for those herds using artificial insemination) to identify the source of the infection may be indicated.

PCR on the PBS sample (containing the head of the Tricamper™) is recommended for detection of T. foetus. i.e. there is no need to collect and submit TFEM for detection of T. foetus via PCR. Note, export protocols may require culture for T. foetus using TFEM.

Contact Us

For assistance or further information contact Customer Service.

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