Sample Collection Guide
Collection of samples from bulls and cows for culture of *Campylobacter fetus* and *Trichomonas foetus*

**Description**

In bulls *Campylobacter fetus* subspecies *venerealis* and *Trichomonas foetus* inhabit the mucosa of the glans penis, prepuce and the distal portion of the urethra. Preputial scrapings (smegma) and preputial washings are suitable for culture. Bulls commonly become persistent carriers of these organisms and are the main reservoir of infection in the herd. A persistent carrier stage in cows is uncommon. In cows, *T. foetus* may colonise the vagina for about a month after infection, and *C. fetus venerealis* may colonise the vagina for a few months after infection. Infertility is more common than abortion, but when abortion does occur, organisms may be cultured from abomasal contents of aborted foetuses and uterine discharges.

Please refer to the Media Instructions for *Campylobacter fetus* and *Trichomonas foetus* for information regarding specific transport media required for this testing.

**Sample collection from bulls**

**Sampling method A – using the Tricamper™: Collection of preputial scrapings**

When using this method, request the smaller 5 ml volumes of phosphate buffered saline (PBS) from the laboratory. Request the larger 20 ml volumes if you are using sampling method B – wet method (collection of preputial washings).

**Equipment available from the laboratory**

1. One Tricamper™ sampling tool per bull.
2. Sterile, screw-capped, 10 ml containers, each containing 5 ml phosphate buffered saline (PBS).
3. Disposable pipettes with 0.5 and 1 ml graduations (to transfer PBS into the transport media).
4. *Campylobacter* enrichment transport medium (CETM).
5. *Trichomonas 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™ (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 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.

Sampling method B - wet method: Collection of preputial washings

When using this wash method, request the larger 20 ml volumes of PBS from the laboratory. Request the smaller 5 ml volumes if you are using sampling method A – using the Tricamper™.

Equipment available from the laboratory

1. Sterile, screw-capped, 30 ml containers, each containing 20 ml phosphate buffered saline (PBS).
2. Disposable pipettes with 0.5 and 1 ml graduations (to transfer PBS into the transport media).
3. Campylobacter enrichment transport medium (CETM).
4. Tritrichomonas foetus enrichment medium (TFEM).

Equipment you need to prepare

1. Sterile insemination pipette connected by plastic or rubber tube to a 20 ml sterile disposable syringe. If more than one bull is tested, use new equipment or disinfect and rinse pipette/syringe between bulls.

Method

1. 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. 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.
2. Withdraw the tip of the pipette close to the orifice, where the washing fluid has accumulated, and suck the fluid into the syringe. Transfer the washings to the PBS container and stand to allow impurities to settle, then, using the pipette provided 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. 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.

We detail two sampling methods for Campylobacter and Tritrichomonas culture, which are recommended either in the Australia New Zealand Standard Diagnostic Techniques (ANZSDP) or in the OIE Terrestrial Manual. Other techniques have also been described, e.g. suction of vaginal mucus by a pipette or collection of vaginal mucus by swab.
Sampling method A: Collection of vaginal mucus by Tricamper™

When using this method, request the smaller 5 ml volumes of PBS from the laboratory.

Equipment available from the laboratory
1. One Tricamper™ per cow.
2. Sterile screw-capped, 10 ml containers, each containing 5 ml phosphate buffered saline (PBS).
3. Disposable pipettes with 0.5 and 1 ml graduations (to transfer PBS into the transport media).
4. *Campylobacter* enrichment transport medium (CETM).
5. *Tritrichomonas foetus* enrichment medium (TFEM).

Method
1. Open the vulva with one hand and insert a Tricamper™ 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. 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 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.

Sampling method B – wet method: Collection of vaginal washings

When using this method, request the larger 20 ml volumes of PBS from the laboratory. However you will only use about 8 ml of this for flushing the vagina. Request the smaller 5 ml volumes from the laboratory if you are using a Tricamper™.

Equipment available from the laboratory
1. Sterile, screw-capped, 30 ml containers, each containing 20 ml phosphate buffered saline (PBS).
2. Disposable pipettes with 0.5 and 1 ml graduations (to transfer PBS into the transport medium).
3. *Campylobacter* enrichment transport medium (CETM).
4. *Tritrichomonas foetus* enrichment medium (TFEM).

Equipment you need to prepare
1. Sterile insemination pipette connected by plastic or rubber tube to a 20 ml sterile disposable syringe. If more than one cow is tested, use new equipment or disinfect and rinse pipette/syringe between cows.

Method
1. Fill the 20 ml syringe with 5-8 ml of PBS. Discard the remaining PBS so that the PBS container is empty. 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.
2. 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
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