

Sample Collection Notes for Genital *Campylobacter* Tests

For the preparation of tubing for the preputial wash (bulls) and vaginal lavage (cows), polythene tubing or a similar product with internal diameter 4 mm, external diameter 6 mm is required.

For obtaining preputial wash samples from the bull, a length of about 20 cm is cut and the end is made blunt by gentle heat in a flame (Care – some tubing may be flammable). For samples from the cow a length of about 45 cm is closed at one end by gentle heating in a flame. In the 3 cm of tubing adjacent to the closed end, make about 16 small holes at various parts of the circumference. This tubing should be sterilised before use.

1. SAMPLE COLLECTION AND CULTURE FROM BULLS

Procedure

Because *Campylobacter* is difficult to isolate and maintain, media should be inoculated immediately and forwarded to the laboratory as soon as possible. Two samples each in Transport and Enrichment Media (TEM), should be submitted to the laboratory from the bull under examination. One should be labelled “U” (unfiltered) and the second labelled “F” (unfiltered).

Preparation of TEM

With care, reconstitute each of the two vials of Freeze Dried Antibiotic Supplement (FDABS) with 1 ml of sterile distilled water and ensure that the contents of the two vials are completely resuspended. Transfer the reconstituted contents of the first vial to the bottle labelled “U”, the second to the bottle labelled “F”.

1.1 Preputial Washings and Culture

The quality of sample is vitally important to the successful diagnosis of *C.fetus* infection. As some infected bulls carry comparatively small number of organisms, a strenuous physical effort should be used to liberate them from the folds of preputial and penile mucosa. *Campylobacters* are usually most numerous in the area of the formix where the mucosa of the prepuce meets that of the penis. In relation to the preputial orifice, the position of the formix depends on the state of retraction of the penis. Normally during preputial washing the penis is fully retracted, in which case the formix will be about 38 cm, (15 inches) behind the orifice. Consequently, the collector should concentrate major effort on an area 23 cm-48 cm (9-18 inches) behind the orifice.

- 1.2 The bull should be restrained so that the operator is safe and has room to move.
- 1.3 If the preputial orifice is grossly dirty, clip the hairs and wash the orifice gently with detergent and dry with clean towels.
- 1.4 Draw about 30 ml of warmed sterile PBS into a 50 ml disposable syringe and attach the tubing.
- 1.5 Before inserting the tube through the preputial orifice, massage the preputial cavity to remove any air. (This helps in the recovery of the fluid).
- 1.6 Introduce the tube into the preputial cavity to a distance of 15-20 cm (6-8 inches). Seal the orifice by gripping above the tubing with the fingers of one hand. Inject the PBS into the prepuce. The tubing and the syringe should remain *in situ* during massage.
- 1.7 With the free hand briskly massage the fluid within the preputial cavity. About 100 vigorous massage movements should be carried out, concentrating on an area 23-40 cm (9-18 inches) behind the orifice.
- 1.8 Gently withdraw the fluid back through the tube into the syringe. Recovery of fluid will be aided by massaging the prepuce towards the preputial orifice and maintaining slight negative pressure in the syringe whilst slowly withdrawing the tube.

- 1.9 Transfer the sample to a universal bottle – the bottle that originally contained the sterile PBS may be used.

NB - If less than 15 ml of fluid is recovered or the sample is grossly diluted with urine – repeat the procedure.

- 1.10 Immediately inoculate the TEM by drawing approximately 5 ml of sample into a syringe. Inoculate the TEM labelled “U” with 1 ml of sample. Attach the filter to the syringe. Gently pass about 2 ml of sample through the filter discarding the filtrate. Continue to pass sufficient sample through the filter to enable 1 ml of filtrate to be inoculated into the TEM labelled “F”.

2. SAMPLE COLLECTION AND CULTURE FROM COWS

Preparation of TEM

With care, reconstitute one vial of FDABS with 1 ml of sterile distilled water for each cow, and ensure that the entire contents of the vial are completely resuspended before adding to the TEM base.

Vaginal Lavage and Culture

- 2.1 The cow should be restrained so that the operator is safe and has room to move.
- 2.2 If the vulva is grossly dirty, the hairs should be gently cropped and the external surface gently washed with detergent and dried with clean towels.
- 2.3 The volume of mucus in the vagina is dependent on the stage of the oestrus cycle, the highest being at oestrus. At other times the mucus is thicker, less voluminous and tends to adhere tightly to the walls of the vagina. Mucus samples taken at oestrus are more likely to yield *C.fetus* on culture.
- 2.4 Fit the perforated tube to a 50 ml disposable syringe.
- 2.5 Fill the syringe and tube with 20-30 ml of PBS so that the air is excluded.
- 2.6 Insert the free end of the tube into the vagina as far as the cervix. Expel the PBS into the vagina with some force.
- 2.7 Draw the liquid back into the syringe and repeat the process several times. Withdraw the fluid by applying a gentle vacuum with the syringe. It may be necessary to move the tube backwards and forwards along the floor of the vagina to find where the liquid has lodged. It should be possible to recover at least as much fluid as has been introduced.
- 2.8 Inoculate one bottle of TEM with 1 ml of sample. (**Note:** TEM marked “F” or “U” may be used as it is not possible to obtain a filtered sample of mucus).
- 2.9 Syringe the remainder of the sample into the emptied PBS universal container for further tests

NB: These sample instructions are taken from the instructions that accompany the sampling kit.

FAIR PROCESSING NOTICE

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