



Diagnostic and Analytical Services

Veterinary Laboratory Service

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USING THE **InPouch™TF** TO SAMPLE FOR BOVINE TRICHOMONADS

Bovine trichomoniasis is a venereally transmitted infection with the flagellated protozoan, *Trichomonas foetus*. The major pathological manifestation of this infection is early embryonic death or abortion in an impregnated cow. The cow shows few other signs of infection. An infected bull is asymptomatic. The organism can only infrequently be found by direct microscopic examination of clinical specimens and serological methods of diagnosis are not reliable. In vitro cultivation is the gold standard but this method has been tedious and sometimes inaccurate.

The **InPouch™TF** system represents an improvement in the culture method for diagnosis of *T. foetus* infection. It has been found to produce reliable results in less time than the standard in vitro method, have fewer problems with contaminating microorganisms, and have significant advantages in both sensitivity and specificity.

DESCRIPTION OF THE SYSTEM AND IT'S PRINCIPLES

The **InPouch™TF** is a self-contained system for the detection by culture of *T. foetus* from bovine preputial or vaginal samples. A strip of wire tape serves as a closure for the pouch. The proprietary medium is selective for the transport and growth of the trichomonad, while inhibiting the growth of contaminating microorganisms which might interfere with reliable diagnosis. The transport and culture vessel is a flexible pouch constructed of clear plastic film which has excellent water-vapor and oxygen-barrier qualities. The resulting reduced-oxygen environment enhances and maintains the desired selective growth characteristics of the medium. Within the pouch are two V-shaped chambers. Approximately 3 ml of medium is contained in the lower chamber, and 1 ml in the upper chamber. The two chambers are separated by a channel that allows liquid to pass through only if pressure is applied. This two compartment system allows for direct observation of a newly collected specimen in the upper chamber before inoculation into the larger volume of the lower chamber for incubation. In either case, the sample can be concentrated by letting the cellular materials settle to the bottom. Microscopic observation of both chambers is done through a plastic viewing frame which is clipped onto the flexible pouch. An inoculum containing 1 to 10 organisms is sufficient to cause a positive test. Transport and off-site testing can be performed easily due to the flexible packaging and integral design of the pouch. The pouch is labelled for animal identification and test result only. The shelf-life of the test, as indicated by label expiration date, is one year at room temperature.

SAMPLING TECHNIQUE*

Specimen collection technique is important regardless of the nutrient medium used for culturing. Use a dry infusion pipette with a 20 cc syringe on both bulls and cows. In the bull, direct the pipette to the distal penis in the sheath. Take the specimen by scraping the mucosa of the distal penis and the fornix area while applying suction with the syringe. In the cow, use the same technique in the anterior vagina or the cervical os. The mucus may be thick, and some persistence may be required to get a thick, slightly yellow material that may stick to the edges of the pipette. A satisfactory sample, from either cow or bull, can also be obtained using a long, guarded swab for collection. The specimen should not be refrigerated or frozen.

* Refer also to the "Collection of Samples from Bulls for Culture of Campylobacter fetus and Tritrichomonas foetus" for wet and dry sampling options.

INOCULATION

Remove pouch from box. Fold pouch back over itself to reduce the crease. Make sure that the liquid in the upper chamber is below the closure tape to prevent fluid from leaking upon opening. Tear open the pouch at the notch just above the closure. Open the pouch, sufficiently to admit the pipette, by pulling the closure tape's middle tabs apart. Insert the specimen pipette tip into the liquid of the pouch's upper chamber. The pouch is inoculated by expelling approximately 0.5 cc and no more than 1.0 cc of the sample out of the pipette with the syringe, into the pouch. If the collected material adheres to the wall of the pipette, the pipette can be rinsed by drawing a small amount of liquid medium back into the pipette with the syringe then flushing the pipette back into the pouch. The production of bubbles should be minimized. If the specimen is on a swab, insert specimen swab into the liquid of the pouch's upper chamber. The contents of the swab should be "milked" out by pressing the tip of the swab between the fingers through the flexible walls of the pouch. Express the contents of the upper pouch chamber into the lower chamber. Roll

down the pouch until the tape is at the top of the medium; the label should still be visible. Fold the wire tape's end tabs to lock the roll. This action helps maintain partial-anaerobiasis. Return to RVL at 22°C.

TRANSPORT

InPouch™TF is an excellent device for the transport of samples. The plastic pouch resists damage in transport and maintains viability during transport. **The specimen should be maintained between 15-37°C.**

TECHNICAL NOTES

Because a large volume of fluid is being scanned, more focusing of the microscope is required when examining the **InPouch™TF** in comparison to a cover slip preparation. Make sure to focus on the liquid and not the textured plastic of the pouch.

InPouch™TF medium suppresses but does not entirely eliminate the growth of the yeast. Using low power (10x), scanning the fields included in the plastic viewer's window should be adequate to determine the presence of trichomonads. When pouches are heavily contaminated with bacteria, gas may be produced.

REAGENTS

The medium contains the following ingredients: trypticase, proteose peptone, yeast extract, maltose and other sugars, amino acids, salts, antifungal and antimicrobial agents in a normal saline phosphate buffer.

STABILITY AND STORAGE

Store the InPouch™TF at room temperature (15-25° Celsius). Shelf life is 1 year from the date of manufacture. Although the plastic film of the pouch is resistant to water vapor loss, some may occur over time. Pouches should be stored vertically, in the dark, in their closed container. If upper chamber is dry or sticky, squeeze fluid from the lower chamber. **The medium should not be frozen.** A pouch with a cloudy medium or precipitate should not be used.

REFERENCES

1. Thomas, et al 1990, *Agri-Practice Journal*, vol 11, pp. 13-17.
2. Borchardt, Normal, Thomas, Harmon, 1992, *Veterinary Medicine*, Feb, pp.104-112.