

# InPouch™ TF

## *Trichomonas foetus* Test

### INTRODUCTION

**Bovine** trichomoniasis is a venereally transmitted infection by the 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 in direct microscopic examination of clinical specimens and serological methods of diagnosis are not reliable.

### PRINCIPLES OF THE PROCEDURE

The InPouch™ TF is a self-contained system for the detection by culture of *T. foetus* from bovine preputial or vaginal samples. The proprietary medium is selective for the transport and growth of the trichomonad, while inhibiting the growth of yeast, mold and bacteria which might interfere with a reliable diagnosis.

The InPouch consists of a high barrier, oxygen resistant, plastic pouch with two V-shaped chambers connected by a narrow passage.

The two-compartment system allows direct observation (**wet mount**) of a newly collected specimen in the upper chamber before expressing the contents into the lower chamber for **culture**.

**An inoculum containing 1 to 10 organisms is sufficient to result in a presumptive positive test.**

Presumptive positive pouches for *T. foetus* can be tested via a PCR procedure to verify the result. Transport and off-site testing can be performed easily due to the flexible packaging and integral design of the pouch.

### REAGENTS

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

### PRECAUTIONS

*For in vitro diagnostic use only. The pouch is labeled for animal identification and test results only. NOTE: Individual State Departments of Agriculture require that only state certified veterinarians collect and read bovine cultures. Only state certified laboratories may read bovine cultures and perform PCR confirmation testing.*

All specimens should be handled according to CDC-NIH recommendations for potentially infectious organisms. (**BIOHAZARD LEVEL 2**).

### DISPOSAL

All InPouches should be autoclaved or disposed by equivalent means. (**BIO SAFETY LEVEL 2**).

### STORAGE & SHELF LIFE

Store uninoculated InPouches at room temperature (18°-25°C) in a horizontal position away from direct sunlight. **Never refrigerate**

**ate/freeze** the InPouches. Product shelf life is 12 months from the date of manufacture.

### PRODUCT DETERIORATION

Do not use an InPouch if medium appears to be cloudy, leaky, dark brown, dry, or if the medium has thick syrup-like consistency

### QUALITY CONTROL

The InPouch™TV diagnostic medium is manufactured in small lots. Each lot is released only after a QC test of viability, doubling time and sterility. QC testing is repeated through the end of shelf life by BioMed. Reference laboratory quality control should use BioMed Diagnostics Quality Control Procedure. (**Doc. No. 100-135**).

### *Trichomonas foetus* LIVE CULTURE

BioMed Diagnostics maintains a live culture of *T. foetus* (clinical isolate) for QC purposes. This live culture (positive control) can be purchased from BioMed Diagnostics. (**Catalog #11-1015**).

To maintain an active culture inoculate a new pouch with one drop (10µl) of the actively growing culture and incubate at 37°C for 24 hours. Subculturing every 3-4 days when the organisms reach a concentration of 1 x 10<sup>5</sup>/ml.

### MATERIALS SUPPLIED

- InPouch™TF pouches
- Package Insert
- Microscopic Clips (optional)

### MATERIALS NEEDED BUT NOT SUPPLIED

- Infusion pipette
- Wooden applicator sticks/cotton-tipped swabs
- Microbial loop
- Disposable gloves
- Microscope with 10x, 20x, 40x objectives
- 20 ml syringes (for bovine samples)

### SPECIMEN COLLECTION - BOVINE

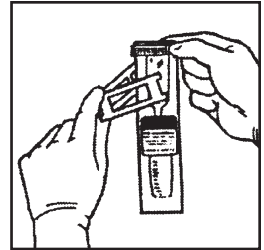
The specimen collection technique is important regardless of the nutrient medium used for culturing.

- Use a clean, dry infusion pipette with a 20 ml syringe on each bull or cow.
- Clip the hair around the prepuccial orifice in bulls.
- If necessary, rinse out the prepuccial cavity with sterile saline (not water) to clean out mud and manure. This will help prevent contamination from non pathogenic intestinal trichomonads and coliform bacteria.
- Direct the pipette to the distal penis in the sheath. Obtain the specimen by scraping the mucosa of the distal penis and the fornix area while applying suction with the syringe.
- For cows, advance the pipette gently to the floor of the vaginal fornix, and patiently aspirate mucus. This may take 20-60 seconds, due to the viscosity of mucus. 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.

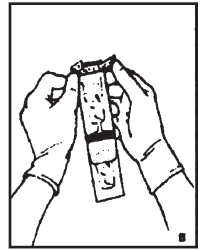
- A satisfactory sample, from either a cow or bull, can also be obtained using a long, guarded cotton swab for collection. **The specimen should not be refrigerated or frozen.**

### INOCULATION

- Remove the pouch from the bag and if necessary, manually express the liquid from one chamber to the other, resulting in approximately 1 ml in the upper chamber. Be 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 by pulling the closure tape's middle tabs apart.
- **Bovine infusion pipette** - insert the specimen pipette tip into the liquid of the pouch's upper chamber and expel 0.5-1.0 cc of the sample into the pouch. If the collected material adheres to the wall of the pipette, rinse the pipette by flushing a small amount of the liquid medium back and forth into the pouch. Minimize the production of bubbles. Dispose of both pipette and syringe.
- **Bovine swab** - insert the swab into the liquid of the pouch's upper chamber. The swab can be "milked" out by pressing the tip of the swab between the fingers through the flexible walls of the pouch. Dispose of the swab.

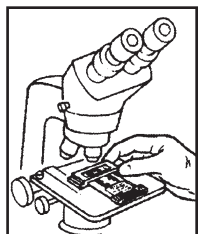
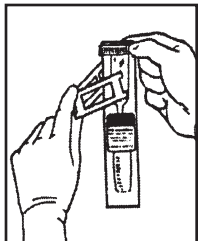


Squeeze the top to close, roll the top to close, roll the top edge down and continue rolling twice. Fold the wire tabs over to prevent the InPouch from reopening. **Use the BioMed label to write animal identification information. Do not cover the chambers**



### LABORATORY PROCEDURES

- **Immediate Specimen Concentration** - It is possible to concentrate the cellular material by standing the pouch vertically or by placing it in a vest or shirt pocket for at least 15 minutes prior to microscopic evaluation. The trichomonads will concentrate at the bottom of the chamber
- **Wet Mount** - immediate microscopic examination. Place the viewing clip horizontally over the upper chamber and close the clip. (Using the clip is optional). Observe with a microscope under low power (10x), using (20x or 40x) if necessary for confirmation.
- **READING TIP:** Trichomonads gravitate to the edges of the InPouch chambers just as they gravitate to the edges of a cover slip on a standard wet mount. If none are seen along



the edges, briefly scan the liquid. Make sure to focus on the liquid and not the textured plastic of the pouch. Do not mistake Brownian motion of small debris particles for evidence of trichomonad activity.

**1 to 10 trichomonads observed is presumptive positive.**

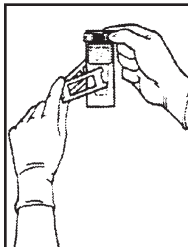
## BOVINE SPECIMEN CULTURING

Express all liquid into the lower chamber. Roll down the pouch until the tape is at the top of the label. Fold the wire tap's end tabs to lock the roll.

**Incubate the pouch vertically at 35°-36°C for 6 days.**

## EXAMINATION OF THE INPOUCH

- **Mixing** - before reading the pouch, pull the pouch up and down across the edge of a table approximately 3-4 times.
- **Microscopic Evaluation** - Place the viewing clip horizontally over the lower chamber and close the clip. (Using the clip is optional). Observe with a microscope.
- **Bovine:** Repeat the evaluations daily for the presence of motile trichomonads for **6 days (after inoculation)**. **Incubate 35° - 36°C.** NOTE: Field studies indicate that if *T. foetus* is present, 98% of InPouches will be positive at 5 days, 2% of InPouches will be positive at 6 days.



## TRANSPORT

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

## SPECIFICITY

InPouch TF medium is effective in culturing *T. foetus*, *T. suis*, *Trichomonas gallinae* and *Pentatrichomonas hominis*.

## LIMITATIONS TO THE PROCEDURE

In bovine samples, *P. hominis* is a contaminant. While differential staining can sometimes distinguish trichomonad species based on the number of flagellae, PCR testing is the best way to distinguish among trichomonads.

The InPouch medium suppresses but does not eliminate yeast and bacterial growth. A build up of gas from bacterial growth can be vented by opening the pouches inside a **BIOHAZARD LEVEL 2 HOOD**.

Too much fecal material can make the medium too cloudy to examine. Subculture if necessary into another InPouch.

## SUBCULTURE PROCEDURE FOR POUCHES WITH FECAL BACTERIA:

Mix the InPouch and express the media into the upper chamber. Inoculate a new InPouch with one drop (10µl) of the overgrown pouch using a glass pasteur pipette. Incubate the new pouch at 25°C and examine microscopically per bovine or feline procedure.

WARNING: This product contains a chemical known to the State of California to cause cancer, birth defects and other reproductive harm.

## REFERENCES

1. Thomas, et al., Agri-Practice Journal, 11:13-17, 1990.
2. Borchardt, Norman, Thomas, Harmon, Veterinary Medicine, Feb: 104-112, 1992

# InPouch™ TF

*Trichomonas foetus* Test

Catalog# 11-1001                      20 Test Kit

Catalog# 11-1003                      100 Test Kit

A SELECTIVE CULTURE SYSTEM  
FOR THE DIAGNOSIS  
*Trichomonas foetus*

For Veterinary Use Only  
For *In Vitro* Use Only

Manufactured by

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**DIAGNOSTICS**

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