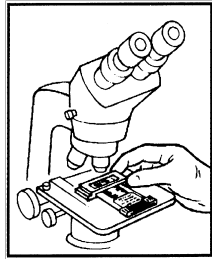


Microscopic Evaluation - Place the viewing clip horizontally over the lower chamber and close the clip. (Using the clip is optional). Observe with a microscope under low power (10x). Use a higher power (20x or 40x) if necessary for confirmation. **READING TIP:** Trichomonads gravitate to the edges of the InPouch™. If none are seen along the edges, briefly scan the liquid. Make sure to focus in the liquid and not the textured plastic of the pouch. Do not mistake Brownian motion of small debris particles for evidence of trichomonad activity.

Repeat the evaluations daily for the presence of motile trichomonads for **2-4 days (after inoculation)**. If no *T. Foetus* is seen, continue to evaluate the InPouch™ culture every other day up to 12 days.



DISPOSAL

All InPouches™ should be autoclaved or disposed of by equivalent means at **BIOSAFETY LEVEL 2**.

Tritrichomonas 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 #11-1115**). Live cultures are available only in the United States.

To maintain an active culture, inoculate a new pouch with one drop (approximately 40µl) of the actively growing culture and incubate at 37°C for 24 hours. They can then be moved to a 32°C incubator or to room temperature. Subculture every 3-4 days when the organisms reach a concentration of 1×10^5 /ml.

Limitations to the Procedure

In feline samples, *P. hominis* and *Giardia* are contaminants, however they do not survive in the InPouch™ media beyond 24 hours. 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 or OUT OF DOORS**.

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

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

References

1. Gookin, et al., Use of commercially available culture system for the diagnosis of *Trichomonas foetus* in cats. J. Am. Vet. Med. Assoc. 2003; 222: 1376-1379.

InPouch™ TF - Feline

Tritrichomonas foetus Test

Catalog No. 11-1107 5 Test Kit
Catalog No. 11-1101 20 Test Kit
Catalog No. 11-1103 100 Test Kit
Catalog No. 11-1115 Live Culture

A SELECTIVE CULTURE SYSTEM FOR
THE DIAGNOSIS OF
Tritrichomonas foetus

For Veterinary Use Only
For *In Vitro Diagnostic* Use Only

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InPouch™ TF - Feline *Tritrichomonas foetus* Test

INTRODUCTION

Recent research has found that *T. foetus* can cause chronic feline diarrhea.¹ The incidence of this infection has been found widely as further research has been conducted.

PRINCIPLES OF THE PROCEDURE

The InPouch™ TF is a self-contained system for the detection of *T. foetus* from feline fecal 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. This 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**.

The InPouch™ is sensitive enough that **an inoculum containing as little as 1 organism is sufficient to potentially 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, protease, peptone, yeast extract, maltose and other nutrients, amino acids, salts, antifungal and antimicrobial agents in a normal saline phosphate buffer.

PRECAUTIONS

For *in vitro* diagnostic use only. **The pouch is labeled for veterinary microbial identification and test results only.** All specimens should be handled according to CDC-NIH recommendations for potentially infectious organisms at **BIOHAZARD LEVEL 2.**

STORAGE AND SHELF LIFE

Never refrigerate or freeze the InPouches™. Store uninoculated InPouches™ at room temperature (18°C to 25°C) horizontally, away from direct sunlight. Product shelf life is 12 months from the date of manufacture. Do not use an InPouch™ if it appears to be cloudy, leaky, dark brown, dry, or if the medium has thick syrup-like consistency.

SPECIMEN COLLECTION

MATERIALS NEEDED

- InPouch™ TF pouches
- Package Insert (this document)
- Microscope clips (optional and sold separately)
- Disposable gloves
- Wooden applicator sticks/sterile cotton-tipped swabs
- Microscope

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

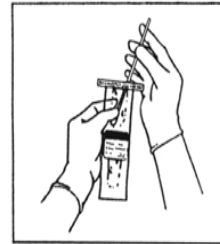
Method 1.....Insert a sterile cotton swab directly into the cat's rectum. It is not necessary to collect feces with the rectal swab since *T. foetus* clings to the cellular lining of the colon. Any feces obtained should only coat the swab. The swab should be free of anything that could kill *T. foetus*, ie. lubricants.

Method 2.....Alternately, with a clean wooden applicator stick or a sterile cotton swab, obtain approximately 0.03grams (smaller than a peppercorn) of feces that has been voided within 1-2 hours of collection.

The specimen should NOT be refrigerated or frozen.

INOCULATION

Step 1.....If necessary, manually express the liquid of the InPouch™ so that there is 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.**

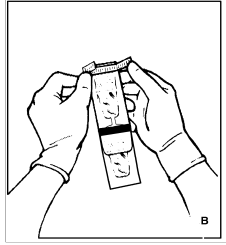


Step 2.....Tear the pouch open at the notch just above the closure. Open the pouch by pulling the closure tape's middle tabs apart.

Step 3.....Insert the swab or stick 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. Avoid placing an

excessive amount of feces in the pouch. Dispose of the swab.

Step 4.....**For wet mount** examination, squeeze the top to close, roll the top edge down twice and fold over the end tabs. **For culture or transport**, express the media into the lower chamber and roll the pouch down until the tape is at the top of the label. Fold the wire tape's end tabs to lock the roll.



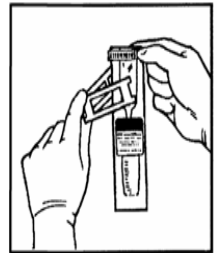
Use the BioMed label to write animal identification information.

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°C.

Laboratory Procedures

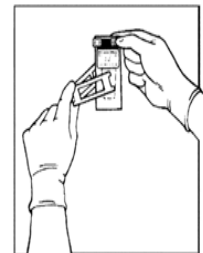
Wet Mount - Immediate microscopic examination. Place the viewing clip horizontally over the upper chamber and close the clip. (Using the clip is optional). Observe with a microscope under low power 10x, using 20x or 40x if necessary for confirmation.



Immediate Specimen Concentration - It is possible to concentrate the cellular material by standing the pouch vertically or by placing it in a shirt pocket for at least 15 minutes prior to microscopic evaluation. The trichomonads will concentrate at the bottom of the chamber.

Incubation and Examination

Incubate the pouch vertically at 35°C - 37°C for 18-24 hours. After initial incubation, keep the pouch at room temperature in the dark. The pouch can be kept in the dark vertically without incubation.



Mixing - before reading the pouch, pull the pouch up and down across the edge of a table approximately 3-4 times.