### References

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## Symbol glossary

biomeddiagnostics.com/l/symbol-glossary

# **Document Revision History**

#### Rev. G, October 2019

New format; added new catalog numbers, reference to online symbol glossary, document revision history; streamlined Procedure; specified chemicals for State of California; specified 18-25°C instead of room temperature; removed "For In Vitro Diagnostic Use Only"; reorganized and retitled some sections

#### Rev. H, March 2020

Updated procedural and evaluation sections, added references 3-5. Updated Logo. Configured formatting to a quad-fold presentation.



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# InPouch **TF - Feline**

A selective culture system for the diagnosis of Tritrichomonas foetus Feline

Catalog No.	12-071-001	5 Tests
Catalog No.	12-071-002	10 Tests
Catalog No.	12-071-004	100 Tests
Catalog No.	12-091-003	LIVE Culture

For Veterinary Use Only.



# Intended Use

InPouch® TF - Feline is a self-contained system for the detection of *T. foetus* in feline fecal samples. The proprietary medium is selective for the transport and growth of the trichomonad while inhibiting the growth of mold, bacteria and yeast which could interfere with diagnosis.

### Description and Principle

*T.* foetus can cause chronic diarrhea in felines. Evidence of this infection has been found widely and further research is being conducted. <sup>1,2</sup>

InPouch devices consists of clear high-barrier, oxygenresistant plastic which is formed into an enclosed pouch in the shape of two "V"- like chambers connected by a narrow passage (cannula). This two-compartment system allows direct (wet mount) observation on a newly inoculated specimen in the upper chamber before expressing it into the lower chamber for culture.

InPouch is sensitive enough that an inoculum containing as little as one organism is sufficient for a presumptive positive test.

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

### **Reagents and Appearance**

InPouch Medium appears clear straw to amber and contains tripticase, protease, peptone, yeast extract, maltose (and other nutrients), amino acids, slats, antifungal and antimicrobial agents in a normal saline phosphate buffer. Final pH of media is  $6.7 \pm 0.1$ .

### Precautions, Safety and Disposal

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

Do not use if package is damaged.

All specimens are to be handled according to CDC-NIH regulations for potentially infectious organisms at Biosafety Level 2.

All InPouch devices are regarded as Biosafety Level 2 and must be destroyed by autoclave sterilization or equivalent means. Once the pouch has been inoculated and resealed, re-open only in a biological safety cabinet. Because of the potential for containing infectious materials, the pouch must be destroyed by autoclaving at 121°C

▲ **WARNING** This product can expose you to chemicals including Iron dextran, which is known to the State of California to cause cancer, and Neomycin, which is known to the State of California to cause birth defects or other reproductive harm. For more information go to **P65Warnings.ca.gov**.

### Storage

Do not freeze or refrigerate the InPouch device. Store any uninoculated pouches horizontally at 18-25°C away from direct sunlight.

# Shelf Life

Product shelf life is 12 months from the date of manufacture. Do not use InPouch if it leaks, appears cloudy, dark brown, dry/sticky or has a syrup-like consistency.

#### Key notes regarding specimen collection

#### Method 1:

Insert sterile cotton swab directly into feline's rectum. It is unnecessary to collect additional 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*; i.e. lubricants.

#### Method 2:

Alternatively, with a clean wooden applicator or swab, obtain approximately 0.03 g of feces (smaller than a peppercorn) that has been voided within 1-2 hours of collection. Freshly voided feces are preferred.

Specimen should not be refrigerated or frozen. No fecal dilution is necessary.

#### Materials Provided

• InPouch® TF - Feline

#### Materials Required but Not Provided

- Sample (see "Key Notes Regarding Specimen Collection")
- Sterile cotton-tipped wooden applicators
- Viewing clip (Optional, Cat. Nos. 10-000-001, 10-000-002)
- Microscope
- Laboratory incubator capable of incubation at 25-37°C

Integrate

Feline and mix fecal

sample in the upper

chamber of the pouch. and mix.

Sample

Inoculate Sample



Insert 0.025-0.05g of fresh feces into the upper chamber of InPouch TF- Feline.

Note: A rectal swab from the inner lining of the colon can also provide enough specimen. Note: Specimen can be directly observed under the microscope at this point.

#### Notes:

- No dilution is necessary when using InPouch TF-Feline thereby saving time and cost of diagnosis.
- Independent non-bias scientific study shows very limited to no growth of common fecal bacteria usually found in other media. This allows for selectivity.

3 Slide the content into the lower chamber of InPouch TF-Feline Mount under the microscope stage and examine for *T. foetus*.

> **Note:** See below for further assistance on *T. foetus* identification.<sup>2</sup>

The use of the fresh fecal sample is

recommended, and fecal sample

For long term use or while in transit,

store fecal material in the InPouch at

18-25°C. T. foetus does NOT survive

should be free of cat litter.

well 4°C to -20°C

chamber.



Remove the pouch from the bag and manually express the liquid so that all the liquid is in the lower that all the liquid is in the lower

> Use the integral white tabs to open and secure the mouth of the pouch open.



If required, incubate inoculated InPouch at 25°C for 48 hours and examine under the microscope for *T. foetus*.

Note: It is recommended that further incubation at 37°C should ONLY be performed after initial incubation at 25°C as described above. Please note that incubation at 37°C is possible. However, it may encourage bacterial growth.

 It is recommended that cats diagnosed with Giardia spp that do not respond to prescribed treatments may be reevaluated for T. foetus infection.

### Incubation

Incubate pouch vertically at 25°C for 48 hours. After initial incubation, further incubation can be performed at 37°C (see step 5 for further details).

## Quality Control

This product has been tested and meets the CLSI (formerly NCCLS) Approved Standard for commercially prepared media (M22-A3). At the time of manufacture, quality control testing is performed on each lot of InPouch TF-Feline. The ability of the media to support growth and demonstrate expected growth, selectivity and morphology is verified by lot. Refer to the CoA for lot-specific information.

# Reading the Result

### Evaluation

Place the (optional) Viewing Clip horizontally over the lower chamber of the inoculated pouch and lock the clip. Observe using a low-powered microscope (10x). A higher power microscope (20-40x) may be needed for confirmation. Trichomonads gravitate to the edges of the InPouch chamber. Briefly scan the edges being sure to focus in on the liquid and not the textured plastic. Do not mistake Brownian motion of microscopic debris as evident Tritrichomonas activity.

#### \*Assistance with T. foetus identification under the microscope

- Giardia spp. have motility like the wavy pattern of a falling leaf.
- Most trichomonads have erratic motion.
- The membrane of trichomonads extend throughout the length of the protozoa compared to *Giardia* spp.
- Other tests may be required to distinguish between Pentatrichomonas hominis and T. foetus in cats.

**Note:** The above recommendations for *T. foetus* microscopic identification are for informational purpose only and users are advised to consult relevant literature.

Repeat evaluations daily for 2 days (post-inoculation) for confirmed presence of Trichomonads. If no viable motions are detected, incubate the sample at 35-37°C every other day for up to twelve (12) days.

Viewing tip: Mix pouch by pulling up and down across the edge of a table 3-4 times before reading the pouch. This helps to disperse the contents evenly throughout the media.

### Limitations

*P. hominis* and *Giardia* are contaminants commonly found in feline samples. These contaminants will not survive within the pouch beyond 24 hours and will not affect product performance. Though uncommon, *P. hominis* can grow. PCR testing may help to distinguish Trichomonads.<sup>2</sup>

The InPouch medium suppresses but does not eliminate yeast and bacterial growth. Gas build-up from bacterial growth may be relieved by opening the pouch out of doors or within a Biosafety Level 2 hood.

**NOTE:** Too much fecal material may cause excessive cloudiness; subculture as necessary into another un-inoculated InPouch TF-Feline.

## United States Only:

#### Tritrichomonas foetus Feline Live Culture

Biomed Diagnostics, Inc. maintains a TF-Feline live culture isolate. This positive-control culture can be purchased from the Biomed catalog (Cat. No. 12-091-003).

Inoculate a new pouch with 40  $\mu$ l (1 drop) of stock every 3-4 days (when growth concentration reaches 1 x 10<sup>5</sup>/mL) to maintain a viable stock culture. Incubate newly inoculated pouches at 37°C for 24 hours; transfer to 32°C (culture is also stable at 18-25°C).