Introduction

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. ^{1,2}

The InPouch device consists of clear high-barrier, oxygen-resistant 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 to potentially result in 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 ± 0.1 .

Precautions, Safety and Disposal

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

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, Chloramphenicol** and **Neomycin**, which are 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 the InPouch if it leaks, appears cloudy, dark brown, dry/sticky or has a syrup-like consistency.

Procedure

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. Jubricants.

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.

Specimen should **not** be refrigerated or frozen.

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 37°C

Prepare InPouch



Remove the pouch from the bag and manually express the liquid so that all the liquid is in the lower chamber. Open the pouch by tearing off the top. There is a pre-formed score to facilitate tearing. Use the integral white tabs to open and secure the mouth of the pouch open.

Inoculate Sample



Insert the sample into the upper chamber of the pouch. Squeeze a small amount of liquid from the lower chamber to the upper pouch chamber to flush the sample. Minimize the introduction of bubbles or foam. Dispose of pipette and syringe.

Integrate Sample



For Wet Mount Analysis: Before expressing sample to lower chamber and prior to incubation, roll the top edge down at least twice and secure the end tabs to seal the pouch. Isolate the sample in the Viewing Clip and observe it in the top chamber microscopically.

Express the entire content of the InPouch into the lower chamber. Avoid trapping air. Roll the pouch top tightly, until the wire-tape is at the top of the label. Fold the wire tape end tabs to seal the pouch.

Incubation

Incubate pouch vertically at 37°C. After initial incubation, store the pouch vertically in the dark at 18-25°C; incubation is not necessary.

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 the InPouch TF-Feline. The ability of the media to support growth and demonstrate expected growth, selectivity and morphology is verified by lot.



Reading the Results

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.

Note: 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 Trichomonas activity.**

Repeat evaluations daily for 2-4 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 is the only way to distinguish Trichomonads.²

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 μ l (1 drop) of stock every 3-4 days (when growth concentration reaches 1 x 10⁵/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).

References

- Gookin. et. al. Use of Commercially Available Culture System for the Diagnosis of Tritrichomonas foetus in Cats. J. Am. Vet. Med. Assoc. 2003; 222 1376-1379.
- Gookin, J. L. et. al. Identification of Pentatrichomonas hominis in Feline Fecal Samples by Polymerase Chain Assay. Vet Parasitology. 2007; 145: 11-15

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

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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

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