Introduction

INTENDED USE

The InPouchTM TF is a self-contained system for the detection of Tritrichomonas foetus from bovine preputial or vaginal samples. The proprietary medium is selective for the transport and growth of *T. foetus*, while inhibiting the growth of other organisms which can interfere with a reliable diagnosis.

EXPLANATION

Bovine Tritrichomoniasis is a venereally transmitted protozoan parasite. The primary pathological manifestation of this infection is early embryonic death or abortion in impregnated cows. Cows show few other symptoms of infection, while bulls are asymptomatic.

PRINCIPALS OF THE PRODUCT

The InPouchTM TF is designed to facilitate and simplify the detection of *T. foetus* as the organism is infrequently be found in direct microscopic examination of clinical specimens and serological methods of diagnosis are not reliable. This device conveniently supports the following user needs in a single-exposure system:

- 1. Ease of inoculation
- Proprietary medium selective for TF growth
- Direct microscopic observation of the sample
- Self-contained culture system
- Direct microscopic observation of the culture
- 6. Incubatory capabilities
- Safe transport and preservation of the specimen
- 8. PCR compatible

REAGENTS

The InPouchTM contains peptones, yeast extract, maltose and other nutrients, amino acids, salts, antifungal and antimicrobial agents in a phosphate buffered saline base constructed to isolate the positive detection of *T. foetus*.

SPECIFICITY

InPouchTM TF medium is known to be effective in culturing T. foetus, T. suis, T. galliniae and P. hominis.

STORAGE AND SHELF LIFE

Do not refrigerate or freeze InPouchTM TF tests. Upon receipt, store at room temperature (18°C - 25°C) horizontally, away from direct sunlight. Do not use expired tests. Do not use an InPouchTM test if the liquid appears to be cloudy, leaky, dark brown or dried.

USING THE TEST

SAMPLE COLLECTION



- Infusion/insemination pipette

Materials needed for the Test:

- 20ml syringe/pipette bulb per bull/cow
- Laboratory incubator & Microscope (≥100X)

Clip the hair around the perpetual orifice in bulls. Flush the perpetual cavity with sterile

saline solution (not water) to clean out mud and manure if necessary (decreases the risk of

overgrowth of non T. foetus bacterium).

Bulls: direct the pipette to the distal penis in the sheath. Scrape the mucosa of the distal penis and the fornix area while applying suction with syringe or bulb to obtain the specimen.

Cows: advance the pipette gently to the floor of the vaginal fornix, and aspirate mucus.

INOCULATION

Sample

Preparation

Collect

Sample



Sample

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.

Insert the sample into the upper chamber of the pouch (0.5 -1.0 mL of specimen). Squeeze a small amount of liquid from the lower to the upper pouch chamber to flush the sample. Minimize the introduction of bubbles or foam. Dispose of both pipette and syringe.

Integrate Sample

Express the entire contents 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 ends tabs to seal the pouch



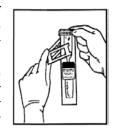
INCUBATION

Incubate the pouch vertically at 35°C - 37°C for up to 6 days. The InPouchTM TF is designed for safe transport, if needed. Inoculated InPouchTM TF tests should be transported within 48 hours after inoculation and maintained at 15°C - 37°C

READING THE RESULTS

MICROSCOPIC EVALUATION

To search for the presence of microorganisms, place a viewing clip horizontally over the lower chamber of the InPouch and close (the clip is optional). Place the InPouchTM on the microscope stage under low power (100x mag.) to look trichomonads. Use a higher power (200x - 400x mag.) if necessary for confirmation.



Observation of 1 to 10 live, motile T. foetus is all that is required for a presumptive positive result. Continue incubation and repeat the microscopic observation daily for six days before a negative result is reported.



Field studies indicate that 98% of positive results will occur within 5 days; up to 2% of positive results may not be detected until the 6^{th} day or incubation/observation.

READING TIPS

For immediate wet mount examination: Before expressing sample to lower chamber and prior to incubation, roll the top edge down twice and fold over the end tabs to seal the pouch: observe the sample in the top chamber microscopically.



- *Tritrichomonas gravitate to the bottom and side edges of the pouch chamber.
- *Verify that your field of focus is in the liquid and not the textured plastic film layer of the pouch.
- *Do not mistake Brownian motion or small particles for evidence of Tritrichomonas activity. T. foetus are relatively large (9-20 µm) and highly motile.

ADDITIONAL PRODUCT NOTES

- 1. NEVER refrigerate or freeze the specimen.
- 2. Complete each label with the sample information
- 3. All specimens should be handled according to CDC-NIH recommendations for Biosafety Level 2 (BSL-2) organisms.

OUALITY CONTROL

The InPouchTM TF is manufactured in accordance with controlled procedures at BioMed. Each lot undergoes an initial QC performance testing prior to release for consumer use. Additional performance testing is repeated at specific intervals throughout the marked shelf-life of each lot to ensure absolute reliability of the product.

The following is recommended for customers who choose to complete independent QC testing of the InPouchTM TF:

- 1. Obtain a sample of viable *Tritrichomonas* organisms in the range 2.0 x 10⁵ 2.0 x 10⁶ live *cells*/mL
- 2. Inoculate three (3) InPouchTM TF diagnostic tests with 1-2 drops of the live culture using a sterile glass Pasteur pipette (~20 40 ul) per the "Inoculation" steps discussed on the reverse side of this insert.
- 3. Incubate the inoculated InPouchTM TF diagnostic tests for 24 hours at 37°C

NOTES ON QUALITY

- 1. Too much fecal material can ruin the test by making the medium too cloudy for examination. When necessary, subculture the suspect InPouchTM tests into another InPouchTM.
- 2. While differential staining can sometimes be of help in *Tritrichomonas* species identification based on the number of flagella, PCR testing is the only reliable means of definitive identification.
- 3. In bovine samples *P. hominis* or other non *T. foetus* protozoa are contaminants.

SAFETY

The $InPouch^{\rm TM}$ TF is for veterinary protozoa identification and test results only.

Consult your local State Department of Agriculture regulations before use. Some states require that only certified veterinarians collect and read bovine TF cultures and/or submit samples for PCR testing.

The InPouch growth 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 BSL-2 rated biological safety cabinet.

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

DISPOSAL

Since InPouchTM TF has potential for containing live, infectious materials, the InPouchTM TF must be destroyed by autoclaving at 121°C for 20 minutes or other suitable means for sterilization and disposal of BSL-2 organisms.

REFERENCES

BonDurant. Vet Clin North Am Food Anim Pract. 1997. 13(2):345-61

Thomas, et al. Agri-Practice. 1990. 11:13-17

Borchardt, et al. Veterinary Medicine. 1992. 11:104-112

Tritrichomonas foetus LIVE CULTURE

Live cultures of *T. foetus* (clinical isolate) for research, training and QC purposes are available (N. American customers only). 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 (approximately 40μ l) 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 x 10^5 /ml

InPouchTM TF

Tritrichomonas foetus Test

Catalog No. 11-1010 Catalog No. 11-1003

10 Test Kit 100 Test Kit

A SELECTIVE CULTURE SYSTEM FOR THE DIAGNOSIS OF BOVINE

Tritrichomonas foetus

InPouch TF Bovine is Supplied in the UK by Vetlab Supplies Ltd

Visit our website

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For Veterinary Use Only

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Biomed Diagnostics, Inc.

PO Box 2366 • White City, OR 97503 tel. (800)-964-6466 • fax. (541) 830-3001 info@biomeddiagnostics.com www.biomeddiagnostics.com