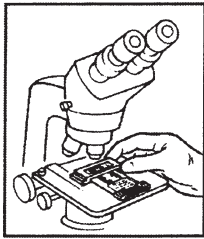


MICROSCOPIC EXAMINATION



Even though the InPouches look like clear broth to the naked eye, under the microscope you will observe what resembles crystals and debris floating about. This is NOT contamination. The crystals are the nutrients for *Trichomonas vaginalis*. Do not mistake Brownian movement of the nutrient particles as evidence of trichomonads.

READING TIP: Trichomonads gravitate to the edges of the InPouch 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 fluid. Be sure to focus on the liquid and not on the textured plastic of the pouch.

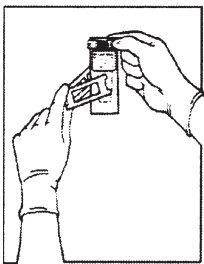
Cloudy uninoculated InPouches may have been exposed to refrigeration or freezing temperatures for longer than 24 hours. The cloudiness may disappear if you allow the pouches to warm up in a 37°C incubator.

The antifungal and antimicrobial ingredients in the InPouch inhibit yeast and bacteria from multiplying. They will be held “in stasis” for several hours.

Report the Wet Mount result per your laboratory protocol.

One *Trichomonas vaginalis* observed is presumptive positive.

CULTURE-INCUBATE AT 37°C



If no trichomonads are observed. Incubate the InPouch™TV at 37°C. Microscopically examine the lower chamber of the pouch for trichomonads every 24 hours for 3 days. As the trichomonads multiply, you will observe a white sediment along the sides and along the bottom of the chamber. The medium can also change to a darker color.

If no trichomonads are observed after 3 days and incubation at 37°C, the test is presumptive negative for *Trichomonas vaginalis*.

URINE/AMNIOTIC FLUID/PROSTATIC FLUID PROCEDURE

Specimen should be less than one hour old. Centrifuge 15cc of the fluid in a **glass tube** at **500 rpm** for **5 minutes**. Remove the supernatant and inoculate a pouch with 1-2 drops of sediment with a **glass pipette**. Observe for trichomonads as in wet mount procedure #1. Incubate the pouch at 37°C for 3 days if no trichomonads are observed.

SEMINAL FLUID PROCEDURE

Seminal fluid should be 30-60 minutes old. Inoculate the pouch with a drop using a **glass pipette**. Observe for trichomonads as in procedure #1. Incubate the pouch at 37°C for 5 days if no trichomonads are observed.

TECHNICAL NOTES

Never centrifuge an InPouch to concentrate the trichomonads! We recommend standing the folded InPouch vertically inside a paper cup or a clean, empty urine cup for up to 15 minutes prior to microscopic examination.

Specificity: For cultivation of *T. vaginalis* only. Other trichomonas species will not survive and replicate at the pH and medium composition found in the InPouch test kit.

Age limitations: There are no patient age limitations on specimens collected.

Culture: *T. vaginalis* replicates within several hours after inoculation into the InPouch system. An inoculum containing 1-10 organisms is sufficient. *T. vaginalis* can grow in the pouches up to 5 days, however, after 3 days the organisms start to die as the nutrients are exhausted.

Limitations to the Procedure: *T. vaginalis* are very “sticky” organisms and they will adhere to plastic containers, plastic tubes, plastic pipettes, Dacron swabs, rayon swabs, condoms, and mucus. **Use only cotton swabs, wire microbiological loops, glass containers, glass tubes and glass pipettes.**

Decreased recovery will occur if patient specimens are contaminated with birth control foams or jellies.

Menses does not interfere with the test.

WARNINGS: This product contains a chemical (concentration is less than 0.01%) known to the State of California to cause cancer, birth defects and other reproductive harm. Antibiotics and antifungal reagents may cause allergic reactions. Take the necessary biohazard precautions during use. Autoclave and dispose the used InPouches in a biohazard container.

An Evaluation of *Trichomonas vaginalis* Culture Viability After 48 Hours at Room Temperature

Trichomonas vaginalis SJC66 was incubated for 48 hours at 37°C in an InPouch™TV test pouch. A Neubauer hemocytometer was used to determine a final dilution of the culture to 7.75 x 10⁹/ml. Four pouches were then inoculated from this dilution of trichomonads. Pouch #1 was inoculated 30 µl, Pouch #2 with 60µl, Pouch #3 with 90µl and Pouch #4 with 120µl.

The pouch densities were:

Pouch #1	2.3 x 10 ⁹ /ml	Pouch #3	6.9 x 10 ⁹ /ml
Pouch #2	4.6 x 10 ⁹ /ml	Pouch #4	9.2 x 10 ⁹ /ml

All four pouches were allowed to remain at room temperature for 48 hours before being placed into an incubator at 37°C for 24 hours. Each was examined microscopically for viability after 24 hours. All were positive for motile trichomonads with Pouch #1 presenting the fewest and Pouch #4 the greatest numbers of organisms.

This demonstrates that a specimen inoculated into the InPouch™TV with viable trichomonads with a minimum of 2.3 x 10⁹/ml would remain viable for a minimum of 48 hours if stored at room temperature.

Kenneth a. Borchardt, Ph.D., Chief Research Scientist
Doc. No. 100-045B

REFERENCES

1. Krieger, J.N. et al., JAMA 1988; 259: 1223-1227
2. Draper, D. et al., Detection of *Trichomonas vaginalis* in Pregnant Women with the InPouch TV Culture System, J. Clin. Microbiology 1993; 31:1016-1018.
3. Krieger, J.N., Trichomoniasis in Men: old issues and new data. Sex Trans. Dis, March-April, 83-96, 1995.
4. Borchardt, K. A. et al. A Clinical Evaluation of Trichomoniasis in San Jose, Costa Rica using the InPouch TV test. Genitourin Med. 1992; 68: 328-330.

SYMBOL KEY

Symbol	Used For	Symbol	Used For
	Batch code		Temperature limitation
	Date of manufacture		Catalog number
	Use by YYYY-MM-DD or YYYY-MM		Caution, consult accompanying documents
	Manufacturer		Authorized representative in the European Community
	In vitro diagnostic medical device		in European community

The label on the InPouch TV test pouch includes a section where patient information can be recorded. The information is: patient name, patient ID, date, clinic and doctor. It is up to the discretion of the practitioner to complete this section or not.

InPouch™ TV

Trichomonas vaginalis test

Catalog# 10-2001 20 Test Box

Catalog# 10-2003 100 Test Box

A SELECTIVE CULTURE SYSTEM FOR THE DIAGNOSIS OF HUMAN

Trichomonas vaginalis



For In Vitro Use Only

STORE THE INPOUCH™TV KITS AT 18°-25°C

PROTECT FROM LIGHT

DO NOT REFRIGERATE

DO NOT FREEZE

Manufactured by



BIOMED DIAGNOSTICS

1388 Antelope Road
White City, OR 97503
1-800-964-6466
(541) 830-3000
fax: (541) 830-3001

www.biomeddiagnostics.com
email: info@biomeddiagnostics.com

InPouch™ TV

Trichomonas vaginalis Test

INTENDED USE

The InPouch™ TV, *Trichomonas vaginalis*, test provides an accurate system for early microscopic identification and culture confirmation of *T. vaginalis* from female and male urogenital sites.

SUMMARY AND EVALUATION

Trichomoniasis is a sexually transmitted infection (STI) caused by the flagellated protozoan, *Trichomonas vaginalis*. It is recognized as one of the prevalent forms of sexually transmitted infections world-wide, in both males and females.¹ The CDC estimates three million cases occur in the U.S. per year. Trichomoniasis may result in premature rupture of membranes in pregnancy and it can also cause nongonococcal urethritis in males.²

PRINCIPLES OF THE PROCEDURE

The InPouch™ TV is a self-contained system for the detection of *T. vaginalis* from female vaginal samples or male urethra/urine samples. The proprietary medium is selective for the transport and growth of *T. vaginalis*, while inhibiting the growth of contaminating microorganisms 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 chamber system permits direct observation (**wet mount**) of a newly collected specimen in the upper chamber before expressing the inoculum into the lower chamber for **culture**.

The sample can be concentrated by letting the cellular material settle to the bottom of the chamber before microscopic observation. **Microscopic observation of the chamber is done with or without using the plastic microscope viewing clip.**

An inoculum containing 1 to 10 trichomonads is sufficient to cause a positive test. Transport and off-site testing can be performed easily due to the flexible packaging and integral design of the InPouch.

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. An unopened InPouch should contain a clear, amber liquid.

PRECAUTIONS


For *in vitro diagnostic use* only. All specimens should be handled according to CDC-NIH recommendations for potentially infectious human serum or other body fluids. (**BIOSAFETY LEVEL 2**).

Trichomonads can be “infected” with bacteria and viruses and transfer these pathogens to people.

DISPOSAL

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

STORAGE & SHELF LIFE

Store uninoculated InPouches at room temperature (18°-25°C)  in a **horizontal position away from direct sunlight**. **Never refrigerate/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 a thick and 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. Reference laboratory quality control should use BioMed Diagnostics, Quality Control Procedure. Doc. No. 100-038

Trichomonas vaginalis LIVE CULTURE

BioMed Diagnostics maintains a live culture of *T. vaginalis* (clinical isolate) for QC purposes. This live culture (positive control) can be purchased from your distributor or from BioMed Diagnostics. (**Catalog #10-2015**.)

To maintain an active culture inoculate a new pouch with one drop (30µl) of the actively growing culture and incubate at 37°C for 24 to 48 hours with subsequent holding at 32°C. The subculture will be viable for two to four days depending on the isolate. Subculturing should be done when the organisms reach a concentration of 1 x 10⁴/ml.

A secondary product, the **InPouch™TV Subculture Medium (Catalog #10-2108)** can be used to maintain a culture for up to seven days.

MATERIALS SUPPLIED

- InPouch™TV pouches
- Package Insert
- Microscope Viewing Clip (optional)

MATERIALS NEEDED BUT NOT SUPPLIED

- Microscope with 10x, 20x, or 40x objectives
- Disposable gloves
- Disposable **glass** pipettes (for urine sediment)
- Disposable **cotton** swabs

SPECIMEN TRANSPORT

Once inoculated and sealed, the InPouch™TV medium can be kept up to 48 hours at room temperature if

necessary. If held longer than 48 hours, incubation at 37°C is required.

Clue cells and yeast cells can also be observed microscopically for several hours after inoculation.

Refrigeration will destroy *T. vaginalis*.

SPECIMENS-USE STERILE COTTON SWABS

- **Vaginal:** collected from posterior fornix.
- **Male Urethral**

SPECIMENS - USE STERILE GLASS PIPETTE

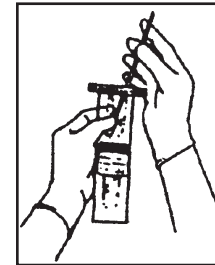
• **Seminal Fluid:** specimen should be 30-60 minutes old. Use a glass pipette to collect a drop of SF and inoculate the InPouch.

• **Male Urine and CSF:** 15 cc of fresh specimen. Centrifuge at 500 rpm for 5 minutes. Decant the supernatant. Inoculate the InPouch with the sediment.

LABELS

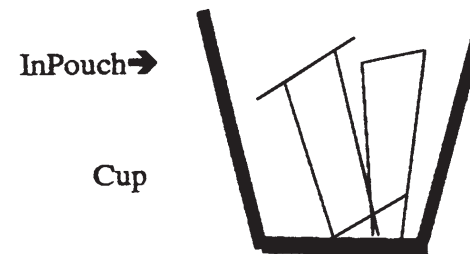
Patient labels should ONLY be placed on top of the BLUE BioMed label. NEVER on the viewing chambers.

InPouch™TV Procedure for the Physician's Office

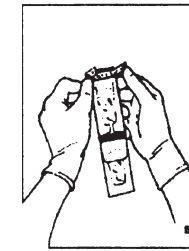


1. To avoid fluid leakage, squeeze the fluid from the top of the InPouch downward toward the bottom of the chamber. Tear off the plastic above the white closure. To admit the **cotton swab**, open the InPouch by pulling the closure tape's middle tabs apart. Milk the swab between the InPouch walls. **Remove the swab and discard.**

HELPFUL HINT: HANDS-FREE METHOD OF TAKING A SPECIMEN USING THE InPouchTV.



NOTE: You do not have to close the InPouch TV immediately. You may place a ready-to-use InPouch TV in a cup. This will free up your hands for other jobs such as the collection and inoculation of specimens. You may wait until you have completed all required test procedures to close TV InPouch.



2. Squeeze the top closed, roll top of upper chamber down one complete roll, keeping the medium in the top chamber, and fold the tabs over to prevent the InPouch from reopening. **Fill in patient information and place patient label over the blue BioMed label - Not on the viewing chamber. DO NOT REFRIGERATE!**

InPouch™TV Test Procedure For The Laboratory

TRANSPORTING TO LABORATORY

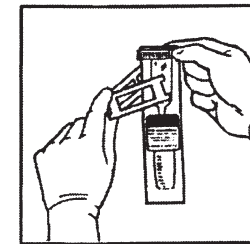
Once inoculated and sealed, the InPouch TV medium can be kept up to **48 hours at room temperature if necessary. If held longer than 48 hours, incubation at 37°C is required.**

SPECIMEN PROCESSING

Process the inoculated pouches according to your laboratory protocol. Place the laboratory label over the patient label on the pouch. Never place a label on either pouch chamber.

WET MOUNT PROCEDURE #1

(5 to 15 minutes after inoculation)



Place the viewing clip horizontally over the upper chamber. Close the clip. (Using the clip is optional). Place the pouch on the stage and scan under 10x and 20x. Confirm under 40x. **Yeast and Clue Cells can also be observed with this technique.**

WET MOUNT PROCEDURE #2

If you cannot perform the wet mount within 15 minutes of inoculation, you can still observe **yeast, clue cells and trichomonads** in the lower chamber. Squeeze the closure strip with the thumb and forefinger. Hold the bottom of the pouch with the other hand and move the medium from the top chamber to the lower chamber by pulling it upward across the edge of the counter/table in a “shoe shine” motion.

Now roll the **EMPTY** upper chamber down to the top of the label, fold the tabs over to prevent the InPouch from reopening.

Place the pouch on the microscope stage and examine as in wet mount procedure #1.