

InPouch™ TV

Subculture Medium

Trichomonas vaginalis Subculture Medium

REF Catalog# 10-2108 10 Test Box
For In Vitro Diagnostic Use Only



1-800-964-6466

White City, OR 97503

INTENDED USE

The InPouch™ TV Subculture pouch is a highly selective growth medium which maintains viable *T. vaginalis* cultures (positive controls) for up to seven (7) days. The positive cultures are used in the Quality Control Procedure for InPouch™ TV, (Doc. No. 100-038).

SUMMARY AND EVALUATION

Trichomoniasis is a sexually transmitted infection caused by the flagellated protozoan, *Trichomonas vaginalis*. It is recognized as one of the prevalent forms of sexually transmitted infections (STI) world-wide, in both males and females.

PRINCIPLES OF THE PROCEDURE

The InPouch™ TV Subculture system represents an improvement in the subculture method for maintaining live cultures of *T. vaginalis*. It has been found to reproduce reliable results, to have fewer problems with contaminating microorganisms, and to save time by reducing the need for frequent subculturing.

The culture vessel is a flexible pouch constructed of clear plastic film that has excellent water vapor and oxygen barrier qualities. The reduced oxygen environment enhances and maintains the desired growth characteristics of the medium.

REAGENTS

The InPouch Subculture 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.

PRECAUTIONS

For *in vitro* diagnostic use only. The Subculture Medium should NOT be used to collect samples directly from patients. Some strains of *T. vaginalis* may be inhibited by the different chemical concentrations in the Subculture Medium. Only trichomonads growing in the InPouch™ TV medium (Catalog No. 10-2001/2003) should be transferred into the Subculture Medium.

All specimens should be handled according to CDC-NIH recommendations for potentially infectious human serum or other body fluids. (**BIO SAFETY 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. (**BIOHAZARD LEVEL 2**).

STORAGE & SHELF LIFE

Store uninoculated Subculture Medium pouches at room temperature (18°C-25°C) in a horizontal position away from sunlight. **Never refrigerate/freeze** the InPouches. Product shelf life is 9 months from the date of manufacture.

PRODUCT DETERIORATION

Do not use a Subculture Medium pouch if the medium appears to be: cloudy, leaky, dark brown, dry, or a thick and syrup-like consistency.

QUALITY CONTROL

The InPouch™ TV Subculture Medium is manufactured in small lots. Each lot is released only after a QC test of viability, doubling time and sterility using the Quality Control Procedure for InPouch™ TV, (**Doc. No. 100-038**).

POSITIVE CONTROLS

BioMed Diagnostics maintains a live culture of *T. vaginalis* Clinical isolate #6. It is used as the positive control for InPouch™ TV medium and for the Subculture Medium at the factory. This live culture can be purchased from your distributor or from BioMed Diagnostics. (**Catalog #10-2015**.) **Doubling Time Normal Range (using Clinical Isolate #6):**

3-10 hours for InPouch™ TV medium

8-24 hours for InPouch™ TV Subculture Medium

Reference laboratories may use their own *T. vaginalis* clinical isolate culture or an ATCC *T. vaginalis* culture as long as the Doubling Times are within the Normal Range.

MATERIALS SUPPLIED

- InPouch™ TV Subculture Medium Pouches
- Package Insert

MATERIALS NEEDED BUT NOT SUPPLIED

- Lab Coat
- Microscope with 10x objective
- Disposable gloves
- Disposable **sterile glass** pipettes/microbiological metal loops
- Biosafety Level 2 Hood
- 37° C incubator
- 32° C incubator (optional)
- Positive control culture
- Microscope viewing clip (optional)

SUBCULTURE PROCEDURE - using *T. vaginalis* live culture Clinical Isolate #6 (positive control).

1. Upon receipt of the positive control pouch, place it in a dark 37° C incubator to restimulate growth. This may take approximately 24-48 hours.
2. The positive control should be subcultured when it is approximately 1×10^5 trichomonads. This number can be determined by observing the InPouch, with a microscope, under 100x magnification and counting 40-50 live organisms/field. Sediment at the bottom of the InPouch indicates heavy trichomonad growth.
3. Mix the positive control pouch by holding the top of the pouch with the thumb and index finger on the right hand and the bottom edge of the pouch with the thumb and index finger of the left hand. Move the pouch twice, vertically up and down, against the edge of a table. The trichomonads will be evenly distributed in the culture medium. Important: Be sure the pouch is adequately sealed before rubbing against the edge of a table.
4. Force a small amount of the medium into the upper chamber.
5. Unfasten the seal.
6. Remove approximately 30µl with a sterile glass pipette/microbiological metal loop.
7. Inoculate a Subculture pouch. **DO NOT USE MORE THAN 30µl!** The extra organisms will metabolize the nutrients resulting in trichomonad overgrowth and the need for frequent subculturing.
8. Roll the top tab down twice to seal the upper chamber.
9. Examine the upper chamber, with a microscope, at 100x, for motile trichomonads.

10. If none are seen, reinoculate the Subculture pouch with another drop from the original positive control InPouch.
11. Force the medium into the lower chamber.
12. Mix the pouch as in Step #3.
13. Roll the Subculture pouch down to the top of the BioMed label and seal with the tabs.
14. Incubate the Subculture pouch (new positive control) in a dark 37° C incubator for 24 hours.
15. Check the Subculture pouch, microscopically, for live trichomonads. If none are seen, reinoculate the pouch with another drop from the original positive control InPouch.
16. Incubate the Subculture pouch (new positive control) in a dark 37° C incubator for another 24 hours.
17. Check the Subculture pouch, microscopically, for live trichomonads.
18. The Subculture pouch should be incubated a minimum of 48 hours, at 37° C, to obtain 1 x 10⁵ trichomonads.
19. Maintain the Subculture pouch, at 32° C, for 5 to 7 days.

SUBCULTURE PROCEDURE - using a clinical *T. vaginalis* specimen

1. Maintain the clinical *T. vaginalis* specimen in a regular InPouch™ TV (Catalog #10-2001/2003).
2. Verify the Doubling Time with the Quality Control procedure. (Doc. No. 100-038)
3. Follow the subculture procedure for live culture, Clinical Isolate #6.

SUBCULTURE PROCEDURE - using ATCC *T. vaginalis* frozen samples

1. Follow the ATCC directions for reconstitution.
2. Inoculate a regular InPouch™ TV (Catalog #10-2001/2003) with the ATCC sample.
3. Incubate the pouch in a dark 37° C incubator until the trichomonads reach 1 x 10⁵.
4. Verify the Doubling Time with the Quality Control procedure. (Doc. No. 100-038).
5. Follow the subculture procedure for live culture, Clinical Isolate #6.











TECHNICAL NOTES

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

Limitations to the Procedure: *T. vaginalis* are very “sticky” organisms and they will adhere to plastic containers, plastic tubes, plastic pipettes, plastic loops. Use only glass containers, glass pipettes, glass tubes or metal microbiological loops.

Reading Tip: *T. vaginalis* gravitate to the edges of the InPouch chamber 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 plastic of the pouch.

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 agents may cause allergic reactions. Take the necessary biohazard precautions during use. Autoclave and dispose of the used Subculture InPouches in a biohazard container.

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 Subculture Medium includes a section where the subculture date and specimen number can be indicated. It is up to the discretion of the practitioner to complete this section or not.

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