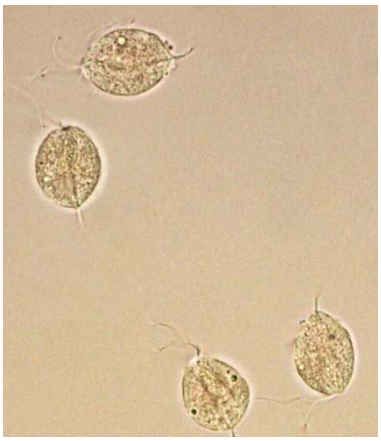
INSTRUCTIONS FOR USE

TRICHOMONAS CPLM SELECTIVE BROTH

Ready-to-use tubes



T.vaginalis: microscopic examination
Medical Laboratory Technologist Channel

1 - INTENDED USE

In vitro diagnostic device. Liquid medium for the isolation and cultivation of *Trichomonas vaginalis* from clinical specimens with mixed flora.

2 - COMPOSITION -TYPICAL FORMULA *

Tryptone	20.000 g
Liver extract	5.000 g
Cysteine HCl	1.500 g
Maltose	1.000 g
Agar	0.500 g
Methylene blue	0.003 g
Chloramphenicol	0.100 g
Horse serum	50 ml
Purified water	1000 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Trichomonas vaginalis, is a flagellate, that lives on the surface of the epithelium of the urogenital tract. It produces trichomoniasis in women, while in men, the infection can be asymptomatic or have characteristics of urethritis, epididymitis, and prostatitis. *Trichomonas vaginalis* infection is the most common non-viral sexually transmitted infection.[1] Worldwide. there are an estimated 250 million cases of *Trichomonas* infection each year, with an overall estimated prevalence of 4,5%.[2]
Culture has greater sensitivity (>80%) than the wet mount method and is considered the gold standard method for the detection of *T.vaginalis*.[2]
Trichomonas CPLM (Cysteine-Peptone-Liver-Maltose Medium) Selective Broth is a modification of the STS Medium of Kupferberg *et al.* for the cultivation of *Trichomonas* spp.[3] The classical formula has been modified by the addition of liver extract and horse serum to improve performance.
Tryptone and liver extract provide carbon, nitrogen, vitamins, and minerals to support the growth of *Trichomonas*; maltose is an energy source for microbial growth. Chloramphenicol, a relatively stable antibiotic, replaces the penicillin and streptomycin recommended for addition to the STS base; it suppresses the growth of most Gram-positive and Gram-negative bacteria. Horse serum is included to provide useful growth factors for *Trichomonas*. Cysteine and agar at low concentration create reducing conditions in the medium that favour the development of *Trichomonas*. Methylene blue is included as an indicator of redox: in the oxidized state it is green in colour, in the reduced state it is colourless.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	yellow with green ring, limpid
Final pH at 20-25°C	6.0 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Trichomonas CPLM Selective Broth W0104050199, EDMA: 14.05.01.90, RDM:1514993/R	Ready-to-use tubes	5513311	20 x 9 mL glass tubes, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator, microscope and laboratory equipment as required, ancillary culture media and reagents for the identification.

7 - SPECIMENS

In women, vaginal secretions are the preferred specimen type for culture, as urine culture is less sensitive. In men, culture specimens require a urethral swab, urine sediment, and/or semen; to improve yield, multiple specimens from men can be used to inoculate a single culture.[4] Specimens must be collected properly and inoculated immediately into the medium. For detailed information, consult appropriate texts.[4-6] Collect specimens before antimicrobial therapy where possible.

8 - TEST PROCEDURE

Bring to room temperature or preferably to 37°C the required tubes.
Inoculate specimens suspected of containing *Trichomonas* organisms into the medium using swabs containing the specimen or by alternative methods, as appropriate.
Incubate tubes at 35 ± 2°C in an aerobic atmosphere for 2-7 days.

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9 - READING AND INTERPRETATION

After 48 h of incubation and again daily, aseptically remove a drop of the culture and place it on a slide and cover with a glass coverslip. Examine under 100x-400x magnification. Do not to mix the culture, but remove the material from the bottom of the tube, with a sterile pipette.
A positive culture is defined as visualization of trophozoites with morphology and motility characteristic of *T.vaginalis.*
Negative result is defined as the absence of motile trichomonads after 7 days of incubation.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
T.vaginalis ATCC 30001	35-37 °C / up to 72h / A	motile organism observed
C.albicans ATCC 18804	35-37 °C / up to 72h / A	growth
E.coli ATCC 25922	35-37 °C / up to 72h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready to use tubes of Trichomonas CPLM Selective Broth is tested for productivity and selectivity. Productivity is tested by inoculating viable cells of *T.vaginalis* ATCC 30001 in test tubes and incubating at 35-37°C for up to 72 hours. The microscopically examination under 400x magnification will display the presence of motile trophozoites.
Productivity is also tested by inoculating the suitable decimal dilutions of *C.albicans* ATCC18804 and incubating at 35-37°C for up to 72 hours. After incubation *C.albicans* will grow developing a turbidity into the tubes.
Selectivity is tested by inoculating more than 1000 CFU of non-target strains *E.coli* ATCC 25922 and *S.aureus* ATCC 25923 in test tubes and incubating at 35-37°C for up to 72 hours. No turbidity shall be observed.

12 - LIMITATIONS OF THE METHOD

- *T. vaginalis* can grow without producing obvious signs of turbidity in the culture medium.
- Culture has a sensitivity of 75%–96% and a specificity of up to 100%.[4,7] A negative results must be viewed cautiously and evaluated in conjunction with clinical symptoms.[2]
- The medium does not contain antifungal agents so yeasts such as *Candida* spp. may grow in the tubes inoculated with the specimens.
- Even if the broth contains chloramphenicol to reduce contamination by vaginal flora, contamination with bacteria may be a major problem. Passage of the cultures after 2–3 days to reduce bacterial contamination may be required to identify *T.vaginalis* definitively.[8]
- Due to the fastidious nature of *T.vaginalis*, the culture will remain viable for a short period of time after reaching the stationary phase.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use tubes be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each tube is for single use only; do not transfer or subdivide the tube content in other containers.
- The tubes cannot to be considered a "sterile product", but a product with controlled bio-contamination, within the limits of the defined specifications.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store tubes in their original pack at 2-8°C away in the dark. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Do not open until ready to use. Minimize exposure to light. Before use, check the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour, precipitates).

15 – REFERENCES

1. McConnaughey M. Life Cycle of Parasites. Reference Module in Biomedical Sciences, 2014.
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4. Centers of Disease Control and Prevention, 2015 Sexually Transmitted Diseases Treatment Guidelines. June 4, 2015.
5. Shimizu RY, Garcia LD. Specimen collection, transport, and processing: parasitology. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
6. Hobbs MM et al. Methods for Detection of *Trichomonas vaginalis* in the Male Partners of Infected Women: Implications for Control of Trichomoniasis. J Clin Microbiol. 2006; 44(11): 3994–3999.
7. Nye MB, Schwebke JR, Body BA. Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. Am J Obstet Gynecol 2009;200: 181–7.
8. Domeika M, Zhurauskaya L,Savicheva A, Frigo N, Sokolovskiy E, Hallén A, Unemo M, Ballard RC. Guidelines for the laboratory diagnosis of trichomoniasis in East European countries. EE SRH Network Journal of the European Academy of Dermatology and Venereology. First published: 02 September 2010.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack This side up
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/74	2021/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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