CPLM TRICHOMONAS BROTH

Dehydrated culture medium

1 - INTENDED USE

Non-selective medium base for the cultivation of *Trichomonas vaginalis*, requiring horse serum and chloramphenicol supplement.

2 - COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	20.000 g
Liver extract	12.000 g
Cysteine HCl	1.600 g
Maltose	1.100 g
Agar	1.000 g
Methylene blue	0.003 g

*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]
CPLM (Cysteine-Peptone-Liver-Maltose Medium) Trichomoas Broth supplemented with horse serum and chloramphenicol 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. 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. Chloramphenicol, a relatively stable antibiotic added to the medium base, replaces the penicillin and streptomycin recommended for addition to the STS Kupferberg medium; it suppresses the growth of most Gram-positive and Gram-negative bacteria. Horse serum is added to provide useful growth factors for *Trichomonas*.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.7 g of CPLM Trichomonas Broth in 950 mL of Ringer's solution or purified water. Bring to the boil with frequent agitation and sterilize by autoclaving at 121 °C for 15 minutes. Cool to 47-50°C and add 50 mL of sterile horse serum and the contents of two vials of Chloramphenicol Antimicrobic Supplement (REF 4240003), reconstituted with 3 mL of a water/ethanol mixture (1:1). Mix well and dispense into sterile tubes using aseptic precautions.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Prepared tubes appearance	yellow with green ring, limpid
Final pH at 20-25 °C	6.5 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
CPLM Trichomonas Broth	Dehydrated medium	4013312	500 g (14 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - 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.

9 - 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.

10 - 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.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

11 - 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, the end user can perform its own Quality Control 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

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.

13 - PRECAUTIONS AND WARNINGS

- The product is for Laboratory use and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product 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 at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging).

15 – REFERENCES

1. McConnaughey M. Life Cycle of Parasites. Reference Module in Biomedical Sciences, 2014.
2. Novak-Weekley SN, Leber AL. Intestinal and urogenital amebae, flagellates and ciliates. *In* Carrol KC, Pfaller MA *et al.* editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
3. Kupferberg AB, Johnson G, Sprince H. 1948. Nutritional requirements of *Trichomonas vaginalis*. Proc Soc Exp Biol Med 1948;67:304-308.
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	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 4	Updated layout and content	2022/05

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it