Instructions for Use

REF - BM0413 Trichomonas Broth (CPLM) with Nystatin

Bottled Media

1. Intended Use

For *in vitro* diagnostic use only. Trichomonas Broth (CPLM) with Nystatin is for the selective isolation of *Trichomonas* spp.

2. Composition*

Ingredient	g/L
Tryptone	20.0
Liver extract	12.0
Maltose	1.1
Cysteine hydrochloride	1.6
Agar	1.0
Methylene blue	0.003
Chloramphenicol	0.1
Horse serum	50.0ml
Nystatin solution	2.0ml

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Trichomoniasis is a common sexually transmitted disease caused by infection with *Trichomonas vaginalis* (a protozoan parasite). Although symptoms vary, most people who have *trichomoniasis* are asymptomatic.

This medium is based on the formula described by Kupferburg *et al.* [(1)]. The superiority of the culture procedure over the wet mount procedure for detecting the presence of *trichomonads* in clinical specimens was demonstrated by Williams [(2)], and Kean and Day [(3)]. Feinberg and Whittington [(4)] demonstrated the greater accuracy of the culture procedure for detecting *trichomonads* in clinical material.

4. Principle

The tryptone and liver extract act as carbon, nitrogen and vitamin sources in this medium. Maltose is a fermentable carbohydrate. The agar and cysteine HCl reduce the oxygen tension in the medium which aids the growth of *trichomonas*. Methylene blue is a redox indictor and allows for the visualisation of any significant oxygen diffusion in the medium. The medium is selective due to the inclusion of two selective agents namely, chloramphenicol and nystatin, to inhibit a wide range of both Gram-positive and Gram-negative bacterial species as well as yeasts and fungi.

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Green liquid	6.5 ± 0.2

6. Materials Provided

BM0413 can be provided in the formats detailed below. Each bottle is labelled or ink-jet printed with (abbreviated) product name, product code, lot number and expiry date. Other volumes and containers may be available upon request.

Product Code	**Product Format**
BM0413-M003-5	50 x 5ml in Thin Wall Bijous
BM0413-M036-2.5	50 x 2.5ml in Polystyrene Tubes
BM0413-R500-500	6 x 500ml in 500ml Sirop Bottles

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms.

8. Specimens

BM0413 Trichomonas Broth (CPLM) with Nystatin is suitable for the testing of the following specimens:

- Clinical: Swabs from the lower genital tract (vulva, vagina, cervix, or urethra) in women, or the urethra in men

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Allow media to come to room temperature or preferably 37°C ± 1°C.

Clinical specimens:

Inoculate the broth with the sample swab and incubate at 37 ± 1°C for 48-72 hours.

Inoculated broths may be examined microscopically after 48 hours incubation and again daily, for the presence of flagellate protozoans by aseptically removing a drop of the culture and place it onto a slide and cover with a glass coverslip. Do not mix the culture broth but remove the material from the bottom of the broth, using a sterile pipette.

A positive culture is defined as visualization of flagellate protozoans with morphology and motility characteristics of *Trichomonas vaginalis.* A negative result is defined as the absence of motile *Trichomonas vaginalis* after up to 7 days of incubation.

10. Quality Control

Organism	Incubation	Result (Specificity)
T. vaginalis (clinical isolate)	Incubate at 37 ± 1°C for 48-72 hours.	Growth and motile
C. albicans (NCPF 3179)	Incubate at 37 ± 1°C for 48-72 hours.	Partial inhibition
S. aureus (NCTC 12981)	Incubate at 37 ± 1°C for 48-72 hours.	Inhibited
E. coli (NCTC 12241)	Incubate at 37 ± 1°C for 48-72 hours. Subculture *E. coli* and *S. aureus* onto E&O PP0280 Tryptone Soya agar and incubate at 37 ± 1°C for 18-24 hours. Subculture *C. albicans* onto E&O PP0240 Sabouraud Dextrose Agar and incubate at 37 ± 1°C for 37 ± 1°C for 24-48 hours.	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

All manufactured lots of the finished product are released for sale after the Quality Control has been performed to check the compliance with the specification. To fully verify BM0413 Trichomonas Broth (CPLM) with Nystatin performance, samples are tested to assess colony morphology and recovery level when incubated at 37 ± 1°C for 48-72 hours in the broth, before *Candida albicans* (NCPF 3179) was subcultured onto PP0240 Sabouraud Dextrose Agar, and *Escherichia coli* (NCTC 12241) and *Staphylococcus aureus* (NCTC 12981) onto PP0280 Tryptone Soya Agar and further incubated at 37 ± 1°C for 24-48 hours. *Trichomonas vaginalis* (clinical isolate) is assessed on motility in the medium. *C. albicans* showed partial inhibition and *S. aureus* and *E. coli* were fully inhibited. Therefore, it can be concluded that BM0413 Trichomonas Broth (CPLM) with Nystatin, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- *T. vaginalis* can grow without showing obvious signs of turbidity in the broth. A negative result should be evaluated in conjunction with clinical symptoms.
- Although the broth contains chloramphenicol and nystatin to reduce background flora contamination with bacteria and fungi may be a problem.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSSH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM0413 in the original bottle with the lid tightly closed at between 2 and 8°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured or out of date. Additionally, do not use medium if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Kupferberg, A., Johnson, G., Sprince, H. (1948) Nutritional Requirements of Trichomonas vaginalis. Proceedings of the Society for Experimental Biology and Medicine. 67(3), pp.304-308.
2. Kean, B. and Day, E. (1954) *Trichomonas vaginalis* infection. an evaluation of three diagnostic techniques with data on incidence. AJOG, 68(6), pp.1510-1518
3. Feinberg, J. and Whittington, J. (1957) A culture medium for *Trichomonas vaginalis* donné and species of *Candida*. J. Clin Pathol, 10(4), pp.327-329.
4. Johnson G. and Trussell R. (1943) Experimental Basis for the Chemotherapy of *Trichomonas vaginalis* Infestations. I. Proc. Soc. Exp. Biol., 54(2), pp.245.

Version History*

001 23/02/23 - New Documents Created

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

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place