INSTRUCTIONS FOR USE

MODIFIED THAYER MARTIN (MTM) MEDIUM

Ready-to-use plates

MTM Medium:
Neisseria gonorrhoeae

1 - INTENDED USE

In vitro diagnostic device. Selective and enriched medium for the isolation and cultivation of *Neisseria gonorrhoeae* from clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Peptocomplex	15 g
Corn starch	1 g
Dipotassium hydrogen phosphate	4 g
Potassium dihydrogen phosphate	1 g
Sodium chloride	5 g
Agar	12 g
80°C heated defibrinated sheep blood	50 mL
Purified water	1000 mL
VCNT Supplement	
Vancomycin	3 mg
Colistin	7.5 mg
Nystatin	12,500 IU
Trimethoprim	5 mg
Biovitex Enrichment Supplement	
Nicotinamide adenine dinucleotide (NAD)	2.5 mg
Cocarboxylase	1 mg
p-aminobenzoic acid	0.13 mg
Thiamine	0.03 mg
Vitamin B12	0.1 mg
L-glutamine	100 mg
L-cystine	11 mg
L-cysteine HCl	259 mg
Adenine	10 mg
Guanine HCl	0.3 mg
Ferric nitrate.$6H_20$	0.2 mg
Glucose	1 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

In 1964 Thayer and Martin[1] formulated a selective medium for the cultivation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*, incorporating haemoglobin, yeast supplement B, polymyxin B and ristocetin into GC Agar. Thayer and Martin improved in 1966[2] the formulation substituting the original antibiotics with vancomycin, colistin and nystatin (VCN). In 1970 Martin and Lester[3] modified the new Thayer Martin Medium by increasing agar and glucose content and by incorporating an additional antibiotic, trimethoprim lactate; this improved medium is called Modified Thayer Martin (MTM) medium.
Biolife Modified Thayer Martin (MTM) Medium is prepared with GC Medium Base, supplemented with defibrinated sheep blood chocolatized at 80°C for 15 minutes, Biovitex and VCNT supplements.
Heat-lysed sheep blood is a good source of both hemin (X factor) and NAD (V factor), that enhance the growth of *Neisseria*[4]. V factor and various other factors such as glutamine, cocarboxylase, cystine etc., enhancing the growth of *N.gonorrhoeae* [5], are supplied by the chemically defined enrichment supplement Biovitex.
Vancomycin inhibits Gram-positive bacterial contamination, nystatin is an anti-fungal agent, colistin inhibits Gram-negative microbial flora and almost all saprophytic *Neisseria* spp., trimethoprim suppresses *Proteus* swarming.[5] Peptocomplex provides carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, dibasic and monobasic potassium phosphates buffer prevents pH changes due to amine production, corn starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth.[5]
Modified Thayer Martin (MTM) Medium is intended for the isolation and cultivation of *N.gonorrhoeae* from clinical specimens, containing a mixed flora of bacteria and/or fungi.[5-8]

4 - PHYSICAL CHARACTERISTICS

Medium appearance	brown, opaque
Final pH at 20-25 °C	7.2 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Modified Thayer Martin (MTM) Medium	Ready-to-use plates	541522	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, CO_2 generators and jars or CO_2 incubator with humidifier, ancillary culture media and reagents for the identification of the colonies.

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

7 - SPECIMENS

Modified Thayer Martin (MTM) Medium can be directly inoculated with specimens from non-sterile human sites contaminated by mixed flora of bacteria and/or fungi (urogenital tract, upper respiratory tract, pus and exudates).[8-11] This medium is not useful for the isolation of *Neisseria* spp. from supposedly sterile sites as cerebrospinal fluid, conjunctival swab, skin biopsy, joint fluid.[5] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information because *Neisseria* spp. are very sensitive to collection and storage procedures.[9]

8- TEST PROCEDURE

Allow plates to come to room temperature. The agar surface should be smooth and moist, but without excessive water.
Process the specimen as soon as possible after it is received in the laboratory to avoid loss of gonococci viability and overgrowth of contaminants.
Roll the swab over one quadrant of the surface then streak the specimen over the other quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Alternatively, since swabs for gonococcal culture may contain only small numbers of organisms, roll the swabs directly on the medium in a large "Z" pattern to sufficiently transfer the specimen; cross-streak the "Z" pattern with a sterile loop.
Incubate at 35-36.5°C in a moist atmosphere supplemented with 3-7% CO_2; cultures should be examined daily for growth and held for a maximum of 72 hours.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
N.gonorrhoeae colonies are variable in size, usually small (0,5-2 mm), moderately convex, raised, granular, glistening, moist, with entire to lobate margins, usually greyish-white to translucent; almost all strains become mucoid after 48 hours.
A Gram staining must be performed on suspected *Neisseria* colonies to confirm the presence of uniform Gram-negative diplococci.
Performance of oxidase test is mandatory for colonies suspected to belong to *Neisseria* that shall be positive for *N.gonorrhoeae*.

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, 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.[12]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
N.gonorrhoeae	ATCC 43069	35-36.5°C / 24-48H / CO_2	good growth
S.epidermidis	ATCC 12228	35-36.5°C / 24-48H / CO_2	inhibited
P.mirabilis	ATCC 43071	35-36.5°C / 24-48H / CO_2	inhibited
C.albicans	ATCC 60193	35-36.5°C / 24-48H / CO_2	growth partially inhibited

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 plates of Modified Thayer Martin (MTM) Medium and of the raw materials used for the production of prepared plates (dehydrated GC Medium Base REF 401520 supplemented with VCNT, REF 4240008 and Biovitex REF 4240009 and chocolatized sheep blood) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique with 2 gonococcal strains: *N.gonorrhoeae* ATCC 43069*, N.gonorrhoeae* ATCC 19424. After incubation at 35-36.5°C for 24-48 hours, with 3-7% of CO_2, the amount of growth is evaluated and recorded. All strains show a good growth with typical morphology. The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-4} of a 0.5 McFarland suspension of the non-target organisms *N.sicca* ATCC 9913, *S.epidermidis* ATCC 12228*, E.coli* ATCC 25922*, P.mirabilis* ATCC 43071*, C.albicans* ATCC 60193. After incubation at 35-36.5°C for 24-48 hours, with 3-7% of CO_2, the growth of non-target strains *S.epidermidis, E.coli, P.mirabilis* is inhibited at the dilution 10^{-1} and the growth of *N.sicca* and *C.albicans* is partially inhibited.

12 - LIMITATIONS OF THE METHOD

- The presence of small particles may sometimes be observed in the agar. However, this phenomenon does not affect the performance of the medium.
- Vancomycin sensitive strains of some auxotypes of *N.gonorrhoeae* (e.g. strains that require arginine, uracil and hypoxanthine for growth) which fail to grow on MTM, have been reported from 3% to 10% of the total isolates.[13,14] Some gonococci are susceptible to trimethoprim too.[15]
- It is recommended that both a selective and a non-selective medium be used when isolating pathogenic *Neisseria* in order to avoid the loss of vancomycin and/or trimethoprim sensitive strains.[5]
- This medium is not useful for the isolation of *Neisseria* spp. from supposedly sterile sites as cerebrospinal fluid, conjunctival swab, skin biopsy, joint fluid for which non-selective media are recommended.[5]
- For the growth of *N.gonorrhoeae* it is necessary that the surface of the plates is moist; if it appears dry, humidify with a few drops of sterile distilled water. Place damp gauze or paper towels in the CO_2 container before incubation or use an incubator with humidifier.[5]
- On this medium *N.gonorrhoeae* grows with smaller and more granular colonies than with non-selective chocolate agar.
- Some saprophytic non-target microorganisms, resistant to antimicrobials present in the medium may grow. *N.lactamica* may grow on this medium with colonies smaller and less moist than gonococci, occasionally with a yellowish tint [5].
- Use dacron or calcium alginate swabs for specimen collection, avoid cotton swabs since they contain fatty acids which are inhibitory for *N.gonorrhoeae*.
- The gonococci are one of the most fragile Gram-negative bacteria. It is recommended that any suspected *Neisseria* containing specimen should be inoculated onto primary isolation medium immediately on collection to avoid any loss in viability and/or overgrowth of contaminants; if this is not possible *N.gonorrhoeae* swabs are better held at 4-6° C for not more than 3 hours.[5]
- The incubator temperature should be set at 35-36.5°C [6] because many strains of *N. gonorrhoeae* will not grow well at 37°C [5].
- Examine plates after 24 hours incubation. At 48 hours the Gram morphology may exhibit atypical forms.

- Many standard protocols[4,7,8,10] describe the use of MTM medium for the detection of meningococcal carriage in oropharyngeal and nasopharyngeal swabs. This application is out of Biolife MTM Medium intended use. The end-user should validate this application before routinely using MTM Medium for *N.meningitidis* detection in clinical specimens.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- 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 this product does not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates 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 plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates 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 plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 – REFERENCES

1. Thayer JD, E. Martin Jr E. A selective medium for cultivation of N. gonorrhoeae and N. meningitidis. Pub. Health Rep. 1964; 79:49.
2. Thayer JD, E. Martin Jr E. Improved medium selective for cultivation of N. gonorrhoeae and N. meningitidis. Pub. Health Rep. 1966; 81:559-562.
3. Martin JE Jr, Lester A. Transgrow, a maedium for transport and growth of N. gonorrhoeae and N. Meningitides. HSMHA Helth Service Rep. 1971: 86:30
4. CDC Lab Manual, meningitides; Annex: Preparation of Media and Reagents, 2016
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. CDC: Morbidity and Mortality Weekly Report (MMWR). Screening Tests To Detect Chlamydia trachomatis and Neisseria gonorrhoeae. Infections Recommendations and Reports. October 18, 2002 / Vol. 51 / No. RR-15
7. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
8. Elias J, Frosh M, Vogel U. Neisseria. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.635.
9. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
10. Public Health England: Standards for microbiology investigations (UK SMI)- Bacteriology: UK SMI B2:2017, UK SMI B9:2015, UK SMI B14:2016; UK SMI B28:2017; B51:2014
11. Vandepitte J, Verhaegen J, P. Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd edition Geneve: World Health Organization Geneva; 2003.
12. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
13. Talbot V. et al. Vancomycin sensitive penicillinase producing Neisseria gonorrhoea. Br.J Ven Dis. 1983; 59:277
14. Mirret S, Reller B, Knapp JS. Neisseria gonorrhoeae Strains inhibited by vancomycin in selective media and correlation with auxotype.J Clin Microbiol 1981; 14: 94
15. Lai-King Ng, Martin IE. The laboratory diagnosis of Neisseria gonorrhoeae Can J Infect Dis Med Microbiol. 2005; 16(1): 15–25.

TABLE OF APPLICABLE SYMBOLS

REF or 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	For single use only	Fragile, handle with care

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

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 2	Cap. 11- Performances characteristics	2021/03
Revision 3	Removal of obsolete classification	2023/03

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
export@biolifeitaliana.it; web: www.biolifeitaliana.it