BBL™ Modified Thayer-Martin (MTM II) Agar

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QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Modified Thayer-Martin (MTM II) Agar is an enriched medium for the selective isolation and cultivation of *Neisseria* species.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Add 0.1 mL of a dilution containing 30–300 CFU/0.1 mL to each plate and spread-inoculate using a sterile glass spreader.
   b. Incubate plates at 35 ± 2 °C in an aerobic atmosphere containing carbon dioxide.
   c. Include Chocolate II Agar plates as nonselective controls for all organisms.
2. Examine plates after 18–24 and 48 h for growth, colony size, and selectivity.
3. Expected Results

CLSI Organisms	ATCC®	Recovery
**Neisseria gonorrhoeae*	43069	Growth
**Proteus mirabilis*	43071	Inhibition (partial)
**Staphylococcus epidermidis*	12228	Inhibition (partial)
Neisseria meningitidis	13090	Growth
Neisseria sicca	9913	Inhibition (partial)
Candida albicans	60193	Inhibition (partial)
Escherichia coli	25922	Inhibition (partial)
Additional Organisms		
Neisseria gonorrhoeae 2 strains)	43070 35201	For both strains, colonies small, opaque, grayish-white to colorless, raised, glistening and smooth

*Recommended organism strain for User Quality Control.

NOTE: User QC testing of exempt media for *N. gonorrhoeae* is strongly recommended by CLSI M22-A3.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.2 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Modified Thayer-Martin (MTM II) Agar is used for the isolation of pathogenic *Neisseria* from specimens containing mixed flora of bacteria and fungi.

V SUMMARY AND EXPLANATION

Carpenter and Morton described an improved medium for the isolation of the gonococcus in 24 h.[1] The efficiency of this medium, GC Agar supplemented with hemoglobin and yeast concentrate, was demonstrated in a study of twelve media then in use for the isolation of this organism.[2] The medium was improved by replacing the yeast concentrate with **BBL™ IsoVitaleX™** Enrichment, a chemically defined supplement developed specifically to aid the growth of gonococci, although it has broad application for other microorganisms, e.g., *Haemophilus*.[3-5]

Thayer-Martin Selective Agar was developed for the primary isolation of *N. gonorrhoeae* and *N. meningitidis* from specimens containing mixed flora taken from the throat, vagina, rectum, and urethra.[4,6,7] Consisting of Chocolate II Agar with vancomycin, colistin and nystatin, it is formulated to minimize the overgrowth of gonococci and meningococci by contaminants, to suppress the growth of saprophytic *Neisseria* species and to enhance the growth of pathogenic *Neisseria*.

Martin et al. modified Thayer-Martin Selective Agar by adding trimethoprim to produce Modified Thayer-Martin Agar.[8] A significantly greater number of positive gonococcal isolates from clinical specimens was reported as compared with Thayer-Martin Selective Agar due to the inhibition of swarming *Proteus* species.[8-10] Because of its improved performance, it is recommended over earlier formulations for the isolation of *N. gonorrhoeae*.[11,12] The original formula contained 20 g/L of agar and 1.5 g/L dextrose (in addition to the dextrose in the **IsoVitaleX** Enrichment). The agar concentration has been changed to approximately 12 g/L; the extra 1.5 g/L of dextrose has been eliminated since the lower dextrose content was found to improve the growth of *N. gonorrhoeae*. **BBL** MTM II was developed by careful selection and pretesting of raw materials to provide enhanced growth of gonococci as well as improved inhibition of *Candida* species.

VI PRINCIPLES OF THE PROCEDURE

Modified Thayer-Martin (MTM II) Agar is based on Chocolate II Agar which contains an improved GC Agar base, bovine hemoglobin and **IsoVitaleX** Enrichment. The GC base contains nitrogenous nutrients in the form of casein and meat peptones, phosphate buffer to maintain pH and corn starch, which neutralizes toxic fatty acids that may be present in the agar. Hemoglobin provides X factor (hemin) for *Haemophilus* species. **IsoVitaleX** Enrichment is a defined supplement which provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, co-enzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

This selective medium contains the antimicrobial agents, vancomycin, colistin, nystatin, (V-C-N Inhibitor) and trimethoprim to suppress the normal flora. Vancomycin is active primarily against gram-positive bacteria. Colistin inhibits gram-negative bacteria, including *Pseudomonas* species, but is not active against *Proteus* species. Nystatin inhibits fungi. Trimethoprim inhibits *Proteus* species.

VII REAGENTS

Modified Thayer-Martin (MTM II) Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	7.5 g	Agar	12.0 g
Selected Meat Peptone	7.5 g	Hemoglobin	10.0 g
Corn Starch	1.0 g	**IsoVitaleX** Enrichment	10.0 mL
Dipotassium Phosphate	4.0 g	V-C-N Inhibitor	10.0 mL
Monopotassium Phosphate	1.0 g	Trimethoprim Lactate	5.0 mg
Sodium Chloride	5.0 g		

*Adjusted and/or supplemented as required to meet performance criteria.

IsoVitaleX Enrichment

Approximate Formula* Per Liter Purified Water

Vitamin B_{12}	0.01 g	Thiamine Pyrophosphate	0.1 g
L-Glutamine	10.0 g	Ferric Nitrate	0.02 g
Adenine	1.0 g	Thiamine Hydrochloride	0.003 g
Guanine Hydrochloride	0.03 g	L-Cysteine Hydrochloride	25.9 g
p-Aminobenzoic Acid	0.013 g	L-Cystine	1.1 g
Nicotinamide Adenine Dinucleotide	0.25 g	Dextrose	100.0 g

*Adjusted and/or supplemented as required to meet performance criteria.

V-C-N Inhibitor

Approximate Formula* Per One Milliliter Restored Solution

Vancomycin	300 µg
Colistin	750 µg
Nystatin	1250 units

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[17-20] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

A variety of swabs and containers have been devised for collecting specimens. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory. Several holding media or transport systems, such as **BBL** specimen collection and transport products, have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Refer to appropriate texts for details of specimen collection and handling procedures.[21,22]

IX PROCEDURE

Material Provided: Modified Thayer-Martin (MTM II) Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. Alternatively, if material is being cultured directly from a swab, proceed as follows:[23]

1. Roll swab directly on the medium in a large "Z" to provide adequate exposure of swab to the medium for transfer of organisms.
2. Cross-streak the "Z" pattern with a sterile wire loop, preferably in the clinic. If not done previously, cross-streaking should be done in the laboratory.
3. Place the culture as soon as possible in an aerobic environment enriched with carbon dioxide.
4. Incubate at 35 ± 2 °C and examine after overnight incubation and again after approximately 48 h.
5. Subculture for identification of *N. gonorrhoeae* should be made within 18–24 h. If shipped after incubation, colonies should be subcultured before performing biochemical identification tests in order to ensure that adequate viability is achieved.

User Quality Control: See "Quality Control Procedures."

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

Typical colonial morphology on Modified Thayer-Martin (MTM II) Agar is as follows:

Neisseria gonorrhoeae	Small, grayish-white to colorless, mucoid
Neisseria meningitidis	Medium to large, blue-gray, mucoid

Colonies may be selected for Gram staining, subculturing or other diagnostic procedures.

XI LIMITATIONS OF THE PROCEDURE

Selective media for pathogenic *Neisseria* may inhibit other pathogenic bacteria; e.g., *Haemophilus*.

The existence of strains of *N. gonorrhoeae* inhibited by the components of V-C-N Inhibitor and trimethoprim lactate have been reported.[25,26]

Some strains of *Capnocytophaga* species may grow on this selective medium when inoculated with oropharyngeal specimens.[27]

XII PERFORMANCE CHARACTERISTICS

In a clinical study conducted at a sexually transmitted diseases clinic, **BBL** Modified Thayer Martin Agar was compared to a new **BBL** selective medium. *Neisseria gonorrhoeae* was recovered on one or both media from 175 (29%) of 607 genital specimens, 3 (3%) of 88 oral specimens, and 6 (29%) of 21 rectal specimens. A total of 166 cultures were positive on MTM for an overall sensitivity of 90% (166/184).[27]

XIII AVAILABILITY

Cat. No.	Description
221567	**BD BBL™** Modified Thayer-Martin (MTM II) Agar, Pkg. of 20 plates
221885	**BD BBL™** Modified Thayer-Martin (MTM II) Agar, Pkg. of 30 plates
221568	**BD BBL™** Modified Thayer-Martin (MTM II) Agar, Ctn. of 100 plates

XIV REFERENCES

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