

THAYER MARTIN AGAR (MODIFIED)

INTENDED USE

Remel Thayer Martin Agar (Modified) is a solid medium recommended for use in qualitative procedures for the isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

SUMMARY AND EXPLANATION

In 1945, Johnston introduced a medium to grow *N. gonorrhoeae* in 24 hours.¹ This was the advent of GC Agar Base. In 1964, Thayer and Martin formulated a selective medium for isolation of *N. gonorrhoeae* and *N. meningitidis* that contained antibiotics capable of suppressing the commensal microbial flora present in the anal canal, vagina, pharynx, and other sites.² The antibiotics used initially, ristocetin and polymyxin B, were replaced in 1966 by vancomycin, colistimethate, and nystatin.³ Since 1970, the addition of trimethoprim to Thayer Martin media to suppress swarming of *Proteus* has gained wide-spread acceptance.⁴ In subsequent years, multiple studies have demonstrated improved recovery rates for pathogenic *Neisseria* spp. using selective media.⁵⁻⁸

PRINCIPLE

Thayer Martin Agar (Modified) contains hemoglobin, which provides the X factor (hemin), and GCHI Enrichment, which provides the V factor, vitamins, amino acids, coenzymes, and dextrose. Vancomycin and colistin are selective agents which inhibit gram-positive cocci and gram-negative bacilli, respectively. Thayer Martin Agar (Modified) contains nystatin which has proven to be effective in the suppression of *Candida albicans*. Trimethoprim lactate has been added to suppress the swarming of *Proteus* species. Dextrose is added to enhance the growth of gonococci. The tablet provided in the JEMBEC™ system liberates carbon dioxide into the enclosed pack when the tablet comes in contact with the water vapor present in the medium.

REAGENTS (CLASSICAL FORMULAE)*

Casein Peptone.....	7.5 g
Meat Peptone.....	7.5 g
Sodium Chloride.....	5.0 g
Dipotassium Phosphate.....	4.0 g
Dextrose.....	1.5 g
Corn Starch.....	1.0 g
Monopotassium Phosphate.....	1.0 g

Colistin.....	7.5 mg
Trimethoprim Lactate.....	6.25 mg
Vancomycin.....	3.0 mg
Hemoglobin Solution.....	350.0 ml
●GCHI Enrichment.....	10.0 ml
Nystatin.....	12,500 U
Agar.....	10.0 g
Deminerlized Water.....	650.0 ml

pH 7.2 ± 0.2 @ 25°C

●GCHI Enrichment:

Vitamin B12.....	0.01 g
L-Glutamine.....	10.0 g
Guanine Hydrochloride.....	0.03 g
Adenine.....	1.0 g
P-Aminobenzoic Acid.....	13.0 g
L-Cystine.....	1.1 g

Glucose.....	100.0 g
NAD.....	0.25 g
Coccarboxylase.....	0.1 g
Ferric Nitrate.....	0.02 g
Thiamine Hydrochloride.....	0.003 g
Cysteine Hydrochloride.....	25.9 g
Deminerlized Water.....	1000.0 ml

*Adjusted as required to meet performance standards.

PROCEDURE

Note: Inoculate the specimen as soon as possible after receipt in the laboratory. Inoculation of selective and nonselective media from specimens known to contain commensal microbial flora has been shown to increase the recovery of pathogenic *Neisseria* spp.^{9,10}

Plate Procedure:

1. Inoculate the plate by rolling the swab over the agar surface in a large "Z" pattern to sufficiently transfer the specimen.
2. Cross-streak the plate using a sterile inoculating loop to achieve isolated colonies.
3. Incubate plate in 3-7% CO₂ at 33-37°C and examine after 24-48 hours. Continue incubation of negative plates for 72-96 hours before reporting as negative.

JEMBEC™ Procedure for Carbon Dioxide Generation:

1. Using forceps, remove a CO₂ tablet from the foil pouch and place in the well of the plate.
2. Inoculate the plate by rolling the swab over the agar surface in a large "Z" pattern to sufficiently transfer the specimen.
3. Cross-streak the plate using a sterile inoculating loop to achieve isolated colonies.
4. Secure the top of the plate tightly and label with the patient information.
5. Place the plate in an environmental maintenance pouch, seal securely, and incubate in an inverted position.

EXPECTED RESULTS

Colonies of *N. gonorrhoeae* are translucent, raised, gray, and may be mucoid. *N. meningitidis* colonies are larger than *N. gonorrhoeae*, bluish-gray in color, and may be mucoid. Test isolates should be examined by Gram stain to verify they are gram-negative diplococci. Additional biochemical and/or serological testing is required for definitive identification, following established laboratory guidelines. Consult appropriate references for further instructions.^{9,10}

QUALITY CONTROL

All lot numbers of Thayer Martin Agar (Modified) have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.¹¹ Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL ORGANISM

Neisseria gonorrhoeae ATCC® 43070
**Neisseria gonorrhoeae* ATCC® 43069
**Neisseria meningitidis* ATCC® 13090
**Candida albicans* ATCC® 60193
**Escherichia coli* ATCC® 25922
**Neisseria sicca* ATCC® 9913
**Proteus mirabilis* ATCC® 43071
**Staphylococcus epidermidis* ATCC® 12228

*CLSI recommended organism

INCUBATION

CO₂, 24-48 h @ 33-37°C
CO₂, 24-48 h @ 33-37°C
CO₂, 24-48 h @ 33-37°C
CO₂, 24-48 h @ 33-37°C
CO₂, 24-48 h @ 33-37°C
CO₂, 24-48 h @ 33-37°C
CO₂, 24-48 h @ 33-37°C
CO₂, 24-48 h @ 33-37°C

RESULTS

Growth
Growth
Growth
Inhibition (partial)
Inhibition (partial)
Inhibition (complete)
Inhibition (partial)
Inhibition (partial)

LIMITATIONS

1. Cultures for pathogenic *Neisseria* should be incubated in 3-7% CO₂. Higher concentrations of CO₂ may be inhibitory to some strains.⁹
2. Organisms other than *N. gonorrhoeae* and *N. meningitidis* may grow on Thayer Martin Agar (Modified). Further testing is required for identification confirmation following established laboratory procedures. Consult appropriate references for further instructions.^{9,10}
3. Media containing antibiotics may be too selective for some strains of pathogenic *Neisseria*. Even though the clinical symptoms or microscopic examination may indicate infection with *Neisseria* spp., isolation of the organism may fail due to overgrowth with commensal microbial flora.^{9,10}

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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