THAYER MARTIN AGAR (IMPROVED)

INTENDED USE

Remel Thayer Martin Agar (Improved) is a solid medium recommended for use in qualitative procedures for 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.[5-7] Martin and Lewis recommended the use of anisomycin, considered a more stable antifungal agent, in place of nystatin to increase the shelf life of the media.[8]

PRINCIPLE

Thayer Martin Agar (Improved) contains hemoglobin, which provides the X factor (hemin), and GCHI Enrichment, which provides the V factor, vitamins, amino acids, coenzymes, and dextrose. The vancomycin is increased over earlier Thayer Martin formulas to provide improved inhibition of gram-positive cocci. Anisomycin is added to suppress the growth of *Candida albicans* and trimethoprim lactate effectively suppresses the swarming of *Proteus* spp. The medium also contains colistin to inhibit most gram-negative organisms, including *Pseudomonas* spp. and an increased concentration of dextrose which allows for better growth of gonococci. The tablet provided in the JEMBEC™ and Pill-Pocket systems 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	Anisomycin	20.0	mg
Meat Peptone	7.5	g	Colistin	7.5	mg
Sodium Chloride	5.0	g	Trimethoprim Lactate	6.25	mg
Dipotassium Phosphate	4.0	g	Vancomycin	4.0	mg
Dextrose	1.5	g	Hemoglobin Solution	350.0	ml
Corn Starch	1.0	g	●GCHI Enrichment	10.0	ml
Monopotassium Phosphate	1.0	g	Agar	10.0	g
			Demineralized Water	650.0	ml

pH 7.2 ± 0.2 @ 25°C

●GCHI Enrichment:

Glucose	100.0	g	Cocarboxylase	0.1	g
Cysteine Hydrochloride	25.9	g	Guanine Hydrochloride	0.03	g
L-Glutamine	10.0	g	Ferric Nitrate	0.02	g
L-Cystine	1.1	g	ρ-Aminobenzoic Acid	0.013	g
Adenine	1.0	g	Vitamin B12	0.01	g
NAD	0.25	g	Thiamine Hydrochloride	0.003	g
			Demineralized 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 likely to contain commensal microbial flora has been shown to increase the recovery of pathogenic *Neisseria* spp. [9,10]

Transgrow Procedure:

1. Hold Transgrow medium in an upright position during inoculation to minimize the loss of carbon dioxide. Remove the bottle cap for only the shortest period necessary. Inoculate the medium by placing the swab at the bottom of the bottle and crossing from side to side over the surface of the medium. Remove and discard the swab.
2. Tighten cap securely after inoculation.
3. If the bottle is to be transported after inoculation, viability of the organism may be extended by incubating the culture for 16-20 hours at 33-37°C prior to shipment.

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% CO_2 at 33-37°C and examine after 24-48 hours. Continue incubation of negative plates for 72-96 hours before reporting as negative.

JEMBEC™ / Pill-Pocket Plate Procedure for Carbon Dioxide Generation:

1. Using forceps, remove the CO_2 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.

(Continued on back)

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 (Improved) 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	INCUBATION	RESULTS
Neisseria gonorrhoeae ATCC® 43070	CO_2, 24-48 h @ 33-37°C	Growth
**Neisseria gonorrhoeae* ATCC® 43069	CO_2, 24-48 h @ 33-37°C	Growth
**Neisseria meningitidis* ATCC® 13090	CO_2, 24-48 h @ 33-37°C	Growth
**Candida albicans* ATCC® 60193	CO_2, 24-48 h @ 33-37°C	Inhibition (partial)
**Escherichia coli* ATCC® 25922	CO_2, 24-48 h @ 33-37°C	Inhibition (partial)
**Neisseria sicca* ATCC® 9913	CO_2, 24-48 h @ 33-37°C	Inhibition (complete)
**Proteus mirabilis* ATCC® 43071	CO_2, 24-48 h @ 33-37°C	Inhibition (partial)
**Staphylococcus epidermidis* ATCC® 12228	CO_2, 24-48 h @ 33-37°C	Inhibition (partial)

*CLSI recommended organism

LIMITATIONS

1. Cultures for pathogenic *Neisseria* should be incubated in 3-7% CO_2. Higher concentrations of CO_2 may be inhibitory to some strains.[9]
2. Organisms other than *N. gonorrhoeae* and *N. meningitidis* may grow on Thayer Martin Agar (Improved). 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 suggest infection with *Neisseria* spp., recovery of the pathogen 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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