Cultech Diagnostics
800-638-2625

Product No. 1295
Revision No. 01

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

MARTIN LEWIS / MARTIN LEWIS AGAR BIPLATE

INTENDED USE

Martin Lewis Agar is an enriched and selective medium used for the isolation of pathogenic *Neisseria* species from specimens containing mixed microbiologic flora.

SUMMARY AND EXPLANATION

Thayer-Martin Selective Agar was developed for the isolation of *N. gonorrhoeae* and *N. meningitidis* from specimens containing mixed flora taken from the throat, vagina, rectum and urethra.[1,2] Thayer-Martin consists of a chocolate agar base to which vancomycin, colistin and nystatin have been added to minimize overgrowth by contaminants, suppress the growth of saprophytic *Neisseria* sp., and enhance the growth of pathogenic *Neisseria.*

Martin, et. al., modified Thayer-Martin Agar by incorporating trimethoprim to produce Modified Thayer-Martin (MTM) medium. Trimethoprim suppresses the growth of swarming *Proteus* sp., thereby increasing the number of gonococci isolated from clinical specimens.[3]

A further modification of these early formulations was developed to improve the isolation of pathogenic *Neisseria* sp. from specimens which contained large amounts of mixed microbial flora. This formulation, Martin-Lewis Agar, contains an increased level of vancomycin for greater inhibition of gram positive organisms. Anisomycin has been substituted for nystatin to improve inhibition of *Candida albicans.*[4]

PRINCIPLES OF THE PROCEDURE

Martin Lewis Agar consists of a chocolate GC agar base, bovine hemoglobin, and a chemically defined enrichment. The GC base provides nitrogenous nutrients, phosphate buffers, and cornstarch to neutralize toxic fatty acids. Hemoglobin provides X factor (hemin). Chemically defined enrichments provide V factor (nicotinamide adenine dinucleotide - NAD), vitamins, amino acids, coenzymes, dextrose, ferric ions and other growth factors necessary for optimal growth of pathogenic *Neisseria* species.

The antimicrobial agents vancomycin, colistin, anisomycin and trimethoprim are incorporated to suppress competing normal flora organisms. Vancomycin actively inhibits gram positive organisms, colistin suppresses gram negative organisms other than *Proteus* sp., anisomycin is effective against fungi and trimethoprim suppresses swarming *Proteus* species.

TYPICAL FORMULA AND APPEARANCE

Appearance = opaque, chocolate brown
(Approximate formula* per liter of processed water)

Pancreatic Digest of Casein	7.5g
Selected Meat Peptone	7.5
Corn Starch	1.0
Dipotassium Phosphate	4.0
Monopotassium Phosphate	1.0
Sodium Chloride	5.0
Agar	13.0
Hemoglobin	10.0
Chocolate Enrichment Solution	10 ml
VCAT Inhibitor Solution	10 ml

*adjusted and/or supplemented to meet performance criteria.

USER QUALITY CONTROL

NCCLS CONTROL ORGANISMS (ATCC STRAINS)

**Neisseria gonorrhoeae* (ATCC 43069)	Growth (small, grayish-white to colorless mucoid colonies)
**Proteus mirabilis* (ATCC 43071)	Inhibition (partial)
**Staphylococcus epidermidis* (ATCC 12228)	Inhibition (partial)
Neisseria meningitidis (ATCC 13090)	Growth (medium to large, blue-grey, mucoid colonies)
Neisseria sicca (ATCC 9913)	Inhibition (complete)
Candida albicans (ATCC 60193)	Inhibition (partial)
Escherichia coli (ATCC 25922)	Inhibition (partial)

*Recommended organism strain for User Quality Control

PRECAUTIONS

This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria.

STORAGE / SHELF LIFE

Plated media should be stored at 2-8°C (36-46°F), media side up, in the unopened or resealed package protected from light. DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened plates to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, plates should be handled in a manner that minimizes product exposure to the environment. Product which has exceeded the assigned expiration date noted on the label should not be used.

Do not use plates that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration. The presence of excessive condensate may indicate plates which have been damaged by exposure to temperature extremes.

SPECIMEN COLLECTION

The quality of culture results depends primarily on the adequacy and condition of the specimen submitted for examination. Proper specimen collection techniques must be followed to ensure the most accurate culture results. Sterile swabs and collection containers should be used. Plates should be inoculated promptly after specimen collection. If a delay in inoculation is unavoidable, transport medium should be employed. Specimens should be collected prior to the initiation of antimicrobial therapy.

Detailed information on proper specimen collection may be obtained from microbiology reference materials.[5,6]

MATERIALS PROVIDED

Martin Lewis Agar BiPlates.

MATERIALS REQUIRED BUT NOT PROVIDED

Incubator maintaining 35-37°C.
CO_2 environment generation (See PROCEDURE)
Ancillary culture media, reagents and laboratory equipment as required.

PROCEDURE

To optimize recovery from clinical specimens, it is recommended that the media be inoculated as follows:

1. Remove the plate from refrigerated storage and allow to warm to room temperature. Make sure that agar surface is free of excessive moisture which could cause confluent organism growth. Label plate with specimen identification number and inoculation date.
2. Roll the swab directly on section labeled "I" in a large "Z" pattern to give adequate exposure of swab to the medium for transfer of organism.

3. Using a sterile inoculating loop, streak across the "Z" pattern in a zig-zag fashion to obtain isolated colonies. Avoid applying excess pressure to the agar surface during inoculation to prevent gouging and splitting of the agar medium.
4. Repeat steps 1,2, & 3 for the side labeled "II".
5. Incubate the plates at 35°C in an aerobic environment enriched with carbon dioxide for up to 72 hours.
6. Remove the plate from the incubator. (Note: The CO_2 atmosphere within the container will dissipate upon opening it). Examination of culture results prior to detection of organism growth or 72 hours of incubation may be accomplished by 1) examining the plate through the unopened jar or bag or 2) using additional CO2 generating systems or repeating the candle extinction jar procedure after container has been opened.
7. Plates should be examined 72 hours before a negative interpretation can be made.
8. Refer to appropriate sources for identification of isolates.

LIMITATIONS OF THE PROCEDURE

Pathogenic *Neisseria* species, especially *N. gonorrhoeae*, are fastidious organisms that exhibit sensitivity to desiccation and temperature extremes. The ability to detect microorganisms by culture techniques can be affected by the following factors: improper specimen collection, storage and inoculation, initiation of antiinfective therapy prior to specimen collection, improper culture incubation temperatures and atmospheres, improper length of culture incubation, and improper storage and handling of culture media.

Some strains of *N. gonorrhoeae,* inhibited by vancomycin and trimethoprim lactate, have been reported. Certain oxidase positive, gram negative bacilli will grow on selective media and produce colonies resembling *N. gonorrhoeae*. Selective media for pathogenic *Neisseria* may be inhibitory to other pathogenic bacteria, e.g., *Haemophilus.*

REFERENCES

1. Martin, J.E., T.E. Billings, J.F. Hackney, and J.D. Thayer. 1967. Primary isolation of *N. gonorrhoeae* with a new commercial medium. Public Health Rep. 82:361-363.
2. Thayer, J.D. and J.E. Martin. 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis* . Public Health Rep. 81:559-562.
3. Seth, A. 1970. Use of trimethoprim to prevent overgrowth by *Proteus* in the cultivation of *N. gonorrhoeae.* Brit. Journ. of Vener. Dis. 46:201-202.
4. Martin, J.E. Jr., and J.S. Lewis. 1977. Anisomycin: improved antimycotic activity in modified Thayer-Martin Medium. Public Health Lab. 35:53-62.
5. Kellogg, D.S. Jr., K.K. Holmes, and G.A. Hill. 1976. Cumitech 4, Laboratory Diagnosis of Gonorrhea. Coordinating ed., S. Marcus, and J.C. Sherris. American Society for Microbiology, Washington, D.C.
6. Eschenbach, D., H.M. Pollock, J. Schachter. 1983. Cumitech 17, Laboratory Diagnosis of Female Genital Tract Infections. Coordinating ed., S.J. Rubin. American Society for Microbiology, Washington, D.C.

USER QUALITY CONTROL PROCEDURES AND INFORMATION

QUALITY CONTROL

HealthLink recommends the following quality control procedures be performed on each batch (batch = same lot, same shipment) of culture media received in the laboratory:

1. Inspect plates according to instructions contained on the "Quality Control Log Sheet." (See also Section "STORAGE/SHELF LIFE")
2. Peel off the lower portion of a product bag label (Quality Control Certificate) for the lot being accepted into the laboratory and affix it to the Quality Control Log Sheet.
3. Initial and date the Quality Control Log Sheet.
4. This media is not exempt from end user quality control testing relating to media growth characteristics according to NCCLS Document M22A - Quality Assurance for Commercially Prepared Microbiological Media. Quality control organisms may be ordered from HealthLink through an authorized distributor. The organisms are listed in "USER QUALITY CONTROL".

Note: Notify Technical Service immediately if media does not meet the inspection criteria.

TECHNICAL SERVICE

HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To receive additional product information, procedural instructions, QA/QC log sheets, or to have technical questions answered, please call between the hours of 9:00 am to 5:00 pm EST.

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