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.\(^3\) 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.\(^5\)

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.\(^6\)

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
(An 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 15.0
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)
Proteus mirabilis (ATCC 43071)
Staphylococcus epidermidis (ATCC 12228)
Neisseria meningitidis (ATCC 13090)
Neisseria sicca (ATCC 9913)
Candida albicans (ATCC 60193)
Escherichia coli (ATCC 25922)

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 JEMBEC Plates (10 each)
10 CO2 Tablets
10 zip lock bags

MATERIALS REQUIRED BUT NOT PROVIDED
Incubator maintaining 35-37°C.
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 JEMBEC Plate from refrigerated storage and allow to warm to room temperature. Agar surface should be free of excessive moisture which could cause confluent organism growth. Label plate with specimen identification number and inoculation date.
2. Roll the specimen swab, or swab immersed in purulent surfaces. (See Diagram 1).

3. Using a sterile inoculating loop, streak in a zig-zag fashion over swabbed area and continue over the entire plate surface for isolation of colonies. Avoid applying excess pressure to the agar surface during inoculation to prevent gouging and splitting of the agar medium. (See Diagram 2.)

4. Place one CO₂ generating tablet into the Jembec plate well. Close the plate and place into a zip lock bag and seal. Note: Do not add water to the tablet.

5. Incubate the plates at 35-37°C for 18 - 72 hours. Note: Pathogens may be detected as early as 18 hours however, it is recommended that inoculated plates be incubated for a total of 72 hours before a negative interpretation can be made.

6. Remove the JEMBEC plate from the incubator. (Note: The CO₂ atmosphere within the zip lock bag will dissipate upon opening the bag.) 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 bag or 2) using an additional CO₂ tablet upon reincubating the opened and examined culture plate.

7. Refer to microbiological texts for identification of isolates.

LIMITATIONS
Note: 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 antifungal therapy prior to specimen collection, improper culture incubation temperatures and atmospheres, improper length of culture incubation and improper material, over storage and handling of culture media 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.

REFERENCES