BBL™ Martin-Lewis Agar
BBL™ Martin-Lewis Agar - Gono-Pak
BBL™ Martin-Lewis Agar - JEMBEC™

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Martin-Lewis Agar is an enriched medium for the selective isolation of *Neisseria* species.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. For the *Neisseria gonorrhoeae* and *N. meningitidis* strains, 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. For all other organisms, use dilutions containing 10^4–10^5 CFU/0.1 mL and spread-inoculate using a sterile glass spreader.
   b. Incubate plates at 35 ± 2°C in an aerobic atmosphere containing 3–5% 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

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)
Neisseria gonorrhoeae (2 strains)	43070 35201	Colonies small, opaque, grayish-white to colorless, raised, glistening and smooth

*Recommended organism strain for User Quality Control.

NOTE: Must be monitored by users, according to 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

Martin-Lewis 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 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.

Also recommended over earlier formulations is Martin-Lewis Agar, a further modification of the earlier formulations developed for the selective isolation of pathogenic *Neisseria*, which is more inhibitory to gram-positive bacteria and yeasts than Thayer-Martin agars.[12,13] The concentration of vancomycin is increased from 3.0 µg/mL to 4.0 µg/mL for greater inhibition of gram-positive bacteria, and anisomycin is substituted for nystatin for improved inhibition of *Candida albicans*. This organism has been shown to inhibit *N. gonorrhoeae*.[14,15]

Gono-Pak is the name given to a selective medium – resealable polyethylene bag - CO_2 generating tablet system described by Holston, et al. for the isolation of *N. gonorrhoeae*. It was found to be comparable to the candle jar method for the isolation of *N. gonorrhoeae* from clinical specimens.[16,17] The Gono-Pak system obviates the need both for a separate carbon dioxide system

and for transferring the specimen from the transport system to a culture plate. It has been reported to be superior to Transgrow (Modified Thayer-Martin Agar with a CO_2-enriched atmosphere in a bottle) as a transport system.[18]

The JEMBEC™ style plate was developed by John E. Martin, Jr., of the Centers for Disease Control in association with Ames Laboratories and was designed to provide a self-contained CO_2 environment through the use of a CO_2-generating tablet placed in a specially designed well provided in the plate.[19]

The JEMBEC system is recommended for the growth and transportation of *Neisseria gonorrhoeae* and, like the Transgrow System, has the advantage over other transport systems of obviating the necessity of transferring the specimen from the transport system to a culture plate.

VI PRINCIPLES OF THE PROCEDURE

Martin-Lewis 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, anisomycin (V-C-A 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. Anisomycin inhibits yeasts. Trimethoprim inhibits *Proteus*.

In the Gono-Pak system, a tablet consisting of a mixture of citric acid and sodium bicarbonate is activated by the moisture (humidity) produced by the culture medium within the sealed plastic bag and generates CO_2 levels sufficient for the growth of *Neisseria gonorrhoeae* on the selective media provided with the system.[17]

In the JEMBEC system, a tablet consisting of a mixture of citric acid and sodium bicarbonate is placed in a well within the plate and is activated by the moisture (humidity) produced by the culture medium within the sealed plastic bag. The CO_2 levels generated are sufficient for the growth of *Neisseria gonorrhoeae* on the selective media provided with the system.[19]

VII REAGENTS

Martin-Lewis 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-A 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-A Inhibitor

Formula Per One Milliliter Restored Solution

Vancomycin	400 µg
Colistin	750 µg
Anisomycin	2.0 mg

Gono-Pak System

The Gono-Pak system contains Martin-Lewis Agar plates, resealable polyethylene bags and CO_2-generating tablets (sodium bicarbonate and citric acid).

JEMBEC System

In addition to the plated medium, the JEMBEC system consists of a resealable polyethylene bag and a CO_2-generating tablet (sodium bicarbonate and citric acid).

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

If excessive moisture is observed, invert the 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"[20-23] 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.

JEMBEC CO_2 tablets and bags may be stored with the plates at 2–8°C or separately from the plates at ambient room temperature.

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

Do not use JEMBEC bags if perforated or defective or tablets if they appear to be damaged (broken tablet or torn foil).

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[24,25] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Martin-Lewis Agar

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

Test Procedure: Observe aseptic techniques.

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:[26]

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.

   a. With the Gono-Pak System:

      Place inoculated plates in the polyethylene bag provided (one or two plates per bag). Cut off the corner of one foil-wrapped CO_2 tablet to expose the tablet and place in the bag. DO NOT ADD WATER TO THE TABLET.

      To seal the bag, simply press down on the "zipper" at the end of the bag with fingers and slide along to the opposite end. Be sure that the bag is sealed completely. After the bag is sealed, incubate in an inverted position (agar bed up) at 35°C for 18–48 h.[11,27]

      To transport the culture after incubation, place the sealed Gono-Pak system in a suitable mailing or shipping container. Care should be taken to protect the culture from extreme heat or cold and to ensure delivery to the testing laboratory as soon as possible.

   b. With the JEMBEC System:

      With sterile forceps, remove a CO_2-generating tablet from its foil wrapper and place it in the specially designed well in the plate. Place inoculated plates in the polyethylene bag provided (one plate per bag). DO NOT ADD WATER TO THE TABLET. Seal the bag by pressing down on the "zipper" at the end of the bag with fingers and slide along to the opposite end. Be sure that the bag is sealed completely. After the bag is sealed, incubate in an inverted position (agar bed up) at 35°C for 18–48 h.[11,27]

      To transport the culture after incubation, place the sealed JEMBEC system in a suitable mailing or shipping container. Care should be taken to protect the culture from extreme heat or cold and to ensure delivery to the testing laboratory as soon as possible.

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."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Typical colonial morphology on Martin-Lewis 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 vancomycin and trimethoprim lactate have been reported.[28,29]

Some strains of *Capnocytophaga* species may grow on this selective medium when inoculated with oropharyngeal specimens.[30]

While the Gono-Pak and JEMBEC systems are an improvement over previous transport systems, optimum recovery of *N. gonorrhoeae* will be obtained by direct inoculation of media with the specimen and immediate incubation in a CO_2-enriched atmosphere at 35°C.

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[24,25,31-34]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
221557	**BBL™** Martin-Lewis Agar, Pkg. of 20 plates
221558	**BBL™** Martin-Lewis Agar, Ctn. of 100 plates
221793	**BBL™** Martin-Lewis Agar, Pkg. of 20 Gono-Pak plates
221804	**BBL™** Martin-Lewis Agar, Pkg. of 10 JEMBEC™ plates
299602	**BBL™** Martin-Lewis Agar, Pkg. of 10 JEMBEC™ plates with label
299630	**BBL™** Martin-Lewis Agar, Ctn. of 160 JEMBEC™ plates with label

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