BD™ Martin Lewis Agar, Modified

INTENDED USE

BD Martin-Lewis Agar, Modified is an enriched medium for the selective isolation of *Neisseria gonorrhoeae* and *N. meningitidis* from clinical specimens containing mixed flora of bacteria and fungi.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.
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 in use for the isolation of this organism.[2] Later on, several improvements of the medium were made. [3-5]
Martin-Lewis Agar is a modification of an earlier formulation for the selective isolation of pathogenic *Neisseria* (*N. gonorrhoeae* and *N. meningitidis*) which is more inhibitory to gram-positive bacteria and yeasts than Thayer-Martin agars.[6,7] In **BD Martin Lewis Agar, Modified**, amphotericin B is substituted for anisomycin or the earlier used nystatin for improved inhibition of *Candida albicans*. This organism has been shown to inhibit *N. gonorrhoeae*.[8,9]

In **BD Martin-Lewis Agar, Modified** nutrients are provided from the Chocolate II Agar base which contains casein and meat peptones as nitrogen sources, phosphates to maintain pH, and corn starch, to neutralize toxic fatty acids that may be present in the agar. Hemoglobin provides X factor (hemin). **BD IsoVitaleX™ Enrichment** is a defined supplement which provides V factor (nicotinamide adenine dinucleotide, NAD) and vitamins, amino acids, coenzymes, glucose, ferric ion, and other factors which improve the growth of pathogenic *Neisseria*.
This medium contains several antimicrobial agents to suppress the normal flora. Vancomycin is active primarily against gram-positive bacteria. Colistin inhibits gram-negative rods, including *Pseudomonas* species, but is not active against *Proteus* species. Trimethoprim inhibits *Proteus*. Amphotericin B inhibits fungi.

REAGENTS

Formulas* Per Liter Purified Water

BD Martin-Lewis Agar, Modified		BD IsoVitaleX Enrichment	
Pancreatic Digest of Casein	7.5 g	Vitamin B_{12}	0.01 g
Selected Meat Peptone	7.5	L-Glutamine	10.0
Corn Starch	1.0	Adenine	1.0
Dipotassium Phosphate	4.0	Guanine Hydrochloride	0.03
Monopotassium Phosphate	1.0	*p*-Aminobenzoic Acid	0.013
Sodium Chloride	5.0	Nicotinamide Adenine Dinucleotide	0.25
Agar	14.0	Thiamine Pyrophosphate	0.1
Hemoglobin	10.0	Ferric Nitrate	0.02
IsoVitaleX Enrichment	10.0 ml	Thiamine Hydrochloride	0.003
Trimethoprim lactate	0.005 g	Cystein Hydrochloride	25.9
Amphotericin B	0.005	L-Cystine	1.1
Colistin	0.0075	Glucose	100.0
Vancomycin	0.004		
pH 7.2 ± 0.2			

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

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

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates in an aerobic atmosphere enriched with CO_2 for 42 to 48 hours at 35 ± 2°C. Read plates after 18 to 24 and after 42 to 48 h of incubation.

Strains	**Growth Results**
Neisseria gonorrhoeae ATCC™ 43069	Growth fair to excellent
Neisseria meningitidis ATCC 13090	Growth good to excellent
Neisseria sicca ATCC 9913	Inhibition partial to complete
Candida albicans ATCC 60193	Inhibition partial to complete
Escherichia coli ATCC 25922	Inhibition partial to complete
Proteus mirabilis ATCC 43071	Inhibition partial to complete, no swarming
Staphylococcus epidermidis ATCC 12228	Inhibition partial to complete
Uninoculated	Chocolate brown, opaque

PROCEDURE

Material Provided

BD Martin-Lewis Agar, Modified (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This is a selective medium for pathogenic *Neisseria* species, especially for the isolation of *Neisseria gonorrhoeae* and can be used for all types of specimens. Frequent specimens include swabs form the genitourinary tract, the rectum, and the oropharynx (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).[6,10] This medium may also be used for the detection of *Neisseria meningitidis* from specimens containing normal flora, e.g. from nasal swabs from carriers in the epidemiological investigation of an outbreak of bacterial meningitis. It must not be used as the only primary isolation medium for *N. meningitidis* from cerebrospinal fluid, but may be used as an additional isolation medium.

Specimen Collection and Transport

Neisseria gonorrhoeae and *N. meningitidis* are sensitive to adverse environmental conditions. Therefore, appropriate transport media must be used for all specimens suspected to contain pathogenic *Neisseria*. Specimens must be sent to the laboratory as fast as possible and must not be older than 24 hours, even if transport media are used. The optimal transport temperature is 20 to 25° C. Do not refrigerate! [6, 10]

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if

material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. A nonselective chocolate agar plate (e.g. **BD Chocolate Agar (GC Agar with IsoVitaleX)** must also be inoculated to provide an indication of *Neisseria gonorrhoeae* sensitive to the selective ingredients of **BD Martin-Lewis Agar, Modified** [11,12], and for the detection of *N. meningitidis* from cerebrospinal fluid. Additionally, the usual media for aerobic culture must be included if considered necessary to detect other pathogens.
Incubate the inoculated media in an aerobic environment enriched with 5 to 10% carbon dioxide and incubate at 35 ± 2°C for 42 to 48 hours or longer if necessary. Read plates after 18 to 24 and 42 to 48 hours.

Results
Typical colonial morphology on **BD Martin-Lewis Agar, Modified** is as follows:
Neisseria gonorrhoeae: Small grayish to transparent colonies.
Neisseria meningitidis: Medium to large, blue-gray, mucoid.
A presumptive identification may be made by performing a gram stain and an oxidase test.[6]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
This medium is used for the selective isolation of pathogenic *Neisseria* spp. from all specimens containing contaminating flora and for the differentiation of *Neisseria gonorrhoeae* and *N. meningitidis* from other *Neisseria* species which are usually inhibited on this medium.
The existence of strains of *N. gonorrhoeae* inhibited by vancomycin and trimethoprim lactate has been reported.[11,12] Therefore, a nonselective chocolate agar plate (e.g. **BD Chocolate Agar (GC Agar with IsoVitaleX)** must also be inoculated. If a high incidence of vancomycin-sensitive strains is known in a certain patient population, **BD GC-Lect™Agar** may be used instead of **BD Martin-Lewis Agar, Modified**.[13]
Do not use this medium as the only isolation medium for *N. meningitidis* from specimens from primarily sterile body sites, such as cerebrospinal fluid. Always include a non-selective chocolate agar plate (see above).
Neisseria lactamica may grow on this medium and may resemble *N. meningitidis*.[6]
Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification. Consult appropriate references.[6]
Strains of *Capnocytophaga* species may grow on this medium when inoculated with oropharyngeal specimens.[13]

REFERENCES

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PACKAGING/AVAILABILITY

BD Martin-Lewis Agar, Modified

Cat. No. 254029 Ready-to-use plated media, 20 plates

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

http://www.bd.com
http://www.bd.com/europe/regulatory/