BD™ Mueller Hinton Chocolate Agar

INTENDED USE

BD Mueller Hinton Chocolate Agar is used for the isolation and cultivation of fastidious bacteria from clinical specimens. It may also be used for the susceptibility testing of *Neisseria gonorrhoeae.*

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Because clinical microbiology laboratories in the early 1960s were using a wide variety of procedures for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents, Bauer, Kirby and others developed a standardized procedure in which Mueller Hinton Agar, a medium originally devised for the isolation of gonococci, was selected as the test medium.[1-4] A subsequent international collaborative study confirmed the value of Mueller Hinton Agar for this purpose because of the relatively good reproducibility of the medium, the simplicity of its formula, and the wealth of experimental data that had been accumulated using this medium.[5]

According to the CLSI, the recommended medium for disc diffusion susceptibility testing of *Streptococcus pneumoniae* is Mueller Hinton Agar with 5% Sheep Blood. The recommended medium for *Haemophilus influenzae* is Haemophilus Test Medium (HTM) Agar. Interpretive criteria are provided in the CLSI Document M100 (M2), which is included with CLSI Document M2, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard.[6]

The recommended medium for *Neisseria gonorrhoeae* is GC Agar with a defined growth supplement. According to other data, Mueller Hinton Agar with hemin and Iso-VitaleX may be used for the routine susceptibility testing of *N. gonorrhoeae* against penicillin and spectinomycin.[7]

Mueller Hinton Agar when supplemented with heated blood or hemoglobin and growth factors (e.g., **BD IsoVitaleX™**) has been recommended as a nonselective medium for the isolation of *Neisseria* and *Haemophilus*.[8]

In **BD Mueller Hinton Chocolate Agar**, beef extract and casein peptone provide nutrients. Starch absorbs toxic compounds such as fatty acids derived from cotton swabs. Hemoglobin provides the X factor. **BD IsoVitaleX** supplies vitamins and growth factors, including the V factor (=NAD) which is necessary for growth of *Haemophilus influenzae*.

REAGENTS

BD Mueller Hinton Chocolate Agar

Formula* Per Liter Purified Water

Beef Extract	2.0 g
Acid Hydrolysate of Casein	17.5
Starch	1.5
Hemoglobin	10.0
IsoVitaleX	10.0 ml
Agar	17.0 g

pH 7.3 +/- 0.2

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

BD IsoVitalex Enrichment contains the following growth factors (formula* per liter purified water):

Vitamin B_{12}	0.01 g
L-Glutamine	10.0
Adenine	1.0

Guanine Hydrochloride	0.03
p-Aminobenzoic Acid	0.013
Nicotinamide Adenine Dinucleotide (NAD)	0.25
Thiamine Pyrophosphate	0.1
Ferric Nitrate	0.02
Thiamine Hydrochloride	0.003
Cystein Hydrochloride	25.9
L-Cystine	1.1
Glucose	100.0

*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. Excessive shrinkage of this medium due to desiccation may lead to false susceptibility results.

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 at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide. Read plates after 18 to 24 and after 42 to 48 hours of incubation.

Strains	**Growth Results**
Haemophilus influenzae ATCC 10211	Good to excellent growth
Neisseria gonorrhoeae ATCC 43069	Fair to excellent growth
Neisseria meningitidis ATCC 13090	Good to excellent growth
Streptococcus pneumoniae ATCC 6305	Good to excellent growth
Uninoculated	Chocolate brown, opaque, may be slightly inhomogeneous

For inoculation and incubation of susceptibility tests, see **Test Procedure**.

Test strain	**Susceptibility Test Disc**	**Zone size* (mm)**
Neisseria gonorrhoeae ATCC 49226	Penicillin P-10	33 - 40
	Spectinomycin SPT-100	25 - 31

* Zone sizes based on results from at least 3 different lots of **BD Mueller Hinton Chocolate Agar**

PROCEDURE

Materials Provided

BD Mueller Hinton Chocolate Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

BD Mueller Hinton Chocolate Agar can principally be used for all types of specimens from infections suspected to contain fastidious organisms, especially but not only for specimens from

primarily sterile body sites (e.g., cerebrospinal fluid, abcesses) and as a subculture medium from blood cultures. Its main use is for the nonselective isolation of *Neisseria, Haemophilus,* and other bacteria that may not grow on routinely used blood agar media, such as Columbia Agar with 5% Sheep Blood. (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).
The use of this medium for routine susceptibility testing of *Neissseria gonorrhoeae* with penicillin G and spectinomycin requires the use of pure cultures. Do not inoculate with the specimen for direct susceptibility testing!

Specimen Collection and Transport
Neisseria gonorrhoeae, *N. meningitidis*, *Haemophilus*, and other fastidious organisms are sensitive to adverse environmental conditions. Therefore, appropriate transport media must be used for all specimens. 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! [9,10]

Test Procedure
For isolation of fastidious organisms, 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.
If the specimen has been collected from a body site containing normal flora, it should also be inoculated onto appropriate selective media, depending on the pathogenic agent to be isolated. For *Neisseria gonorrhoeae,* a **BD Martin-Lewis Agar, modified** or **BD GC-Lect™ Agar** plate, and for *Haemophilus*, a **BD Chocolate Agar with IsoVitaleX and Bacitracin** plate should be included.
Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide. Read plates after 18 to 24 and after 42 to 48 hours of incubation.

For routine susceptibility testing of *N. gonorrhoeae*, the isolate, which must be a pure culture, is suspended in **BD Trypticase™ Soy Broth** to match the turbidity of the McFarland 0.5 standard. Within 15 min after adjusting the turbidity of the inoculum, immerse a sterile swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streakings to obtain even inoculation.
Apply the discs by means of an antimicrobial disc dispenser, using aseptic precautions. Deposit discs so that the centers are at least 24 mm apart. It is preferable to deposit penicillin discs so that they are not less than 10 mm from the edge of the Petri dish. After discs have been placed on the agar, tamp them with a sterile needle or forceps to make complete contact with the medium surface. This step is not necessary if the discs are deposited using the **BD Sensi-Disc™** 6- or 8-place self-tamping dispenser.
Within 15 minutes after the discs are applied, invert the plates and incubate them in an aerobic atmosphere enriched with 5% carbon dioxide at 35°to 37° C for 20 to 24 hours.

Results
Typical colonial morphology on **BD Mueller Hinton Chocolate Agar** is as follows:

Haemophilus influenzae	Small, moist, pearly with a characteristic "mousy" odor
Neisseria gonorrhoeae	Small, grayish-white to colorless, mucoid
Neisseria meningitidis	Medium to large, blue-gray, mucoid
Streptococcus pneumoniae	Small, flat or larger mucoid greenish colonies, medium surrounding colonies may be greenish

Susceptibility tests: Zones must be read from the top of the plate. Penicillin susceptibility should be confirmed with a beta-lactamase test, e.g. **BD Cefinase™** test.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Mueller Hinton Chocolate Agar is an enriched, non-selective medium on which fastidious and non-fastidious bacteria, including normal flora, will grow. Therefore, it is recommended to inoculate specimens also onto appropriate selective media.
The term “fastidious bacteria” relates to bacteria that do not grow or do not grow well on normally used primary isolation media containing sheep blood, e.g. *Haemophilus,* pathogenic *Neisseria*, and several other organisms. For detailed descriptions of the type of specimens that must be inoculated onto this medium and of the type of organisms for which this medium is used for isolation, consult the references.[10,11]
This medium has not been tested to support growth of nutritionally variant streptococci.

The number and types of bacterial species occurring as infectious agents is very large. Therefore, before this medium is routinely used for rarely isolated or newly described micro-organisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question.
Susceptibility test zone sizes found on this medium are not in complete agreement with those mentioned in CLSI Standard M2[6] which have been recorded from GC Chocolate Agar with a defined growth supplement.

REFERENCES

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PACKAGING/AVAILABILITY

BD Mueller Hinton Chocolate Agar

Cat. No. 254035	Ready-to-use Plated Media, cpu 20
Cat. No. 254082	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

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