



Mueller Hinton Agar + 2% Glucose + Methylene Blue

Medium for susceptibility testing of yeasts.

INTENDED PURPOSE

Mueller Hinton Agar + 2% Glucose + Methylene Blue is a plated medium used for Antifungal Susceptibility Testing of Yeasts with the disk diffusion method.

DESCRIPTION

Mueller Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue dye is the agar medium recommended by CLSI for disk diffusion testing of select *Candida* spp.

TYPICAL FORMULA*

(g/l)

Beef Extract	2.0
Acid Hydrolysate of Casein	17.5
Starch	1.5
Glucose	20.0
Methylene Blue	0.0005
Agar	17.0
Final pH 7.3 ± 0.1 at 25°C	

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

METHOD PRINCIPLE

Acid hydrolysate of casein and beef extract supply amino acids, nitrogen, minerals, vitamins, carbon and other nutrients which support the growth of microorganisms. Starch acts as a protective colloid against toxic molecules which can be present in the medium. Hydrolysis of starch during autoclaving supplies a little amount of glucose, which is a source of energy. Glucose serves as an energy source for fungal cultures while Methylene blue enhances zone edge definition. Agar is the solidifying agent.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Test tubes, inoculating loops, sterile cotton swabs, physiological solution (0.85% (NaCl) saline), 0.5 McFarland turbidity standard, incubator, quality control organisms, antifungal agent disks.

TEST PROCEDURE

Following CLSI guidelines, Mueller Hinton Agar with Glucose-Methylene Blue plates are inoculated with an adjusted colony suspension in 0.85% (NaCl) saline from overnight cultures, adhering to the 15-15-15-minute rule (suspension used within 15 minutes, disks applied within 15 minutes of inoculation, and incubation within 15 minutes of applying the disks). The antifungal disks are applied to the agar surface. Plates are incubated in an inverted position at 35 ± 2°C for 24 hours in ambient air.

For more details, please refer to the current CLSI Disk Diffusion Susceptibility Testing Process for Yeasts.

Note:

1. The disk diffusion susceptibility testing procedure requires the use of pure cultures. Mueller Hinton Agar with Glucose-Methylene Blue must be inoculated with pure calibrated strains obtained by culture on a Petri plate.
2. If excess surface moisture is present, the agar plates should be dried in an incubator or laminar flow hood with the lids ajar until the excess moisture has evaporated (usually 10 to 30 minutes). The surface should be moist, but with no droplets on the agar surface or the Petri plate cover.

INTERPRETING RESULTS

If the plate was satisfactorily streaked and the inoculum was prepared correctly, the resulting inhibition zones will be uniformly circular and there will be a semiconfluent lawn of growth. Read results at 24 hours. If insufficient growth is observed (e.g., pinpoint colonies or a "haze" across the surface) after incubating for 24 hours, read at 48 hours for voriconazole only.

Interpret the zone diameter results by referring to the current CLSI document M60 and report the organism as susceptible, intermediate or resistant to the agents that have been tested.

STORAGE

Store at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration. Avoid quick temperature shifts to prevent condensation.

SHELF LIFE

6 months

QUALITY CONTROL

Appearance of medium: Slightly opalescent, amber to light green.

The agar depth must be approximately 4 mm.

Expected Cultural Response:

		<i>Candida albicans</i> ATCC® 90028	<i>Candida krusei</i> ATCC® 6258	<i>Candida parapsilosis</i> ATCC® 22019	<i>Candida tropicalis</i> ATCC® 750
Antimicrobial Agent	Disk content	Inhibition Zone Diameter (mm)*			
Posaconazole	5 µg	24-34	23-31	25-36	23-33
Voriconazole	1 µg	31-42	16-25	28-37	-

* CLSI Recommended QC Ranges after 24-hour Incubation.

PERFORMANCE CHARACTERISTICS

Performance testing of Mueller Hinton Agar + 2% Glucose + Methylene Blue was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

The performance of the test depends not only on the activity of the disks, but also on other factors such as the use of a standardized inoculum, of appropriate quality control strains, suitable pre-tested culture media and on an adequate storage temperature for the disks.

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

See ordering info below. There may be additional product ref. numbers as well. For an updated listing of available products, visit [liofilchem.com](https://www.liofilchem.com)

Product	Format	Packaging	Ref.
Mueller Hinton Agar + 2% Glucose + Methylene Blue	Plate 90 mm	20 (2 x 10) plates	11510

Revision History

Revision	Release Date	Change Summary
0	2023-09-15	Document creation











This IFU document and the SDS are available from the online Support Center:

[liofilchem.com/ifu-sds](https://www.liofilchem.com/ifu-sds)

References

1. CLSI. Performance Standards for Antifungal Susceptibility Testing of Yeasts. 2nd ed. CLSI supplement M60. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
2. CLSI. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts. 3rd ed. CLSI guideline M44. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
3. Bauer AW, Kirby WM, Sherris JL and Turck M (1966) Am. J. Clin. Pathol., 45:493.
4. Mueller JH and Hinton J (1941) Proc. Soc. Exp. Biol. Med., 48:330.

Table of Symbols

	Batch code
	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult instructions for use
	Do not reuse
	Keep away from sunlight



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