

Mueller Hinton II Agar | Ready-to-use Media

a product by **Biomed MDX**



Rev: 0

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REF FP90M2001

Intended Use:

A medium for drug susceptibility testing of common, rapidly growing bacteria as standardized by the Clinical and Laboratory Standards Institute (CLSI).

Principle of the Procedure:

This medium provides essential nutrients for microbial growth. Casein and beef extract hydrolysates supply amino acids and other nitrogenous compounds. Starch acts as a protective colloid. During autoclaving, a small amount of dextrose is produced from starch hydrolysis, serving as an energy source. Agar solidifies the medium.

Product Summary:

Mueller Hinton II Agar was originally developed for the cultivation of pathogenic *Neisseria*.¹ However, these organisms are now commonly isolated on selective media. Because clinical microbiology laboratories in the early 1960s used a wide variety of procedures for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents, Bauer, Kirby and others developed a standardised procedure in which Mueller Hinton II Agar was selected as the test medium.^{2,3} A subsequent international collaborative study confirmed the value of Mueller Hinton II Agar for this purpose because of the relatively good reproducibility of the medium, the simplicity of its formula, and the wealth of experimental data accumulated using this medium.⁴ The CLSI has written a performance standard for the Bauer-Kirby procedure and this document should be consulted for additional details.⁵ The procedure is recommended for testing rapidly growing aerobic or facultatively anaerobic bacterial pathogens, such as staphylococci, members of the Enterobacteriaceae, aerobic gram-negative rods; e.g., *Pseudomonas spp.* and *Acinetobacter spp.*, enterococci and *Vibrio cholerae*. The procedure is modified for testing fastidious species; i.e., *H. influenzae*, *N. gonorrhoeae* and *S. pneumoniae* and other streptococci. Mueller Hinton II Agar is manufactured to contain low levels of thymine and thymidine,^{6,7} and controlled levels of calcium and magnesium.⁸⁻¹⁰ Thymine and thymidine levels of raw materials are determined using the disc diffusion procedure with trimethoprim-sulfamethoxazole (SXT) discs and *Enterococcus faecalis* ATCC 29212. Calcium and magnesium levels are controlled by testing raw materials and supplementing with sources of calcium and/or magnesium as required to produce correct zone diameters with aminoglycoside antibiotics and *Pseudomonas aeruginosa* ATCC 27853.¹¹

Formulation *per Liter

Beef Extract	2.0g
Acid Hydrolysate of Casein	17.5g
Starch	1.5g
Agar	17.0g

pH 7.3 +/- 0.1

*Adjust and/or supplemental as required to meet performance criteria

Procedure

Materials Provided

90mm Mueller Hinton II Agar.

Materials Required but Not Provided

Ancillary culture media, reagents, and laboratory equipment as required.

Test Procedure

- For routine susceptibility tests, the inoculum may be prepared by making a direct saline or broth suspension of colonies selected from an 18- to 24-h nonselective nutrient agar plate, e.g., blood agar.
- Pick three to five colonies from fresh nonselective agar plate and suspend in the broth. (Colonies should not be more than 24hrs).
- Adjust the suspension to the turbidity of the 0.5 McFarland standard.
- Within 15 minutes, dip the sterile swab into the suspension.
- Inoculate the surface of the plate uniformly on the plate to achieve uniform growth.
- Allow inoculum to be absorbed.
- Place the appropriate discs onto the respective cultures. Positioned the discs in the centers.
- Incubate plates aerobically at $35 \pm 2^{\circ}\text{C}$ within 15 min.
- Observe the plates by measuring the complete zones of inhibition diameters by using a caliper.
- Zone sizes should fall within the ranges of acceptable zone diameter quality control limits specified by the Clinical and Laboratory Standards Institute (CLSI).
- These limits are published in CLSI M100™ Performance Standards for Antimicrobial Susceptibility Testing.

Results

The diameters of the zones of inhibition surrounding the antibiotic discs should be measured and compared to the reference values provided in the Clinical and Laboratory Standards Institute (CLSI) document M100™. The susceptibility of specific organisms can then be categorized as resistant, intermediate, or susceptible.

Quality Control

Inoculate representative samples with the following strains. Incubate the inoculated plates at $35 \pm 2^{\circ}\text{C}$ for 18 to 24 hrs. to allow colonies to develop on the medium.

Strains	ATCC®
<i>Escherichia coli</i>	25922
<i>Pseudomonas aeruginosa</i>	27853
<i>Staphylococcus aureus</i>	25923
<i>Enterococcus faecalis</i>	29212
Uninoculated plate	-

Zone sizes and MIC ranges should fall within the acceptable ranges specified by the ISO/TS 16782 Technical Specification and/or CLSI document M100™. These ranges are periodically updated, so refer to the latest CLSI guidelines for up-to-date recommendations.

Transportation:

Temperature fluctuations may occur during transportation. However, these fluctuations do not affect the performance, quality, or safety of the media.

Storage and Shelf Life:

Upon receipt, store plates at 2 to 8°C, in their original sleeve wrapping until just before 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.

Warning and Precautions:

For in vitro diagnostic use. For Professional Use Only. Do Not Reuse.

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

Limitations of the Procedure

This medium is for laboratory use only and is not intended for the diagnosis of disease or other conditions. Colonies should be identified using appropriate methods.

Reference

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3. Ryan, K.J., F.D. Schoenknecht, and W.M.M. Kirby. 1970. Disc sensitivity testing. *Hospital Practice* 5:91-100.
4. Ericsson, H.M., and J.C. Sherris. 1971. Antibiotic sensitivity testing. Report of an international collaborative study. *Acta Pathol. Microbiol. Scand. Sec. B, Suppl.* 217.
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9. Pollock, H.M., B.H. Minshew, M.A. Kenny, and F.D. Schoenknecht. 1978. Effect of different lots of Mueller-Hinton agar on the interpretation of the gentamicin susceptibility of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 14:360-367.
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11. Thornsberry, C., T.L. Gavan, and E.H. Gerlach. 1977. Cumitech 6, New developments in antimicrobial agent susceptibility testing. Coordinating ed., J.C. Sherris. American Society of Microbiology, Washington, DC.
12. International Organization for Standardization. 2016. Clinical laboratory testing — Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing (ISO/TS 16782:2016).
13. Clinical and Laboratory Standards Institute (CLSI). 2024. Performance Standards for Antimicrobial Susceptibility Testing, 34th ed. CLSI document M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Packaging Symbol

Symbol	Definition
	Catalogue number
	Research Use Only
	Batch code
	Date of manufacture
	Temperature limit
	Use-by date
	Keep away from sunlight
	Do not re-use
	Fragile, handle with care
	Consult instructions for use or consult electronic instructions for use
	Do not use if packaging damaged and consult instructions for use
	Manufacturer

Further Information:

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