Mueller Hinton Fastidious Broth

Liquid medium for MIC determination of fastidious organisms.

INTENDED PURPOSE

Mueller Hinton Fastidious Broth is used for the cultivation and antimicrobial susceptibility testing (AST) of fastidious bacteria isolated from clinical specimens.

This medium is recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for MIC determination by the broth microdilution method of *Streptococcus* spp. (including *S. pneumoniae*), *Haemophilus influenzae, Moraxella catarrhalis, Listeria monocytogenes, Campylobacter jejuni* and *coli, Pasteurella multocida, Corynebacterium* spp., *Aerococcus sanguinicola* and *urinae, Kingella kingae* and several other fastidious organisms.

DESCRIPTION

Mueller Hinton Fastidious Broth (MH-F broth) consists of cation-adjusted MH broth supplemented with 5% lysed horse blood and 20 mg/L β-NAD.

Broth dilution procedures (macrodilution and microdilution), are used to measure quantitatively the *in vitro* activity of an antimicrobial agent against a given bacterial isolate. To perform the test, a series of tubes/wells are prepared with a broth medium to which various concentrations of the antimicrobial agents are added. The tubes/wells are then inoculated with a standardized suspension of the test organism. After incubation at 35 ± 1°C, the tests are examined, and the MIC is determined.

TYPICAL FORMULA* (Per Litre of Purified Water)

Beef Extract	3.0 g
Acid Hydrolysate of Casein	17.5 g
Starch	1.5 g
β-NAD	0.02 g
Lysed Horse Blood	50.0 ml
Final pH 7.3 ± 0.1 at 25°C	

*Formula may be adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Beef extract and acid hydrolysate of casein supply amino acids, nitrogenous, minerals, vitamins, carbon, and other nutrients to support the growth of microorganisms. Starch acts as a protective colloid against toxic substances that may be present in the medium. In addition, hydrolysis of starch during autoclaving provides a small amount of glucose which is a source of energy. Horse blood and β-NAD are a further growth supplementation for fastidious microorganisms.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Test tubes/trays, inoculating loops, swabs, incubator, quality control organisms.

SPECIMENS

The medium is not intended for the direct inoculation of biological samples. It must be inoculated with pure strains obtained by culture on Petri plate.

TEST PROCEDURE

1. Prepare antimicrobial agents in serial two-fold dilutions in MH-F broth.
2. Prepare a standardized suspension of the test organism using either the direct colony suspension or growth method.
3. Add the suitable volume of the adjusted inoculum to each tube/well containing the antimicrobial agent in the dilution series, and mix.
4. Incubate the tubes or trays aerobically at 35 ± 1°C for 16-20 hours.

INTERPRETING RESULTS

After incubation the presence of turbidity indicates growth of the organism. The lowest concentration of antimicrobial agent showing no growth is the MIC of that organism for that agent. Interpret the MIC by referring

to the current EUCAST interpretative criteria, and report the organism as susceptible, intermediate or resistant to the agents that have been tested.

STORAGE

Store at 2-8°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.

SHELF LIFE

4 months.

QUALITY CONTROL

Appearance of Medium: Clear without precipitate, ruby red

Expected Cultural Response:

Control strain		Inoculum	Incubation	Specification
Streptococcus pneumoniae	ATCC® 49619	≤100 CFU	16-20 h/ 35 ± 1°C	Good growth
Haemophilus influenzae	ATCC® 49766			

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of MH-F broth was carried out using the QC strains recommended by EUCAST. Visible turbidity of the broth was observed with all organisms.

LIMITATIONS

The efficacy of this medium has not been established for all microorganisms that might be isolated from clinical specimens. If growth is inadequate, i.e., turbidity that cannot be seen by the naked eye, the MIC values may not be valid. Always include a growth control tube or well that contains the inoculated medium but no antimicrobial agent. If no growth is seen, repeat testing or use an alternative procedure.

In vitro susceptibility of an organism to a specific antimicrobial agent does not mean that it will be effective as a therapeutic agent *in vivo*. Consult appropriate references for details on interpretation of results.

WARNING AND PRECAUTIONS

1) **For *in vitro* diagnostic use (IVD).**
2) **For laboratory professional use only.**
3) Operators must be trained and have certain experience. Please read the instructions carefully before using the product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
4) Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.
5) Do not use if the product or packaging appears to be damaged.
6) Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
7) Handle all specimens as if infectious using safe laboratory procedures. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
8) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
9) Do not mix reagents of different batches. Please use the product within the validity period.
10) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled
11) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
12) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.
13) When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

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 fo symbols at the end of this document.

Product	Format	Packaging	Ref.
Mueller Hinton Fastidious Broth	Tube	20 x 3.6 ml	27507
Mueller Hinton Fastidious Broth	Tube	20 x 11 ml	21105

This IFU document and the SDS are available from the online Support Center:
liofilchem.com/ifu-sds

References

1. Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version 7, January 2022
2. ISO 20776-2:2021. Clinical laboratory testing and *in vitro* diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 2: Evaluation of performance of antimicrobial susceptibility test devices against reference broth micro-dilution
3. EUCAST reading guide for broth microdilution. Version 4.0, 2022. http://www.eucast.org
4. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org
5. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 12.0, 2022. http://www.eucast.org
6. CLSI. Method for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Table of Symbols

LOT	Batch code
REF	Catalogue number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult Instruction For Use
	Do not reuse

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CE IVD