ENGLISH

Cefepime-enmetazobactam FPE 0.004/8-64/8

INTENDED PURPOSE

Cefepime-enmetazobactam FPE 0.004/8-64/8 is an *in vitro* quantitative method for antimicrobial susceptibility of clinical isolates tested on agar media using overnight incubation.

Cefepime-enmetazobactam FPE 0.004/8-64/8 consists of a predefined gradient of cefepime-enmetazobactam used to determine the MIC of the antibiotic against the following microorganisms:

Gram-negative bacteria
Enterobacterales

DESCRIPTION

MTS (MIC Test Strip) are a gradient test used to determine the minimum inhibitory concentration (MIC) of selected organisms to indicate appropriate patient treatment and for identifying resistance patterns. The MIC is the minimum inhibitory concentration of an antimicrobial drug that will inhibit the growth of microbes under standardized *in vitro* conditions.

Cefepime-enmetazobactam FPE 0.004/8-64/8 is made of special high-quality paper impregnated with a predefined concentration gradient of antibiotic across 15 two-fold dilutions like those of a conventional MIC method.

Cefepime concentration ranges from 0.004 to 64 µg/mL, while the concentration of **enmetazobactam** is fixed at 8 µg/ml.

KIT CONTENT

MTS is supplied in 3 different packaging options (no additional reagents are included):

- The 10-test pack contains 10 strips individually packed in desiccant envelops.
- The 30-test pack contains 30 strips individually packed in desiccant envelops.
- The 100-test pack contains 100 strips in a canister with a desiccant built into the lid.

This instruction sheet is available from **www.liofilchem.com/MTS**

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as:

- sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors

- suspension medium - McFarland turbidity standard - agar plate medium (validated by the media manufacturer for use with antimicrobial susceptibility testing, 90- or 150-mm plates)

- forceps - incubator - quality control organisms

Note: The medium to be used as well as the inoculum suspension will depend on the organism under investigation, see the MTS Application Guide for specific recommendations.

PRINCIPLE OF THE METHOD

When MTS is applied onto an inoculated agar surface, the preformed exponential gradient of antimicrobial agent diffuses into the agar for over an hour. After incubation, a symmetrical inhibition ellipse centered along the strip is formed.
The MIC is read directly from the scale in term of µg/mL at the point where the edge of the inhibition ellipse intersects the strip MTS.

SPECIMEN COLLECTION AND PREPARATION

MTS gradient tests are not for use directly with clinical or other specimens. The product is used to indicate appropriate patient treatment against infections caused by microorganisms that can be isolated from clinical samples of adult, juvenile and pediatric patients. There are no different indications for use according to sample source.
The microorganism to be tested must first be isolated on a nonselective culture medium, such as blood agar or tryptic soy agar (TSA). In case of mixed culture, selected colonies should be purified by subculturing. Differential media harboring chromogenic or fluorogenic substrates should not be used for the subculture. It is recommended that cultures be no more than 24 hours old unless additional incubation is required to achieve sufficient growth.

TEST PROCEDURE

Handling

Before using the MTS from an unopened package, visually inspect to ensure the package is intact. Do not use the strips if the package has been damaged. When removed from the refrigerator/freezer, allow the package or storage container to reach room temperature for about 30 minutes. Moisture condensing on the outer surface must evaporate completely before opening the package. Use forceps or a similar device to pick up a strip.

When using MTS from a canister, replace the lid immediately after use and store as outlined under STORAGE.

Inoculum Preparation

Suspend well-isolated colonies from an overnight agar plate into the suspension medium to achieve the recommended McFarland standard. If the inoculum concentration is correct, a confluent lawn of growth will be obtained after incubation. If insufficient growth occurs, the testing should be repeated.
McFarland turbidity standards do not guarantee the correct number of viable cells in the suspension. In order to verify that your procedure gives the correct inoculum density in terms of CFU/mL performing regular colony counts is recommended. An acceptable inoculum should give approximately 1-2 x 10^8 CFU/mL.

Inoculation

Dip a sterile swab in the broth culture or in a diluted form thereof and squeeze it on the wall of the test tube to eliminate excess liquid. Streak the swab over the entire sterile agar surface. Repeat this procedure by streaking 2 more times, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. Allow excess moisture to be absorbed so that the surface is completely dry before applying MTS.

Use well-defined, high-quality media for AST that supports good growth. The brand chosen should have good batch-to-batch reproducibility to ensure that accurate and reliable MIC values are obtained.

The agar medium should have a depth of 4.0 ± 0.5 mm, a pH of 7.3 ± 0.1 and all other quality specifications should be fulfilled. Refer to the media manufacturer's instructions for more information.

Application

Apply the strip to the agar surface with the scale facing upwards and code of the strip to the outside of the plate, pressing it with a sterile forceps on the surface of the agar and ensure that whole length of the antibiotic gradient is in complete contact with the agar surface. Once applied, do not move the strip.

Incubation

Incubate the agar plates in an inverted position at the appropriate temperature, atmosphere and time.

Refer to the MTS Application Guide for specific incubation instructions.

READING THE RESULTS

After the required incubation period, and only when an even lawn of growth is distinctly visible, read the MIC value where the relevant inhibition ellipse intersects the strip. Do not read the plate if the culture appears mixed or if the lawn of growth is too light or too heavy.

For bactericidal drugs, like cefepime-enmetazobactam, read the MIC at the point of complete inhibition of all growth. Haze and macrocolonies or microcolonies within 3 mm from the strip should be read as growth.

Growth along the entire gradient, i.e. no inhibition ellipse, indicates that the value is greater than or equal to (≥) the highest value on the scale. An inhibition ellipse that intersects below the lower end of the scale is read as less than (<) the lowest value. Intersection between two scale segments should be rounded up to the higher value. An MIC of 0.125 µg/mL is considered the same as 0.12 µg/mL for reporting purposes.

NOTES:

- Excessively wet plates prior to inoculation, insufficient drying before applying strips and/or unevenly streaked surfaces may give non confluent growth or jagged ellipse edges. Repeat the test if MIC endpoints are difficult to read. In the case of uneven MIC intersections, read the higher value. Repeat the test if the discrepancy is >1 dilution.
- Occasionally, certain antimicrobial agent/microorganism combinations may give unusual results. In these cases, judgment of the MIC endpoint may be difficult for the inexperienced personnel. However, individuals can be trained through regular use of quality control strains, MTS reading guides and comparison with experienced personnel to correctly assess MIC endpoints.

Procedures specific to Cefepime-enmetazobactam FPE 0.004/8-64/8 are summarized in the following table:

Storage	Temperature at –20°C
Organism	Enterobacterales
Medium	Mueller Hinton Agar
Inoculum	Suspension in saline (0.85% NaCl) to 0.5 McFarland standard (1 if mucoid)
Incubation	Agar plates in inverted position at 35 ± 2°C for 16-20 hours in ambient atmosphere
Reading	Interpret the MIC as 100% inhibition

INTERPRETATION OF THE RESULTS

To categorize the result, typically as susceptible, intermediate or resistant, refer to current MIC breakpoints (below).

Since MTS generates MIC values which fall between two-fold dilutions for interpretation, an MTS MIC value which falls between standard two-fold dilutions must be rounded up to the next standard upper two fold value before categorization. For example a *E. coli* cefepime-enmetazobactam MIC of 0.19 µg/mL is reported as 0.25 µg/mL.

The MIC obtained should be interpreted according to current EUCAST interpretive criteria (see below).

Antimicrobial agent	**Organism**	**EUCAST MIC Criteria (µg/ml)**	
		S≤	**R>**
Cefepime-enmetazobactam	Enterobacterales	4/8	4/8

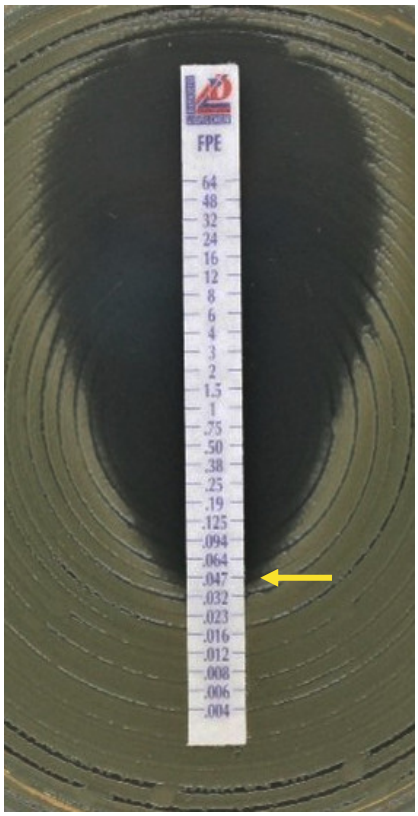
Disclaimer: This breakpoint table might be out-of-date and does not replace EUCAST published guidelines, which always should be consulted before MIC categorization.

NOTES:

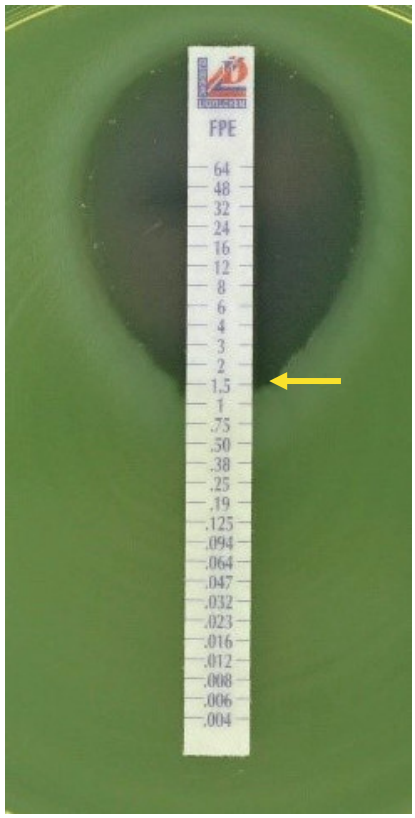
- As with all AST data, MTS results are *in vitro* values only and may provide an indication of the organism's potential *in vivo* susceptibility. The use of results to guide therapy selection must be the sole decision and responsibility of the attending physician. Their judgement should be based on the medical history and knowledge of the patient, pharmacokinetics/pharmacodynamics of the antimicrobial agent, and clinical experience in treating infections caused by the particular microbial pathogen. The drug, dose and dosing regimen must also be considered.
- For details of specific interpretive limitations and/or limitations on the clinical use of an antimicrobial agent in various therapeutic situations, please refer to the tables and footnotes of MIC interpretive standards in the latest CLSI and EUCAST documents.

Cefepime-enmetazobactam FPE 0.004/8-64/8 Reading Guide

Example 1:
E. coli, FPE MIC = 0.047 µg/mL, reported as 0.064 µg/mL

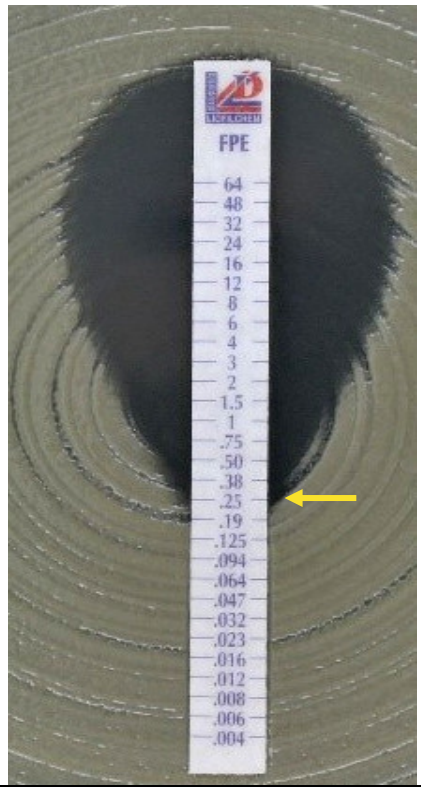


Example 2:
P. aeruginosa, FPE MIC = 1.5 µg/mL, reported as 2 µg/mL



Example 3:
E. coli, FPE MIC 0.023 µg/mL, reported as 0.032 µg/mL

Example 4:
K. pneumoniae, FPE MIC = 0.25 µg/mL



USER QUALITY CONTROL

To check the performance of MTS reagents, media and procedure, test the quality control strain(s) as shown below. Results are considered satisfactory if the quality control result(s) fall within the expected range(s).
Patient isolate results should not be reported if the quality control results are outside of this stated QC range. MIC results for a QC strain that fall a half dilution below the lower QC limit should be rounded up to the next upper two-fold value which would establish QC compliance. MIC results that are a half dilution above the upper limit would be rounded up to the next upper two-fold value which would result in non-QC compliance.

Antimicrobial agent	Control strain		MIC QC range (µg/ml)
Cefepime-enmetazobactam	*Escherichia coli*	ATCC® 25922	0.03/8 – 0.12/8 [a,b]
	Pseudomonas aeruginosa	ATCC® 27853	0.5/8 – 2/8 [a]
	Escherichia coli	ATCC® 35218	0.008/8 – 0.06/8 [a]
	Klebsiella pneumoniae	ATCC® 700603	0.12/8 – 0.5/8 [a]
	Escherichia coli	NCTC 13353	0.03/8 – 0.12/8 [a,b]

a) CLSI M100-Ed34
b) Addendum (May 2024) to EUCAST breakpoint tables v.14.0

PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of Cefepime-enmetazobactam FPE 0.004/8-64/8 was determined by evaluating the agreement of the AST system result with the result generated for the same isolate with the broth microdilution (BMD) reference method. To assess accuracy, Essential Agreement (EA) was calculated. EA occurs when the MIC of the MTS and the reference method agree exactly or is within ± 1 dilution of each other.

Bias

Bias of the method is the evaluation of test device results to determine whether the results that differ from the reference method are significantly skewed or predominantly in one direction.

A total of 300 clinical isolates were tested by three operators. The following table summarizes performance data from these studies.

Antimicrobial agent	Organism	N	% EA	% Bias
Cefepime-enmetazobactam	Enterobacterales	300	97.3	23.7

N, Number of isolates **EA**, Essential Agreement

Reproducibility

100.0 % of Cefepime-enmetazobactam FPE 0.004/8-64/8 results (4 *E. coli* and 3 *P. mirabilis 1 K. pneumoniae, 1 M. morganii* and *1 E. aerogenes* tested in triplicate by 3 operators on 3 days) were within a doubling dilution of reference microdilution results.
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Repeatability

100.0 % of Cefepime-enmetazobactam FPE 0.004/8-64/8 (4 *E. coli* and 3 *P. mirabilis 1 K. pneumoniae, 1 M. morganii* and *1 E. aerogenes* tested in triplicate) were within a doubling dilution of reference microdilution results.

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.

WARNINGS AND PRECAUTIONS

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

STORAGE

Unopened foil packages and canisters: On receipt, store MTS at −20°C to +8°C until the given expiry date. Some MTS (e.g. carbapenems) should be stored frozen at −20°C. Check the drug label for the specific storage temperature.
Opened canisters: MTS in canister can be used for up to 2 months from first opening (record the date on which the canister was open) and must be stored at the label storage temperature. Before using the remaining strips, check the expiry date indicated on the packaging. Do not store near sources of heat and do not expose to excessive temperature variations.
Protect MTS from moisture, heat and direct exposure to strong light at all times.

DISPOSAL OF USED MATERIAL

After use, MTS and material that has come into contact with the sample must be decontaminated and disposed of in accordance with guidelines used in the laboratory for decontamination and disposal of potentially infected material.

SUGGESTIONS FOR TROUBLESHOOTING

For out-of-range QC, first repeat the test with a pure culture or a freshly subcultured QC strain. If the issue is unresolved, follow this guidance for additional suggestions for troubleshooting out-of-range QC results and unusual clinical isolates results.

Observation	Probable Cause	Comments/Suggested Actions
MIC too low	Inoculum too light	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Check steps in inoculum preparation and incubation procedure. Perform colony count check of growth control well immediately after inoculation and before incubation (*E. coli* ATCC® 25922 closely approximates 5 x 10^5 CFU/ml)
MIC too high	Inoculum too heavy	
MIC too high	Antimicrobial agent is degrading	Use alternative lot. Check STORAGE and package integrity

In case of other malfunctions or defects, contact immediately Liofilchem (*) or the local representative.

In case of incident associated with the device, notify immediately Liofilchem (*) or its local representative and the National Competent Authority.

*Please login to https://www.liofilchemstore.it/login.php (user ID and password required) and click on Complaint.

REFERENCES

1. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2024.
2. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 12th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2024.
3. CLSI. Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st ed. CLSI guideline M52. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
4. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. 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 14.0, 2024. http://www.eucast.org.
6. ISO 20776-1:2019. Clinical laboratory testing and *in vitro* diagnostic test systems -- Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices -- Part 1: Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious disease.
7. 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

A Summary of Safety and Performance (SSP) will be available on Eudamed (subject to Eudamed availability).
This summary is also available on request at liofilchem@liofilchem.com

Product	µg/mL	Code	Packaging	Ref.
Cefepime-enmetazobactam	0.004/8 – 64/8	FPE	10	920981
			30	92098
			100	920980

CE 0123 IVD

Table of Symbols

IVD	*In Vitro* Diagnostic Medical Device
REF	Catalogue number
LOT	Batch code
	Do not reuse
CE 0123	Identification number of notified body
	Manufacturer
	Use by
Σ	Contains sufficient for <n> tests
	Consult instructions for use
	Upper limit of temperature

Revision History

Revision	Release Date	Change Summary
0	26 Sep 2024	Document creation

This document is also available from the online Support Center: **liofilchem.com/ifu-sds**

For other language translations, please contact your local Liofilchem representative or **liofilchem.com**

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CE IVD