MTS™ Piperacillin-tazobactam 0.016/4-256/4

Technical Sheet

INDICATIONS FOR USE/INTENDED USE

The MTS™ (MIC Test Strip) Piperacillin-tazobactam TZP 0.016/4-256/4 is a quantitative method intended for the *in vitro* determination of antimicrobial susceptibility of bacteria. MTS™ consists of specialized paper impregnated with a pre-defined concentration gradient of an antimicrobial agent, which is used to determine the minimum inhibitory concentration (MIC) in µg/mL of antimicrobial agents against bacteria as tested on agar media using overnight incubation and manual reading procedures.

Piperacillin/tazobactam has been shown to be active both clinically and *in vitro* against the non-fastidious aerobic organisms listed below according to the EMA or FDA label for this antimicrobial agent.

MTS™ TZP 0.016/4-256/4 generates a stable concentration gradient for piperacillin (0.016-256 µg/mL) in the presence of a fixed concentration of tazobactam (4 µg/mL). It can be used to determine the MIC of Piperacillin-tazobactam against the following microorganisms:

Gram-negative bacteria
Enterobacterales
Pseudomonas aeruginosa
Acinetobacter spp.

DIRECTIONS FOR USE

Storage

Unopened foil packages and canisters: On receipt, store MTS™ TZP 0.016/4-256/4 at –20°C to +8°C until the given expiry date.

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.

Handling

Before using 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 or 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.

Materials Required but Not Provided:

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

  (see the guide below for specific instructions)
- Sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors
- Forceps
- Incubator (35 ± 2°C)
- Quality control organisms
- Additional technical information from **www.liofilchem.com**

Inoculm Preparation

Suspend well-isolated colonies from an overnight agar plate into the suspension medium to achieve the turbidity of 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. 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™.

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.

Application Guide for MTS™ TZP 0.016/4-256/4	
Organism	*Enterobacterales, Pseudomonas aeruginosa, Acinetobacter baumannii*
Medium	Mueller Hinton Agar
Inoculum	Suspension in saline (0.85% NaCl) to 0.5 McFarland (1 if mucoid)
Incubation	Agar plates in inverted position at 35 ± 2°C for 16-20 hours (20-24 h for *A. baumannii*) in ambient atmosphere

Reading the MIC

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 piperacillin-tazobactam, read the MIC endpoint at complete inhibition of 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.

Results Interpretation
To categorize the result, typically as susceptible, intermediate or resistant, refer to current MIC breakpoints published by the CLSI, EUCAST and/or your national reference group (MIC interpretative criteria for defining categories are shown below). Always round up MTS™ half dilution values to the next upper two-fold value before categorization. For example a *E. coli* Piperacillin-tazobactam MIC of 0.75 µg/mL is reported as 1 µg/mL (see reading guide section for example pictures).

Eliminating Used Material
After use, MTS™ and the material that comes into contact with the sample must be decontaminated and disposed of in accordance with current laboratory techniques for the decontamination and disposal of potentially infected material.

QUALITY CONTROL

Quality control strains recommended by CLSI and EUCAST are used according to the method as outlined under DIRECTIONS FOR USE.

CLSI Interpretation					EUCAST Interpretation			MIC QC Ranges (µg/mL)	
Organism	MIC Criteria (µg/mL)				Organism	MIC Criteria (µg/mL)			
	S ≤	SDD	I	R ≥		S ≤	R >		
Enterobacterales	8	16	-	32	*Enterobacterales*	8	16	*E. coli* ATCC® 25922	1-4
P. aeruginosa	16	-	32-64	128	*Pseudomonas* spp.	0.001[1]	16	*P. aeruginosa* ATCC® 27853	1-8
Acinetobacter spp.	16	-	32-64	128				*E. coli* ATCC® 35218	0.5-2
								K. pneumoniae ATCC® 700603	8-32

(1) For *Pseudomonas* species, the EUCAST susceptible breakpoint for piperacillin-tazobactam (S≤0.001 mg/L) is set to report all wild-type isolates as "susceptible, increased exposure". Therefore, isolates with MICs ≤0.016 (the lowest concentration on the piperacillin-tazobactam MTS) should be interpreted as "susceptible, increased exposure" according to EUCAST breakpoints.

PERFORMANCE CHARACTERISTICS

The performance of MTS™ TZP 0.016/4-256/4 has been established by comparison to the broth microdilution (BMD) reference method following CLSI M07 and ISO 20776-1 standards. Performance was evaluated using the following indices: essential agreement (EA), category agreement (CA), minor error (mE), major error (ME), and very major error (VME).

Organism	N	% Essential Agreement	% Category Agreement (CLSI breakpoints)	% Category Agreement (EUCAST breakpoints)
Enterobacterales	200	96.0	91.0	91.0
Pseudomonas aeruginosa	60	96.7	91.7	95.0
Acinetobacter spp.	20	100	95.0	---

Essential agreement (EA) was defined as agreement between MTS™ and BMD methods ± 1 doubling dilution.

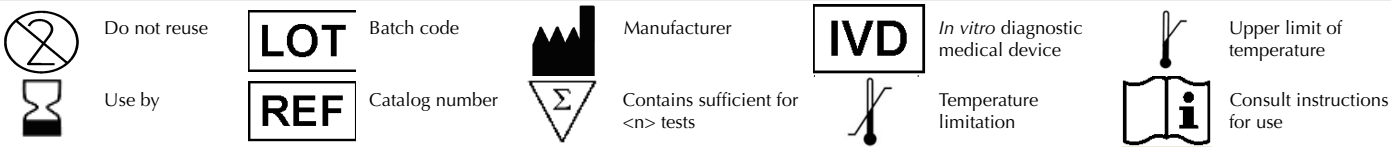
The following errors resulted when MTS™ TZP MICs were 1 doubling dilution apart from the BMD MICs:

- Applying CLSI breakpoints, 1 very major error (VME) and 2 major errors (MEs) were found among the *Enterobacterales* while for *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* 5 and 1 minor errors (mEs), respectively.
- Applying EUCAST breakpoints, 1 VME and 2 MEs were observed among the *Enterobacterales*. For the *P. aeruginosa* isolates, only 3 mEs were found.

REFERENCES

1. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.
2. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org.
3. 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.
4. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
5. ISO 20776-1:2006. Clinical laboratory testing and in vitro diagnostic test systems. Susceptibility testing of infection 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 diseases. ISO, Geneva, Switzerland.

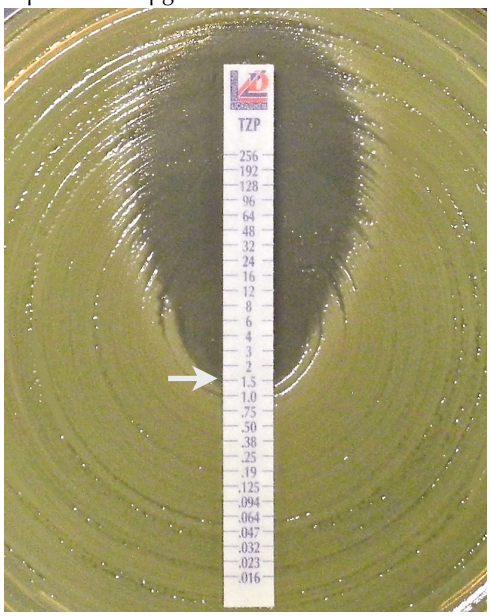
TABLE OF SYMBOLS



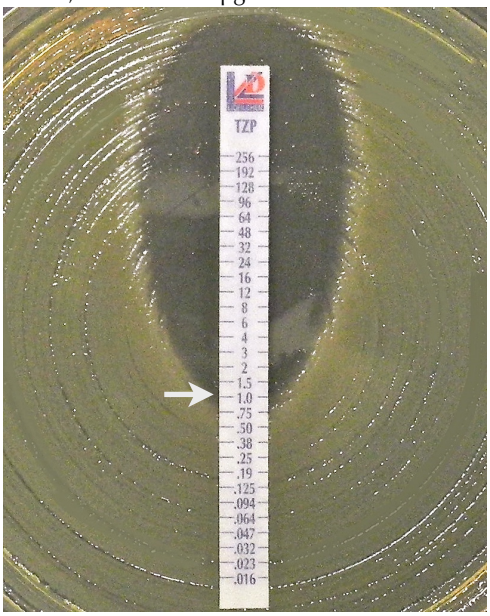
MTS™ Piperacillin-tazobactam Reading Guide

Note: Interpret the MIC as 100% inhibition

Example 1:
E. coli, TZP MIC = 1.5 µg/mL,
reported as 2 µg/mL



Example 2:
E. coli, TZP MIC = 1 µg/mL



Example 3:
K. pneumoniae, TZP MIC = 12 µg/mL,
reported as 16 µg/mL

Example 4:
P. aeruginosa, TZP MIC = 3 µg/mL,
reported as 4 µg/mL

Description		µg/mL	Code	Packaging	Ref.
MTS™	Piperacillin-tazobactam	0.016/4 - 256/4	TZP	10 30 100	921081 92108 921080

This document is available from **liofilchem.com/MTS**

LIOFILCHEM® s.r.l.
Via Scozia, 64026 Roseto degli Abruzzi (TE) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.com

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