



SUMMARY AND EXPLANATION OF THE TEST

The Liofilchem[®] MTSTM (MIC Test Strip) are gradient tests used to determine the minimum inhibitory concentration (MIC) of select bacteria to indicate appropriate patient treatment and for identifying resistance patterns. The MIC is the minimum inhibitory concentration of an antibiotic that will inhibit the growth of bacteria under standardized *in vitro* conditions. Broth and agar dilution MIC procedures based on two-fold serial dilutions of antibiotics are the reference methodologies; expected reproducibility of which is within ± 1 two-fold dilution (¹).

PRINCIPLE OF THE METHOD

MTSTM are 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. When the MTSTM 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 terms of μ g/mL at the point where the edge of the inhibition ellipse intersects the strip MTSTM.

REAGENTS

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

- The 10-test box contains 10 strips individually packed in desiccant envelops and an instruction sheet.
- The 30-test box contains 30 strips individually packed in desiccant envelops and an instruction sheet.
- The 100-test box contains 10 desiccant envelops, each containing 10 strips, and an instruction sheet. The 100-test pack also contains a storage tube.

DIRECTIONS FOR USE

Storage

<u>Unopened foil packages</u>: On receipt, store MTSTM at -20° C to $+8^{\circ}$ C until the given expiry date. Some MTSTM (e.g. carbapenems) should be stored frozen at -20° C. Check the drug-specific MTSTM supplement for the specific storage temperature.

<u>Opened foil packages</u>: Leftover MTSTM from an opened foil package (valid for 100 strip pack only, as the 10 and 30 strip packs contain individually packed strips) must be stored at 2-8°C in the airtight tube, containing desiccant, provided in the pack for no more than 7 days. Do not store near sources of heat and do not expose to excessive temperature variations.

Handling

Before using the MTSTM 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, 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.

Precautions

The MTSTM is not classified as being hazardous according to current regulations but fall within the specific field of application where a safety data sheet must be supplied because they can cause phenomena of sensitization in sensitive subjects if they come into contact with the skin. The MTSTM is a disposable product. The MTSTM is only for diagnostic *in vitro* use and is intended for professional use. They must be used in the laboratory by properly trained operators using approved aseptic and safety methods for pathogenic agents.

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

(The medium to be used as well as the inoculum suspension will depend on the organism under investigation, see the MTS[™] Supplement for more information)

- Sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors
- Forceps
- Incubator $(35 \pm 2^{\circ}C)$
- Quality control organisms
- Additional technical information from www.liofilchem.com

Inoculum 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 (a 0.5 McFarland approximately corresponds to 1-2 x 10⁸ CFU/mL for *E. coli*) performing regular colony counts is recommended.

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 MTSTM.

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 drug-specific MTSTM Supplement for specific incubation instructions.

Eliminating Used Material

After use, MTSTM 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.

Reading the MIC

Observe where the relevant inhibition ellipse intersects the strip and read the MIC at complete inhibition (unless otherwise instructed in the drug-specific MTSTM Supplement). Growth along the entire gradient i.e. no inhibition ellipse indicates that the value is greater than or equal to (\geq) 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. An MIC of 0.125 µg/mL is considered the same as 0.12 µg/mL for reporting purposes. See the appropriate MTSTM product supplements for example specific drug/organism photographs. Also consult the MTSTM Photographic Guide.

Result Interpretation

To categorize the result according to the interpretive criteria, refer to the appropriate MTS^{TM} product supplement for the specific antimicrobial agent interpretive criteria. Since MTS^{TM} generates MIC values which fall between two-fold dilutions for interpretation, an MTS^{TM} 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 *S. aureus* vancomycin MIC of 1.5 µg/mL is reported as 2 µg/mL.

QUALITY CONTROL

To check the performance of the MTSTM result, test the quality control strain(s) as shown in the appropriate MTSTM product supplement. Patient isolate 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.

LIMITATIONS

Refer to the drug-specific MTS™ Supplement.

EXPECTED VALUES

Expected results for susceptibility tests will vary based on location and institution. Organism resistance patterns will be directly related to the population of organisms at each site.

PERFORMANCE CHARACTERISTICS

Refer to the drug specific MTS[™] Supplement.

REFERENCES

1. 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.

GLOSSARY OF TERMS



MTSTM (MIC Test Strip), International Patent

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For all inquiries please fill out the form at https://www.liofilchem.com/en-us/contacts.html

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Indications for Use/Intended Use

The Liofilchem[®] MTSTM(MIC Test Strip) is a quantitative method intended for the *in vitro* determination of antimicrobial susceptibility of bacteria. MTSTM 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.

The MTSTM Plazomicin at concentrations of 0.016 – 256 μ g/mL should be interpreted at 16-20 hours of incubation.

MTS™ PLZ can be used to determine the MIC of plazomicin against the microorganisms listed in the table below:

Plazomicin Activity According to the FDA Label			
Clinical and in vitro	<i>in vitro</i> only		
Enterobacter cloacae	Citrobacter freundii		
Escherichia coli	Citrobacter koseri		
Klebsiella pneumoniae	Klebsiella (Enterobacter) aerogenes		
Proteus mirabilis	Klebsiella oxytoca		
	Morganella morganii		
	Proteus vulgaris		
	Providencia stuartii		
	Serratia marcescens		

Specifications

Antibiotic code: PLZ MIC range: 0.016-256 µg/mL Antibiotic group: Aminoglycoside

Directions for Use

Reading

Follow the MTS[™] package insert instructions. Procedures specific to MTS[™] Plazomicin:

Storage	Temperature between -20°C and +8°C
Organism	Enterobacteriaceae
Medium	Mueller Hinton Agar
Inoculum	Suspension in saline (0.85% NaCl) to 0.5 McFarland standard
Incubation	Agar plates in inverted position at $35 \pm 2^{\circ}$ C for 16-20 hours in ambient atmosphere

FDA plazomicin interpretive criteria (µg/mL)

100% inhibition

Use the following breakpoints to categorize the result according to the interpretive criteria (i.e. susceptible or resistant). An MTSTM 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 *K. pneumoniae* plazomicin MIC of 0.19 µg/mL is reported as 0.25 µg/mL (see reading guide for example pictures).

Bacterial Species	Susceptible	Intermediate	Resistant	
Enterobacteriaceae	<2	4	>8	

US FDA Ref: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210303Orig1s000lbl.pdf

Quality Control range (μg/mL) (CLSI M100S Performance Standards for Antimicrobial Susceptibility Testing, 28th Edition) To check the performance of the MTSTM Plazomicin, media and procedure, test *E. coli* ATCC 259225 and *P. aeruginosa* ATCC 27853 according to the method as outlined in the MTSTM package insert. Results are considered satisfactory if they fall within the following ranges:

Quality Control StrainAcceptable MIC Range (µg/mL)Escherichia coli, ATCC® 259220.25 – 2	
Escherichia coli, ATCC [®] 25922 0.25 – 2	
Pseudomonas aeruginosa, ATCC [®] 27853 1 – 4	
Performance Characteristics	
N Agreement Agreement	
Enterobacteriaceae ² 538 97.0 96.1	

¹ For the plate inoculation procedure, one testing site utilized a plate rotator (Retro C80) to assist even distribution of inoculum. There was no difference in performance for the site using the plate rotator as compared to sites using the manual plate inoculation method.

² MTSTM Plazomicin MIC values tend to be in exact agreement or at least one double dilution lower when testing *C. freundii, C. koseri, K. aerogenes, E. coli, M. morganii, P. stuartii, P. vulgaris* and *S. marcescens* compared to the CLSI reference broth microdilution.

<u>Reproducibility</u>

99.6% of MTSTM Plazomicin results for non-fastidious Gram-negative bacteria (3 *E. coli,* 3 *K. pneumoniae,* 2 *E. cloacae,* 1 *P. mirabilis* and 1 *P. vulgaris* tested in triplicate at 3 sites on 3 days) were within a doubling dilution of reference broth microdilution results.

Limitations

The ability of MTS™ Plazomicin to detect resistant isolates with the following drug/bacterial species combinations is unknown because resistant isolates were either not available or an insufficient number was encountered at the time of comparative testing:

Plazomicin: Citrobacter freundii, Citrobacter koseri, Klebsiella (Enterobacter) aerogenes, Klebsiella oxytoca, Providencia stuartii, Proteus vulgaris, Serratia marcescens

Characterization of 16S rRNA methyltransferases, aminoglycoside modifying enzymes (AMEs), altered efflux and loss of outer membrane porins was not available for organisms at the time of comparative testing, and therefore the performance of MTS^{TM} Plazomicin for non-fastidious Gram-negative bacilli with these resistance mechanisms is unknown for the following:

Enterobacteriaceae

The safety and efficacy of plazomicin in treating clinical infections due to Gram-negative organisms other than *E. coli, K. pneumoniae, E. cloacae* and *P. mirabilis* may not have been established in adequate and well-controlled clinical trials. The clinical significance of susceptibility information in such instances is unknown.

MTS[™] Plazomicin Reading Guide

Note: Read the MIC at complete inhibition

Example 1: *E. coli,* plazomicin MIC = 0.5 μg/mL



Example 3:

E. cloacae, plazomicin MIC = 0.75 μg/mL, reported as 1 μg/mL



Example 2: *K. pneumoniae*, plazomicin MIC = $0.38 \mu g/mL$, reported as $0.5 \mu g/mL$



Example 4: *C. freundii*, plazomicin MIC = 0.38 µg/mL, reported as 0.5 µg/mL



PRESENTAT	ION	μg/mL	Code	Packaging	Ref.
MTSTM	Plazomicin	0.016 - 256	PLZ	10 30	920701 92070
				100	920700

MTS™ (MIC Test Strip), International Patent

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