Liofilchem® MTS™

SUMMARY AND EXPLANATION OF THE TEST

The Liofilchem® MTS™ (MIC Test Strip) are gradient tests used to determine the minimum inhibitory concentration (MIC) of select 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. 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.

PRINCIPLE OF THE METHOD

MTS™ 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 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 terms of µg/mL at the point where the edge of the inhibition ellipse intersects the strip.

For detection of resistance mechanisms such as extended-spectrum beta-lactamase (ESBL) and carbapenemase, double-sided gradient MTS™ carrier the appropriate diagnostic reagents. Resistant bacteria are identified by comparing the inhibition on both sides of the strip.

REAGENTS

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

DIRECTIONS FOR USE

Storage

Unopened foil packages: 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. Products can always be stored lower than the maximum temperature specified.

Opened foil packages: Leftover MTS™ 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 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.

Precautions

The MTS™ is not classified as being hazardous according to current regulations. The MTS™ is a disposable product. The MTS™ 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

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.

- Sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors
- Forceps
- Incubator (35 ± 2°C)
- Quality control organisms (CultiControl™)
- 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 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 no 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 that supports good growth. The brand chosen should have good batch-to-batch reproducibility to ensure that accurate and reliable MIC values are obtained.

Ensure that the agar plate has depth of 4.0 ± 0.5 mm, pH 7.3 ± 0.1 and fulfills quality specifications.

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.

MTS™ testing conditions for most common organisms are shown in the following guide. For further information on specific applications, please consult MTS™ documents available at www.liofilchem.com/MTS

Organism group	Agar media	Inoculum		Incubation		
		Suspension	Turbidity	Temperature	Atmosphere [6]	Time [8]
Aerobes	Mueller Hinton [2, 3, 4, 5]	0.85% NaCl	0.5 McFarland (1 if mucoid)	35 ± 2°C	ambient	16-20 hours [9]
ORSA/ORSE	Mueller Hinton + 2% NaCl (MTS™ oxacillin only)	0.85% NaCl	0.5 McFarland	35 ± 2°C	ambient	24 hours ORSA 48 hours ORSE
Anaerobes	Brucella Blood	Brucella broth or Mueller Hinton broth	1 McFarland	35 ± 2°C	80-85 N_2 / 5-10% CO_2 / 10% H_2 [7]	24-48-72 hours depending on the species
Haemophilus influenzae	HTM (CLSI) MH-F (EUCAST)	Mueller Hinton broth or HTM broth	0.5 McFarland (1 if mucoid)	35 ± 2°C	5% CO_2	20-24 hours
***Streptococcus pneumoniae* and Streptococci [1]**	Mueller Hinton + 5% blood (CLSI) MH-F (EUCAST)	Mueller Hinton broth	0.5 McFarland (1 if mucoid)	35 ± 2°C	5% CO_2	20-24 hours
Neisseria gonorrhoeae	GC-agar base + defined supplements	Mueller Hinton broth	0.5 McFarland	35 ± 2°C	5% CO_2	20-24 hours

1 Includes beta-haemolytic Streptococci groups A, B, C and G and Viridans group *S. mutant, S. mitis, S. sanguis* and *S. bovis*.

2 For trimethoprim and trimethoprim/sulfamethoxazole, ensure that the brand and batch of agar has a low thymine/thymidine content to minimise antagonism of the activity of trimethoprim and sulphonamides.

3 The inherent calcium content in Mueller Hinton agar may vary between brands and batch to batch. Perform quality control of agar plates on a batch to batch basis to qualify it for use, particularly for testing of daptomcyin.

4 The inherent manganese content in Mueller Hinton agar may vary between brands and batch to batch. Perform quality control of agar plates on a batch to batch basis to qualify it for use, particularly for testing of tigecycline.

5 The performances of macrolides and aminoglycosides MTS™ with aerobe microorganisms have been validated and are guaranteed with the **Liofilchem** and **BBL/BD** Mueller Hinton II Agar only.

6 The activity of macrolides, lincosamides, streptogramins, aminoglycosides, quinolones, penicillins and tetracyclines can be affected by the pH decrease consequent to the incubation in 5% CO_2 for fastidious organisms. Please be aware that differences in results can be obtained between systems that are incubated in ambient and in CO_2-enriched air.

7 Ensure that an efficient anaerobic system is used to achieve rapid anaerobiosis to avoid false resistant results with metronidazole.

8 Ensure the agar plate is incubated for the recommended period before reading, especially for delayed expression of resistance and slow growing and fastidious organisms.

9 MTS™ vancomycin results are interpreted at 24 hours of incubation for Staphylococci and Enterococci.

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.

NOTES:

- Antimicrobial drugs can be either "-static" (e.g. bacteriostatic, fungistatic) or "-cidal" in their interactions with target organisms and this needs to be considered for determining correctly the MIC endpoint. For bactericidal drugs, e.g. beta-lactams, 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. For bacteriostatic drugs, e.g. trimethoprim-sulfamethoxazole, in case of trailing endpoints, read at 80% inhibition, i.e. the first point of significant inhibition as judged by the naked eye. Consult the MTS30 document for more information.
- 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. See the appropriate MTS™ technical sheets for example specific drug-organism photographs. Also consult the MTS™ Photographic Guide.
- 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.

Result 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. An overview of CLSI and EUCAST interpretative criteria is provided in **Table 1** (online). 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 *S. aureus* vancomycin MIC of 1.5 µg/mL is reported as 2 µg/mL.

For resistance detection tests, which are phenotypic confirmation methods not intended for standard MIC determination, read the MTS™ result according to the specific instructions in the product technical sheet.

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.

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

To check the performance of the MTS™ result, test the quality control strain(s) as shown in **Table 1** (online). 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™ Technical Sheet.

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™ Technical Sheet.

REFERENCES

1. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; latest edition. CLSI supplement M100.
2. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; latest edition. CLSI standard M07.
3. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard, latest edition. CLSI document M11.
4. Clinical and Laboratory Standards Institute. Performance Standards for Antifungal Susceptibility Testing of Yeasts, latest edition. CLSI supplement M60.
5. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; latest edition. CLSI standard M27.
6. Clinical and Laboratory Standards Institute. Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi; latest edition. CLSI supplement M61.
7. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; latest edition. CLSI standard M38.
8. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters; latest version.
9. The European Committee on Antimicrobial Susceptibility Testing. Antifungal Agents. Breakpoint Tables for Interpretation of MICs; latest version. EUCAST documents available at www.eucast.org

GLOSSARY OF TERMS

Do not reuse	LOT	Batch code		Manufacturer	IVD	*In vitro* diagnostic medical device	Upper limit of temperature
Use by	REF	Catalog number	Σ	Contains sufficient for <n> tests		Temperature limitation	Consult instructions for use

For more information on specific applications, drugs and drug-organism combinations, visit:

Liofilchem.com/MTS

MIC Test Strip
International Patent

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CE IVD

In USA, available for products noted as "FDA Cleared" in the MTS™ Catalog.