MTS™ Technical Sheet **MBL**

Imipenem/Imipenem+EDTA (IMI/IMD) and Meropenem/Meropenem+EDTA (MRP/MRD)
For *in vitro* detection of Metallo Beta-Lactamases.

INTENDED USE

The Liofilchem® MTS™ (MIC Test Strip) MBL strips consisting of Imipenem (IMI)/Imipenem+EDTA (IMD) or Meropenem (MRP)/ Meropenem+EDTA (MRD) are designed to detect Metallo Beta-Lactamases (MBL).
Positive phenotypes should be sent to a reference laboratory for confirmation with genotypic methods.

CONTENTS OF THE PACKAGES

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 box also contains a storage tube.

COMPOSITION

MTS™ MBL strips are made of special featured paper carrier.
In the Imipenem/Imipenem+EDTA strips IMI code indicates the imipenem (4-256 µg/mL or 0.125-8 µg/mL) gradient and IMD code indicates the imipenem (1-64 µg/mL or 0.032-2 µg/mL) plus a constant level of EDTA.
In the Meropenem/Meropenem+EDTA strips MRP code indicates the meropenem (0.125-8 µg/mL) gradient and MRD code indicates the meropenem (0.032-2 µg/mL) plus a constant level of EDTA.

GATHERING AND KEEPING SAMPLES

The colonies that are to test are taken up by culture media that have been previously swabbed with the sample under examination. In the case of mixed colonies the bacterial strains must be purified before inoculation.

PRINCIPLE

The test is set up using a standard MTS™ procedure. The presence of MBL is indicated by a reduction of the IMI or MRP value by ≥3 $\log_2$ dilutions in the presence of EDTA or the appearance of a phantom zone or deformation of the IMI or MRP ellipse.

TEST PROCEDURE

Before using MTS™ MBL strips 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 –20°C 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:

- Mueller Hinton II Agar plates (ref. 10031)
- Sterile saline (0.85% NaCl) (ref. 20095)
- Sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors
- Forceps
- 0.5 McFarland turbidity standard (ref. 80400)
- Incubator (35 ± 2°C)
- Quality control organisms
- Additional technical information from **www.liofilchem.com**

Inoculum preparation

Suspend well-isolated colonies from an overnight agar plate into saline to achieve a 0.5 McFarland standard turbidity (1 McFarland if mucoid).
A confluent or almost confluent lawn of growth will be obtained after incubation, if the inoculum is correct.
In order to verify that your procedure gives the correct inoculum density in terms of CFU/mL, 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. Alternatively, use a rotation plater to efficiently streak the inoculum over the agar surface. Allow excess moisture to be absorbed so that the surface is completely dry before applying MTS™ MBL strips.

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 35 ± 2°C for 16-20 hours in ambient atmosphere. Extend the incubation for up to 48 hours in case of slow growing Gram negative non-fermenters.

EVALUATING THE RESULTS

Reading

When bacterial growth is clearly visible, read the IMI or MRP and IMD or MRD values* where the relevant inhibition ellipses intersect the strip.
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 reading scale.
An inhibition ellipse that intersects below the lower end of the scale is read as less than (<) the lowest value.
When mutant colonies are present in the inhibition ellipse, read the values where these colonies are inhibited.
For IMI and MRP values in the high range, inhibition ellipses may be very small or not clearly distinguishable.
Occasionally, an extra zone (phantom zone) may be seen between the IMI/IMD sections or between the MRP/MRD sections.
The IMI/IMD and MRP/MRD inhibition ellipses may also be deformed at the tapering ends.
The presence of a phantom zone or ellipse deformation indicate MBL production and is caused by the EDTA diffusion from the IMD or MRD section to IMI or MRP section, respectively.

* Important: the MTS™ MBL should be used for confirmation of MBL production only and is not intended for the determination of the Minimum Inhibitory Concentration.

Interpretation
Ratio of IMI/IMD or MRP/MRD of ≥8 or ≥3 log_2 dilutions indicates MBL production. Phantom zone or deformation of the ellipse is also positive for MBL regardless of the IMI/IMD or MRP/MRD ratio. Send all MBL positive strains to a reference laboratory for confirmation with genotypic testing.

Examples of how to interpret results and ratios for IMI/IMD and MRP/MRD:

IMI/IMD	128/12 = 10.7	= MBL +	MRP/MRD	4/0.25 = 16	= MBL +
IMI/IMD	>256/<1 = >256	= MBL +	MRP/MRD	>8/0.032 = >250	= MBL +
IMI/IMD	64/<1 = >64	= MBL +	MRP/MRD	2/0.032 = 62.5	= MBL +
IMI/IMD	64/>64 = <1	= MBL -	MRP/MRD	<0.025/<0.032 = 0.78	= MBL -
IMD	>256/>64 or <4/<1	= Non Determinable	MRP/MRD	>8/>2 = 4	= Non Determinable

QUALITY CONTROL

Quality control should be performed as outlined under PROCEDURE to check the quality of MBL strips, Muller Hinton II agar and the procedure used.

For IMI/IMD, *P. aeruginosa* ATCC® 27853 can serve as a negative control and *S. maltophilia* ATCC® 13636 (intrinsic MBL production) as a positive control.

For MRP/MRD, *K. pneumoniae* ATCC® 700603 can serve as a negative control and *K. pneumoniae* ATCC® BAA-2146 as a positive control.

Alternatively, another MBL-positive strain from your laboratory or from an outside reference source could be used as a positive control.

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.

STORAGE

The unopened package of MTS™ MBL should be stored at –20°C until the given expiry date. Leftover strips from an opened package 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.

Description		µg/mL	Code	Packaging	Ref.
MTS™	Imipenem/Imipenem+EDTA	4-256 / 1-64	IMI/IMD	10 30 100	921621 92162 921620
MTS™	Imipenem/Imipenem+EDTA	0.125-8 / 0.032-2	IMI/IMD	10 30 100	921661 92166 921660
MTS™	Meropenem/Meropenem+EDTA	0.125-8 / 0.032-2	MRP/MRD	10 30 100	921651 92165 921650

TABLE OF SYMBOLS

LOT Batch code	IVD *In Vitro* Diagnostic Medical Device	Manufacturer	Use by
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult accompanying documents

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