MIC Test Strip Technical Sheet **MBL**

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

INTENDED USE

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

MIC Test Strip 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 MIC Test Strip procedure. The presence of MBL is indicated by a reduction of the IMI or MRP M.I.C. 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 MIC Test Strip 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

Inoculum preparation

Suspend well-isolated colonies from an overnight agar plate into saline to achieve a 0.5 McFarland standard turbidity (1 Mc-Farland 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 MIC Test Strip MBL strips.

Application

Apply the strip to the agar surface with the M.I.C. 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 M.I.C. values where the relevant inhibition ellipses intersect the strip.
Growth along the entire gradient i.e. no inhibition ellipse indicates that the M.I.C. is > the highest value on the reading scale.
An inhibition ellipse below the gradient indicates a M.I.C. < the lowest value on the scale.
When mutant colonies are present in the inhibition ellipse, read the M.I.C. where these colonies are inhibited.
For IMI and MRP M.I.C. 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.

Interpretation
MIC 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 M.I.C. 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 = <1	= MBL -
IMI/IMD	64/>64 = <1	= MBL -	MRP/MRD	<0.025/<0.032 = 3.9	= MBL -
IMD	>256/>64 or <4/<1	= Non Determinable	MRP/MRD	>8/>2 = >4	= Non Determinable

QUALITY CONTROL

Quality control according to specifications in table 1 should be performed as outlined under PROCEDURE to check the quality of MBL strips, Muller Hinton agar and the procedure used.
P. aeruginosa ATCC® 27853 can serve as a negative control for MBL and be used to check the imipenem or meropenem component on the strip.
There are no commercially available MBL positive control strains, therefore MBL genotype positive reference strain such as *S. maltophilia* ATCC® 13636 (intrinsic MBL production) or one available in your laboratory or from an outside reference source can be used as a positive control.
The bioavailable content of zinc in Mueller Hinton may vary between batches and brands and can affect the M.I.C. values of carbapenems and thus MBL testing.

Table 1. Quality control specifications for MIC Test Strip MBL IMI/IMD and MRP/MRD strips on Mueller Hinton agar plates (ref. 10031)

Strain	M.I.C. (µg/ml)				MBL interpretation
	Imipenem (IMI)	Imipenem + EDTA (IMD)	Meropenem (MRP)	Meropenem + EDTA (MRD)	
P. aeruginosa ATCC® 27853	≤4 [1)]	1-4	0.25-1	0.25-1	Negative
S. maltophilia ATCC® 13636	64-256	1-4	≥8	0.032-0.125	Positive

Note: [1)] M.I.C. value below the strip range.

PRECAUTIONS

The MIC Test Strip cannot be classified as being hazardous according to current legislation but fall within the specific field of application where a safety data sheet must be supplied because they can cause phenomena of sensitisation in sensitive subjects if they come into contact with the skin.
MIC Test Strip are disposable products. MIC Test Strip are only for diagnostic *in vitro* use and are 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

All unopened packages and unused MIC Test Strip MBL strips must be stored at -20°C or the temperature denoted on the package until the given expiry date. Unused strips must be stored in an airtight storage container with color indicating desiccant. The batch number and expiry date should be clearly marked on the package and/or storage container.
Protect MIC Test Strip MBL strips from moisture, heat and direct exposure to strong light at all times.
Prevent moisture from penetrating into or forming within the package or storage container. MIC Test Strip MBL strips must be kept dry.

PRESENTATION

DESCRIPTION		µg/mL	CODE	packaging	REF.
MIC Test Strip	IMIPENEM / IMIPENEM + EDTA	4-256 / 1-64	IMI/IMD	10	921621
				30	92162
				100	921620
MIC Test Strip	IMIPENEM / IMIPENEM + EDTA	0.125-8 / 0.032-2	IMI/IMD	10	921661
				30	92166
				100	921660
MIC Test Strip	MEROPENEM / MEROPENEM + EDTA	0.125-8 / 0.032-2	MRP/MRD	10	921651
				30	92165
				100	921650

TABLE OF SYMBOLS

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

MIC Test Strip, Patent No. 1395483

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Download the video of the MTS Synergy Applicator System procedure: https://dl.dropbox.com/u/46181555/MTS%20crossing%20set.mov

MIC Test Strip Technical Sheet **Synergy Testing**

Specimen
Cystic fibrosis, multiple drug-resistant organisms, extreme drug resistant organisms, critical specimens, critical infections, critical patients, limited therapy options.

Procedure
Medium: See specific organism for appropriate agar media (e.g. MHA/aerobes, RPMI/fungi)
Inoculum: Suspension in saline (or broth) to 0.5 McFarland (ref.80400) or 1 McF (ref.80401) depending on bacteria. Inoculate normally by sterile swab.
Incubation: 35 ± 2 °C (or other) / ambient (or other) / 24-48 hours (or other) depending on the specific organism.
Interpretation of results: Bactericidal drugs: interpret the M.I.C. at complete inhibition of growth including microcolonies, hazes and isolated colonies. For bacteriostatic drugs, read at 80% inhibition when trailing is seen. When bactericidal is combined with bacteriostatic, read each agent according to their specific category.

Literature
MTS Pack insert, product labels, MTS Application Guide, MTS Interpretative Criteria and Quality Control , MTS Technical Sheets.

Definitions
MIC_A MIC of drug A alone
MIC_B MIC of drug B alone
MIC_{AB} MIC of drug A in combination with B
MIC_{BA} MIC of drug B in combination with A

Interpretation
Fractional Inhibitory Concentration Index (FIC Index) calculations:

FIC Index (Fractional Inhibitory Concentration Index) calculations:
FIC Index = $MIC_{AB}/ MIC_A + MIC_{BA}/ MIC_B$
MIC_{AB} = MIC of A in the presence of B; MIC_{BA} = MIC of B in the presence of A.

Interpretation	**FIC**
Synergy	≤ 0.5
Additive	> 0.5 and ≤ 1.0
Indifference	> 1 and ≤ 4.0
Antagonism	> 4.0

Results

	Single drug		Combination		FIC index	Interpretation
	MIC_A	MIC_B	MIC_{AB}	MIC_{BA}		
Strain 1						
Strain 2						

MTS Synergy Applicator System		
Product	**REF**	Σ
MTS Synergy Applicator Platform	96860	1
MTS Synergy Delivery Tool	96870	10 Tests

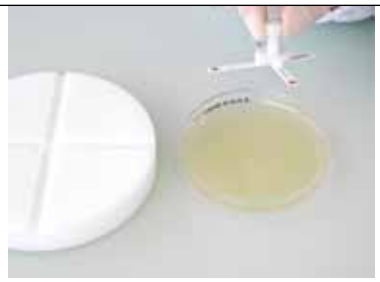
Download the video of the MTS Synergy Applicator System procedure: https://dl.dropbox.com/u/46181555/MTS%20crossing%20set.mov

Method

1. Perform standard M.I.C. of drugs A and B prior to synergy set-up.
2. Use the "MTS Synergy Applicator System"* for the synergy testing.
3. Take a MTS (MIC Test Strip) of the first antibiotic (A) with the tweezers and place it on the MTS Synergy Applicator Platform according to position 1.
4. Adjust the MTS (antibiotic A) such that the the MIC value of the first antibiotic (MIC_A) is positioned at the base intersection.

5. Take a MTS of the second antibiotic (B) with tweezers and place it on the base according to position 2.

6. Adjust the second MTS (antibiotic B) such that the MIC value of the second antibiotic (MIC_B) is positioned at the base intersection and intersects MTS-antibiotic A at its MIC value.



7. Use the MTS Synergy Delivery Tool, press hard onto the two carefully positioned MTS (A and B) and move them to the agar plate.

8. Carefully place the MTS Synergy Delivery Tool (with MTS_A and MTS_B) on the agar.
9. Wait until the strips are completely moistened by surface of the agar.

10. Remove the MTS Synergy Delivery Tool from the agar plate leaving MTS_A and MTS_B positioned at 90°. If necessary, use the tweezers to push the strips onto the agar surface.
11. Finally incubate according to the standard MTS procedure for the specific microorganism.

References

- CLSI M100-S22, 2012. Performance Standards for Antimicrobial Susceptibility Testing.
- CLSI M7-A9, 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.
- EUCAST. Breakpoint tables for interpretation of MICs and zone diameters Version 2.0, January 2012.

* MTS Synergy Applicator System *PATENT PENDING*: A device for standardising the *in-vitro* synergy testing of two antibiotics through the method of crossing the gradient strips. (Liofilchem, 2012).

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