MIC Test Strip Technical Sheet **KPC**

Ertapenem/Ertapenem + Boronic Acid (ETP/EBO) and Meropenem/Meropenem + Boronic Acid (MRP/MBO)
For *in vitro* detection of *Klebsiella pneumoniae* Carbapenemase producing bacteria.

INTENDED USE

MIC Test Strip KPC strips consisting of Ertapenem (ETP)/Ertapenem + Boronic Acid (EBO) or
Meropenem (MRP)/Meropenem + Boronic Acid (MBO) are designed to detect *Klebsiella pneumoniae* Carbapenemase (KPC) producing bacteria. 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 KPC strips are made of special featured paper carrier.
In the Ertapenem/Ertapenem + Boronic Acid strips ETP code indicates the ertapenem (0.125-8 µg/mL) gradient and EBO code indicates the ertapenem (0.032-2 µg/mL) plus a constant level of boronic acid.
In the Meropenem/Meropenem + Boronic Acid strips MRP code indicates the meropenem (0.125-8 µg/mL) gradient and MBO code indicates the meropenem (0.032-2 µg/mL) plus a constant level of boronic acid.

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 KPC is indicated by a reduction of the ETP or MRP M.I.C. by ≥3 $\log_2$ dilutions in the presence of boronic acid or the appearance of a phantom zone or deformation of the ETP or MRP ellipse.

TEST PROCEDURE

Before using MIC Test Strip KPC 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.

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 ETP or MRP and EBO or MBO 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 ETP 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 ETP/EBO sections or between the MRP/MBO sections.
The ETP/EBO and MRP/MBO inhibition ellipses may also be deformed at the tapering ends.
The presence of a phantom zone or ellipse deformation indicate KPC production and is caused by the boronic acid diffusion from the EBO or MBO section to ETP or MRP section, respectively.

Interpretation
MIC ratio of ETP/EBO or MRP/MBO of ≥8 or ≥3 log_2 dilutions indicates KPC production. Phantom zone or deformation of the ellipse is also positive for KPC regardless of the ETP/EBO or MRP/MBO ratio. Send all KPC positive strains to a reference laboratory for confirmation with genotypic testing.

Examples of how to interpret M.I.C. results and ratios for ETP/EBO and MRP/MBO:

ETP or MRP M.I.C. (µg/ml)	EBO or MBO M.I.C. (µg/ml)	ETP/EBO or MRP/MBO	KPC Phenotype
4	0.25	16	+
>8	0.032	>250	+
2	0.25	8	+
3	1	3	–
<0.125	<0.032	<3.9	–
>8	>2	---	Non Determinable

QUALITY CONTROL
Quality control according to specifications in table 1 should be performed as outlined under PROCEDURE to check the quality of KPC strips, Muller Hinton agar and the procedure used.
K. pneumoniae ATCC® BAA-1706 can serve as a negative control for KPC and be used to check the ertapenem or meropenem component on the strip. As a positive control can be used *K. pneumoniae* ATCC® BAA-1705 (intrinsic KPC production) or one available in your laboratory or from an outside reference source.

Table 1. Quality control specifications for MIC Test Strip KPC ETP/EBO and MRP/MBO strips on Mueller Hinton agar plates (ref. 10031)

Strain	M.I.C. (µg/ml)				KPC interpretation
	Ertapenem (ETP)	Ertapenem + Boronic Acid (MBO)	Meropenem (MRP)	Meropenem + Boronic Acid (MBO)	
K. pneumoniae ATCC® BAA-1706	0.25-1.5	0.125-0.5	<0.125	0.032-0.125	Negative
K. pneumoniae ATCC® BAA-1705	4-16	0.032-0.125	4-16	0.016-0.064	Positive

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 KPC 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 KPC 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 KPC strips must be kept dry.

PRESENTATION

DESCRIPTION		µg/mL	CODE	packaging	REF.
MIC Test Strip	ERTAPENEM / ERTAPENEM + BORONIC ACID	0.125-8 / 0.032-2	ETP/EBO	10 30 100	921681 92168 921680
MIC Test Strip	MEROPENEM / MEROPENEM + BORONIC ACID	0.125-8 / 0.032-2	MRP/MBO	10 30 100	921671 92167 921670

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

MIC Test Strip, Patent No. 1395483

LIOFILCHEM® s.r.l.
Via Scozia zona ind.le, 64026 Roseto degli Abruzzi (Te) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.net liofilchem@liofilchem.net

CE IVD

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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

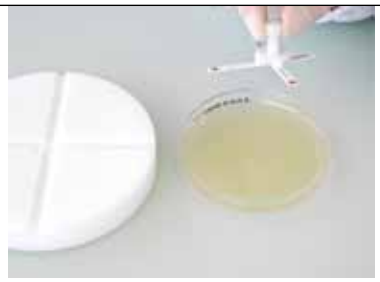
Download the video of the MTS Synergy Applicator System procedure: https://dl.dropbox.com/u/46181555/MTS%20crossing%20set.mov

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).

LIOFILCHEM® s.r.l.

Via Scozia zona ind.le, 64026 Roseto degli Abruzzi (Te) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.net liofilchem@liofilchem.net

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