MIC Test Strip Technical Sheet **KPC**

Ertapenem/Ertapenem+Phenylboronic acid (ETP/EBO) and
Meropenem/Meropenem+Phenylboronic 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+Phenylboronic acid (EBO) or Meropenem (MRP)/Meropenem+Phenylboronic 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+Phenylboronic 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 phenylboronic acid.
In the Meropenem/Meropenem+Phenylboronic 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 phenylboronic 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 value by ≥3 $\log_2$ dilutions in the presence of phenylboronic 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
- Additional technical information from www.liofilchem.net

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 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, MRP, EBO and MBO 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 scale.
An inhibition ellipse that intersects below the lower end of the scale is read as less than (<) the lowest value.
Ignore haze and mutant colonies in the ellipse.
For ETP 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 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 phenylboronic acid diffusion from the EBO or MBO section to ETP or MRP section, respectively.

* Important: the MIC Test Strip KPC should be used for confirmation of KPC production only and is not intended for the determination of the Minimum Inhibitory Concentration.

Interpretation
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 results and ratios for ETP/EBO and MRP/MBO:

ETP or MRP (µg/ml)	EBO or MBO (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 should be performed as outlined under PROCEDURE to check the quality of KPC strips, Muller Hinton II 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.

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
The unopened package of MIC Test Strip KPC 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.

PRESENTATION

DESCRIPTION		µg/mL	Code	Packaging	Ref.
MIC Test Strip	Ertapenem/Ertapenem+Phenylboronic acid	0.125-8 / 0.032-2	ETP/EBO	10 30 100	921681 92168 921680
MIC Test Strip	Meropenem/Meropenem+Phenylboronic 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

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CE IVD