# MIC Test Strip Technical Sheet **AmpC**

Synergic Inhibitory effect of Cloxacillin and Boronic acid

For *in vitro* detection of AmpC β-Lactamase-producing Enterobacteriaceae.

## INTENDED USE

This test is proposed for the phenotypical detection of the AmpC resistance. It can also confirm the suspicion of the AmpC presence combined with porin loss in enterobacterial strains with decreased susceptibility to carbapenems.

- Cefotetan (CTT)/Cefotetan + Cloxacillin (CXT)
- Ertapenem (ETP)/Ertapenem + Cloxacillin (ECX) AND Ertapenem (ETP)/Ertapenem + Boronic acid (EBO)

The first strip is designed to detect AmpC β-Lactamase-producing Enterobacteriaceae while the other two strips have to be used in combination to confirm the suspicion of the contemporary presence of AmpC and porin loss in Enterobacteriaceae with decreased susceptibility to carbapenems. 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 AmpC strips are made of special featured paper carrier.

In the Cefotetan/Cefotetan + Cloxacillin strips CTT code indicates the cefotetan (0.5-32 µg/mL) gradient and CXT code indicates the cefotetan (0.5-32 µg/mL) plus a constant level of cloxacillin.

In the Ertapenem/Ertapenem + Cloxacillin strips ETP code indicates the ertapenem (0.125-8 µg/mL) gradient and ECX code indicates the ertapenem (0.032-2 µg/mL) plus a constant level of cloxacillin.

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.

## BACTERIAL ISOLATES

The test should be performed on pure cultures (or selected subcultures) of the organism to be tested.

## PRINCIPLE

Different Enterobacteriaceae can harborAmpC β-lactamase genes, that can be either chromosomal (*Enterobacter* species, *Citrobacter freundii* etc.), or plasmid-encoded. Strains with AmpC genes can develop resistance during treatment with cephalosporins. The AmpC resistance is characterized by the resistance to 1st, 2nd and 3rd generation cephalosporins (with the partial exception of cefepime). Inhibitors of class A enzymes such as clavulanic acid, sulbactam, and tazobactam have much less effect on AmpC β-lactamases, while they are inhibited by cloxacillin and boronic acid. Of interest, the presence of AmpC enzymes simultaneously with decreased levels of production of outer membrane porins can result in decreased susceptibility to carbapenems.

The tests are set up using standard MIC Test Strip procedure.

- The presence of **AmpC** is indicated by a reduction of the cefotetan M.I.C. by ≥3 $\log_2$ dilutions in the presence of cloxacillin (CXT) or the appearance of a phantom zone or deformation of the CTT ellipse.
- The possible presence of **AmpC and porin loss** is indicated by a reduction of the ertapenem M.I.C. by ≥3 $\log_2$ dilutions in the presence of both cloxacillin (CXT) and boronic acid (EBO) or the appearance of a phantom zone or deformation of the ETP ellipse.

## TEST PROCEDURE

Before using MIC Test Strip AmpC 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.
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 previously prepared suspension 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 16-18 hours in ambient atmosphere.

## EVALUATING THE RESULTS

**Reading**
When bacterial growth is clearly visible, read the M.I.C. values where the relevant inhibition ellipses intersect the strip.
Phantom zone or ellipse deformation may also appear.
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 colonies (heteroresitant strains) are present in the inhibition ellipse, be aware to carefully read the M.I.C. where these colonies are inhibited.
For CTT and ETP M.I.C. values in the high range, inhibition ellipses may be very small or not clearly distinguishable.

**Interpretation**
MIC ratio of CTT/CXT of ≥8 or ≥3 $log_2$ dilutions indicates AmpC production.
MIC ratio of ETP/ECX and ETP/EBO of ≥8 or ≥3 $log_2$ dilutions could indicate AmpC production with porin loss.
Phantom zone or deformation of the ellipse is also interpreted as positive. Indeed, the presence of a phantom zone or ellipse deformation is caused by the cloxacillin diffusion across the strip and indicates synergy of the inhibitor with the cephamycin or carbapenem.
When in a strip both MIC values are above the test ranges the results is indeterminate, as well as if the inhibition ellipse do not close at any level of both part of the strip.
Send all AmpC positive strains to a reference laboratory for confirmation with genotypic testing.

## QUALITY CONTROL

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

- *K. pneumoniae* ATCC® 700603 (ESBL positive ) can serve as a negative control for **AmpC** and can be used to check the cefotetan component on the strip. As a positive control can be used *K. pneumoniae* ATCC® BAA-1144 or one available in your laboratory or from an outside reference source.
- *K. pneumoniae* ATCC® BAA-1706 (KPC negative ) can serve as a negative control for **AmpC combined with decreased porin expression** an can be used to check the ertapenem component on the strips. As a positive control can be used *E. cloacae* CCUG 59627 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

All unopened packages and unused MIC Test Strip AmpC 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 AmpC 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 AmpC strips must be kept dry.

## PRESENTATION

| DESCRIPTION | | µg/mL | CODE | packaging | REF. |
|---|---|---|---|---|---|
| MIC Test Strip | CEFOTETAN / CEFOTETAN + CLOXACILLIN | 0.5-32 / 0.5-32 | CTT/CXT | 10<br>30<br>100 | 921641<br>92164<br>921640 |
| MIC Test Strip | ERTAPENEM / ERTAPENEM + CLOXACILLIN | 0.125-8 / 0.032-2 | ETP/ECX | 10<br>30<br>100 | 921691<br>92169<br>921690 |
| MIC Test Strip | ERTAPENEM / ERTAPENEM + BORONIC ACID | 0.125-8 / 0.032-2 | ETP/EBO | 10<br>30<br>100 | 921681<br>92168<br>921680 |

## 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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