Etest®

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For *in vitro* Detection of MBL (Metallo ß-Lactamases) in Gram-negative bacteria (IP/IPI)

DISCLAIMER (US ONLY) : The contents of this document do not in any way indicate or imply new *in vitro* diagnostic uses of Etest, outside those which are FDA-cleared for certain antibiotics and organism groups.

INTENDED USE

The Etest MBL IP/IPI strip consisting of Imipenem (IP)/ Imipenem + EDTA (IPI) is designed to detect Metallo ß-Lactamases (MBL) in Gram-negative bacteria. Positive phenotypes should be sent to a reference laboratory for confirmation with genotypic methods.

SUMMARY

MBL are class B enzymes whose activity is dependent on divalent cations like zinc or cadmium. Thus, MBL activity can be antagonised by metal chelators such as EDTA. Different MBL genotypes have been identified in various species of bacteria, such as the VIM, IMP and NDM which are being increasingly reported.

PRINCIPLE

The Etest MBL IP/IPI strip (Figure1) consists of a thin, plastic carrier (5 x 60 mm) calibrated with reading scales in µg/mL on one side (a) while the opposite surface carries two predefined gradients (b). IP stands for imipenem (4-256 µg/mL) and IPI imipenem (1-64 µg/mL) plus a constant level of EDTA. The test is set up using a standard Etest procedure. The presence of MBL is reflected by a reduction of the IP Inhibitory Concentration (IC) by ≥ 3 $\log_2$ dilutions in the presence of EDTA or the appearance of a phantom zone or deformation of the IP ellipse (**READING AND INTERPRETATION**, Figures 2, 3 and 4).

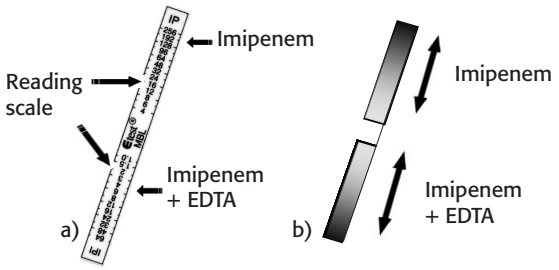

Figure 1. Configuration of Etest MBL IP/IPI strip

REAGENTS

- 30 or 100 Etest strips
- 1 package insert

STORAGE

All unopened packages and unused Etest MBL strips must be stored as indicated on the packaging until the given expiry date. Unused strips must be stored in an airtight storage container with active desiccant. The batch number and expiry date should be clearly marked on the storage container. Protect Etest MBL strips at all times from moisture, heat and direct exposure to strong light.

Prevent moisture from penetrating into or forming within the package or storage container. Etest MBL strips must be kept dry with active dessicant.

HANDLING

When removed from the 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.

Before using Etest strips from the package, visually inspect that the package is intact. Do not use the strips if the package has been damaged.

When handling Etest MBL strips manually, grip only the strip at the area Etest MBL. **Do not touch the surface of the strip with the antibiotic gradient i.e. the side opposite the IC scale**.

PRECAUTIONS AND WARNINGS

- Etest MBL is intended for *in vitro* diagnostic use only.
- Although the procedure is straightforward, proper use of the system requires the judgement of skilled personnel, trained in microbiology and antibiotic susceptibility testing techniques.
- Aseptic procedures should be observed at all times when handling bacterial specimens and established precautions against microbiological hazards strictly adhered to.
- Agar plates should be sterilised after use, before discarding.
- Occasionally, static electricity can cause two or more strips to stick together. Make sure that you separate the strips and apply only one at a time onto the agar surface.
- Because of the instantaneous release of reagents, Etest MBL strips cannot be moved, once in contact with the agar surface.
- Please read the package insert thoroughly before using Etest MBL for the first time.

PROCEDURE

Materials required but not provided

- Mueller Hinton[1)] agar plates (depth of 4.0 ± 0.5 mm)
- Sterile saline (0.85% NaCl)
- Sterile loops, swabs (sterile, non-toxic and not too tightly spun), test tubes, pipettes, scissors, Retro C80™
- Manual applicator or Nema C88™
- 0.5 McFarland turbidity standard
- Incubator (35 ± 2 °C)
- Quality control organisms
- Storage container with active desiccant capsules
- Etest information available at www.biomerieux.com/techlib

IMPORTANT - [1)] The inherent zinc content in Mueller Hinton agar may vary between brands and batch to batch. Perform quality control of agar plates on a batch to batch basis to qualify it for use.

Inoculum preparation

Emulsify well isolated colonies from an overnight agar plate in saline to achieve a turbidity equivalent to 0.5 McFarland standard (1 McFarland if mucoid). When the inoculum is correct, a confluent or almost confluent lawn of growth will be obtained after incubation. Perform regular colony counts **to verify that your procedure gives the correct inoculum density in terms of CFU/mL.**

Inoculation

Dip a sterile, non-toxic and not too tightly spun swab into the inoculum suspension. Remove excess fluid by pressing the swab against the inside wall of the test tube. Swab the entire agar surface three times, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. Alternatively, use Retro C80 (rota-plater, bioMérieux SA) to efficiently streak the inoculum over the agar surface. Allow excess moisture to be absorbed for about 15 to 20 minutes so that the **surface is completely dry before applying the Etest MBL strip.**

Application

Open the Etest package as described under **HANDLING**. With a pair of forceps, grip the strip and place it on a dry clean surface or load a manual applicator tray. Use a manual applicator or Nema C88 to apply the strip on to the inoculated agar surface. Always place the strip on the agar with the reading scale facing upward i.e. towards the opening of the plate, and the reagent side onto the agar surface. If incorrectly placed upside down, no ellipse will form because the reagents cannot diffuse across the non-porous plastic strip. Make sure the whole length of the strip is in complete contact with the agar surface. If necessary, remove air pockets by pressing gently on the strip with forceps, always moving from the minimum concentration upwards. Small bubbles under the strip will not affect results. Once applied, the strip cannot be moved because of the instantaneous release of reagents into the agar.

Incubation

Incubate at 35 ± 2 °C for 16-20 hours in an ambient atmosphere. For slow growing Gram-negative non-fermenters, extend the incubation for up to 48 hours.

READING AND INTERPRETATION

Reading

When bacterial growth is clearly visible, read the IP and IPI IC values[1)] where the respective inhibition ellipses intersect the strip.

Growth along the entire gradient i.e. no inhibition ellipse indicates that the IC is > the highest value on the reading scale. An inhibition ellipse below the gradient indicates an IC < the lowest value on the scale. When mutant colonies are present in the inhibition ellipse, read the IC where these colonies are inhibited.

For IP IC values in the high range, inhibition ellipses may be very small or not clearly discernable. Occasionally, an extra zone (phantom zone) may be seen between the IP/IPI sections (Figure 3). The IP or IPI inhibition ellipses may also be deformed (Figure 4). The presence of a phantom zone or ellipse deformation indicates MBL production and is due to EDTA diffusing across from the IPI section towards IP. Different growth-inhibition patterns are illustrated in Figures 2-4.

[1)] **IMPORTANT:** Etest MBL is primarily a diagnostic tool and imipenem IC values from Etest MBL at the upper and lower ranges may not be substantially equivalent to MICs from a standard Etest Imipenem strip.

Different growth-inhibition patterns:

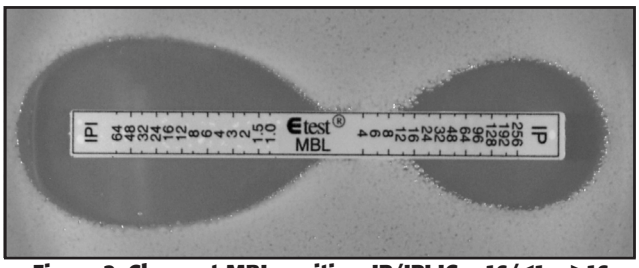

Figure 2. Clear cut MBL positive: IP/IPI IC = 16/<1 = >16

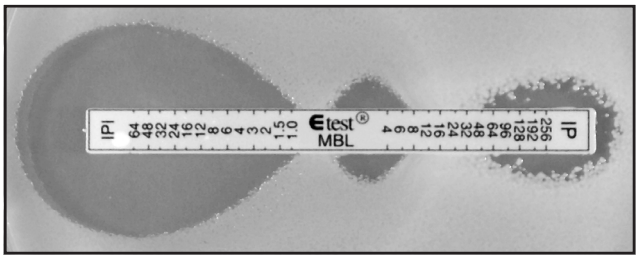

Figure 3. Phantom zone between IP/IPI is indicative of MBL

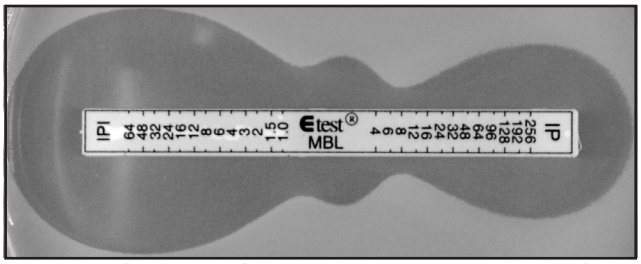

Figure 4. Deformation of the IP or IPI ellipse is indicative of MBL

Interpretation

IC ratio of IP/IPI 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 IP/IPI ratio. Send all MBL positive strains to a reference laboratory for confirmation with genotypic testing.

Examples of how to interpret IC results and ratios:

IP/IPI	128/12 = 10.7	= MBL +
IP/IPI	>256/<1 = >256	= MBL +
IP/IPI	64/<1 = >64	= MBL +
IP/IPI	64/>64 = <1	= MBL -
IP/IPI	>256/>64 or <4/<1	= ND[1)]

Note: [1)] ND = 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 IP/IPI strips, Muller Hinton agar and the procedure used. *P. aeruginosa* ATCC 27853 can serve as a negative control for MBL and *S. maltophilia* ATCC 13636 (intrinsic MBL production) 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. The bioavailable content of zinc in Mueller Hinton may vary between batches and brands and can affect the IC values of carbapenems and thus MBL testing.

Table 1.
Quality control specifications for Etest MBL IP/IPI strip on MHA

Strain	IC (µg/ml) Imipenem (IP)	IC (µg/ml) Imipenem + EDTA (IPI)	MBL Interpretation
P. aeruginosa ATCC® 27853	≤4 [1)]	1-4	Negative
S. maltophilia ATCC 13636	64-256	1-4	Positive

Note: [1)] IC value below the strip range.

PERFORMANCE CHARACTERISTICS

Several *in vitro* studies compared the performance of Etest MBL IP/IPI strip to genotypic methods using characterised MBL-positive strains and negative controls producing other classes of ß-lactamases. Over 300 strains belonging to Gram-negative aerobes and anaerobes were tested. Performance data is summarised in Table 2.

Table 2.
Etest MBL IP/IPI performance compared to genotype methods

Sensitivity[1)] = 96.4% (217/225)	Specificity[2)] = 95.4% (291/305)

Notes:

[1)] Sensitivity = Etest MBL phenotype / reference MBL genotype.
[2)] Specificity = Correct results with Etest / reference results for all strains.

IMPORTANT OBSERVATIONS

1. Strains *of Aeromonas* spp. and *Enterobacteriaceae* producing low basal levels of MBL may not be detected by Etest MBL IP/IPI. Etest MBL MP/MPI may be used in this case for *Enterobacteriaceae*.
2. Strains with non determinable (ND) results should be further investigated.
3. Only Mueller Hinton agar of a suitable brand can be used.
4. Isosensitest or other isotoic media with low zinc levels cannot be used.
5. Etest MBL IP/IPI is for phenotype testing and not for imipenem MIC determinations *per se.*

REFERENCES AND BIBLIOGRAPHY

1. Walsh *et al.* Evaluation of a New Etest for Detecting Metallo-ß-Lactamases in Routine Clinical Testing. JCM, Aug. 2002. p. 2755-2759.
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3. Walsh *et al*. Metallo-ß-lactamases: the quiet before the storm? Clin Microbiol Rev 2005; 18:306-25.
4. Gales *et al.* Emergence of an IMP-like metallo-enzyme in an *Acinetobacter baumannii* clinical strain from a Brazilian teaching hospital. DMID, 45 (2003) 77-79.
5. Edwards *et al*. Assay for metallo beta-lactamases in *B. fragilis*. Br. J. Biomed. Science, vol 55 Sept, 1998.
6. Poirel *et al*. Characterization of VIM-2, a carbapenem-hydrolyzing metallo ß-lactamase and its plasmid- and integron-borne gene from a *P. aeruginosa* clinical isolate in France. Antimicrobial Agents and Chemotherapy., vol. 44, Apr. 2000.

WARRANTY AND DISCLAIMER

EXPRESSED LIMITED WARRANTY AND DISCLAIMER

bioMérieux SA expressly warrants that Etest MBL will detect MBL, if the procedures, precautions and important observations indicated in the package insert are strictly complied with.
bioMérieux SA makes no other warranties, expressed or implied, including the implied warranty of merchantability or fitness for particular purpose.
Any change or modification of the product instructions may affect results. bioMérieux SA shall not be liable for any damages resulting from product tampering, the variance in transportation, stated storage, handling, testing procedures, precautions and other instructions of the most recently revised version of the package insert.

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bioMérieux SA
RCS LYON 673 620 399
69280 Marcy-l'Etoile
France
Tel. 33 (0) 4 78 87 20 00
Fax 33 (0) 4 78 87 20 90
www.biomerieux.com

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