

Principal Etest® MBL

Metallo β -lactamases (MBLs) are Ambler class B enzymes whose activity is dependent on divalent cations like zinc or cadmium, so their activity can be inhibited by metal chelators such as ethylenediaminetetraacetic acid (EDTA).

To detect a MBL using the Etest® MBL (MBL IP/IPI) strips, you compare differential inhibition of a carbapenem alone relative to the same carbapenem plus EDTA in the presence of a MBL.

The Etest MBL is primarily a diagnostic tool and the imipenem MIC values at upper/lower ranges may not be equivalent to an MIC reading of a Etest imipenem strip.

Supply list

- Sterile cotton tipped swabs
- 0.85% physiological saline
- Turbidity meter or prepared 0.5 McFarland Standard
- Sterile loop (5 or 10 μ l) or a sterile swab
- Mueller Hinton agar plate
- Incubator $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- Etest MBL strips
- Positive NDM ATCC BAA-2146 or other known positive strain (18-24 hr culture)
- Negative *P. aeruginosa* ATCC 27853 (18-24 hr culture) or other known negative strain.
- Test organisms: 18-24 hr subculture

Quality Control

Perform quality control with each run.

Day 1 Testing

1. Prepare a 0.5 McFarland dilution of the control and each test organism in saline. Streak a lawn of the suspension for each isolate to a Mueller Hinton agar plate, swab 3 times rotating the plate 60° each time.
2. Allow the surface to dry 3-5 minutes.
3. Place an Etest MBL strip centered across the plate using forceps or Etest grip with the reading scale facing upward.
4. Incubate overnight at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ aerobically 16-24 hours.

Day 2 Reading and interpretation of results

1. After 16-24 hours of incubation, with good growth present, read the IP and IPI values at the inhibition of growth that intersects the strip.
2. Growth along the entire strip, i.e. "no inhibition", indicates an MIC greater than the highest value.
3. Growth inhibition below the lowest gradient MIC indicates a value lower than the reading scale.
4. The MIC ratio of IP/IPI greater than 8 or ≥ 3 log, indicates MBL production.
5. A Phantom zone or deformation of the ellipse indicates MBL production regardless of the IP/IPI ratio.

Different growth-inhibition patterns:

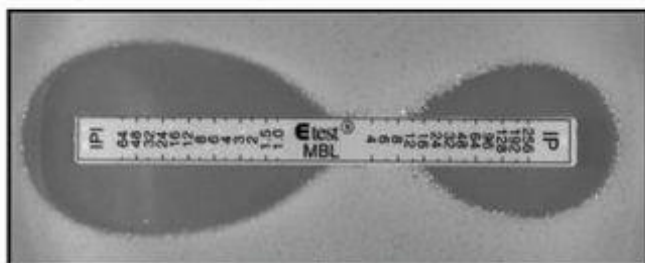


Figure 2. Clear cut MBL positive: IP/IPI IC = 16/<1 = >16

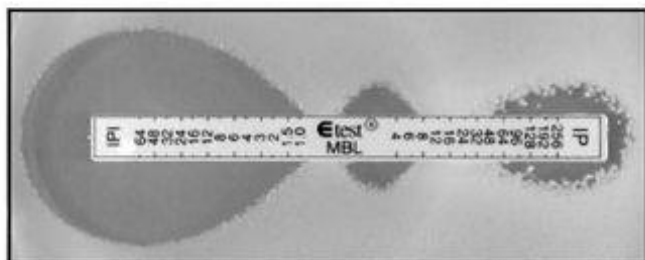


Figure 3. Phantom zone between IP/IPI is indicative of MBL

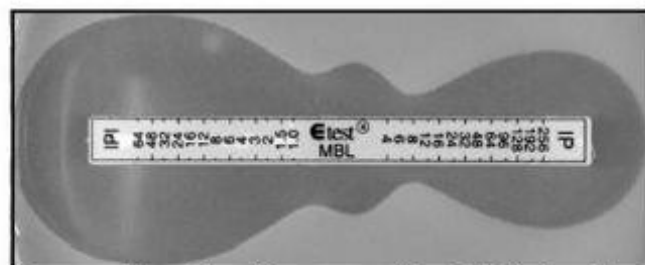


Figure 4. Deformation of the IP or IPI ellipse is indicative of MBL