

Other Methods of Carbapenemase Detection: An Overview

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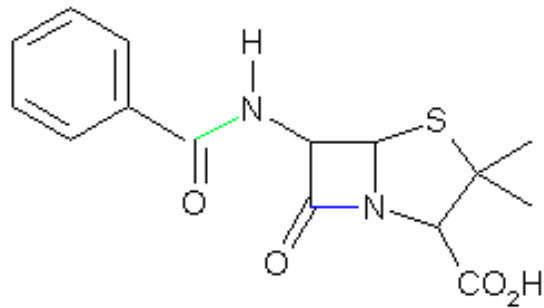
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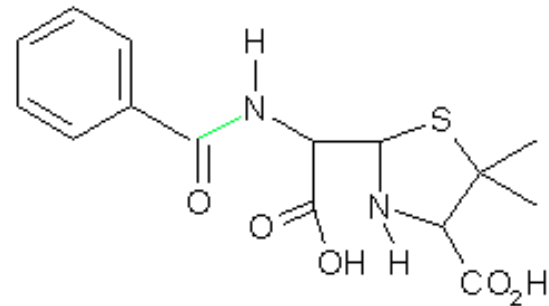
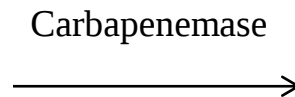
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Carbapenemases



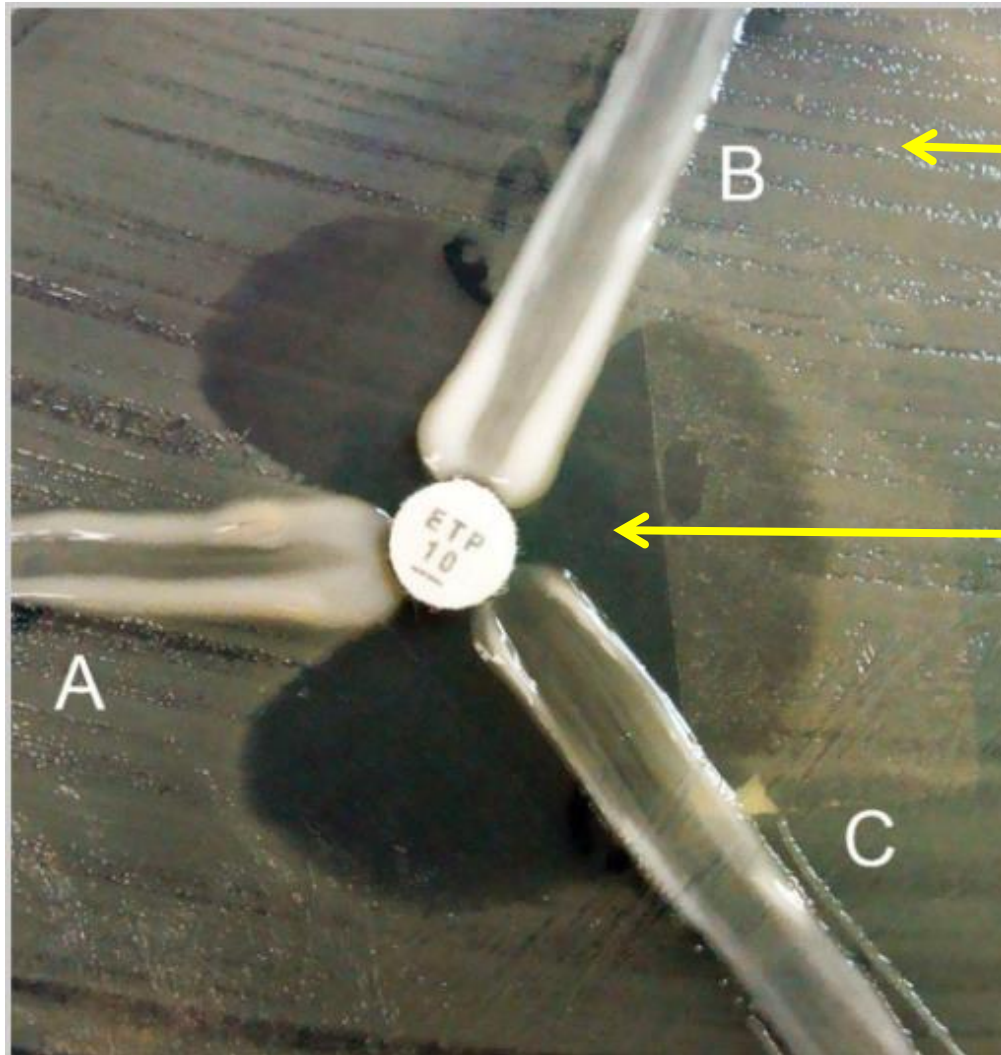
Active Beta-Lactam Ring



Inactive Beta-Lactam Ring
(hydrolyzed)

MODIFIED HODGE TEST

B - Carbapenemase-producing *E. coli* strain



Lawn of Carbapenem-susceptible *E. coli*

Inhibition of Carbapenem-susceptible *E. coli*

C - Carbapenem-resistant, non-carbapenemase producing organism

A - Test Organism

Basic Principle of Modified Hodge

- 1.) Gather materials (reagents, tubes, plates).
- 2.) Prepare a 0.5 McFarland Solution of the *E. coli* carbapenem-susceptible strain (ATCC 25922).
- 3.) Inoculate plate and allow to dry.
- 4.) Place the ertapenem (or meropenem) disk in the middle of the plate.
- 5.) Using a loop, pick 3-5 colonies of test or QC organisms.
- 6.) Inoculate each in a straight line out from the edge of the ertapenem (or meropenem) disk.
- 7.) Incubate at 35°C, ambient air 16-20 hours.
- 8.) Inspect plate and interpret.

Testing Details – Modified Hodge

- **Cost of Test:** ~\$1.00 (@ ISDH)
- **Kit Shelf Life:** ~2-3 months (Mueller-Hinton plate)
- **Time to Test:** 18-24 hours
- **Hands on Time:** ~20 minutes
- **Additional Materials:** Mueller Hinton plate, meropenem or ertapenem disk, Tweezers/Forceps, alcohol pad, McFarland standard/turbidity meter, cotton swabs, tubed saline, control organisms

Limitations of Testing - MHT

- For use only with *Enterobacteriaceae*
- Less sensitive for MBLs
 - M100-S24: “the sensitivity of the test for detecting NDM-type carbapenemases is low (ie, 11%).”
- Some carbapenemase-negative isolates demonstrate a minor indentation (may be more prominent with chromosomal-AmpC producers)

MBL ETEST

Basic Principle of MBL Etest

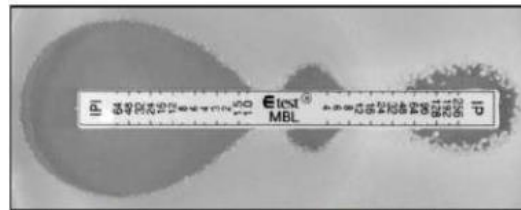
- 1.) Gather materials (reagents, tubes, plates).
- 2.) Prepare a 0.5 McFarland Solution of the test organism.
- 3.) Inoculate plate and allow to dry.
- 4.) Place the etest disk in the middle of the plate.
- 5.) Incubate at 35°C, ambient air 16-18 hours.
- 8.) Inspect plate and interpret.

Interpretation of the MBL Etest

Interpretation	Result
Positive	IP/IPI ratio ≥ 8
	or Phantom zone present
	or Deformed IP or IPI ellipse
Negative	IP/IPI ratio ≤ 8
Indeterminate	IP/IPI $>256/>64$
	or IP/IPI $<4/<1$



Positive



Positive
(Phantom Zone)



Positive
(IPI Ellipse)

Testing Details – MBL

- **Cost of Test:** \$7.66 (@ ISDH)
- **Kit Shelf Life:** ~3 years
- **Time to Test:** 18-24 hours
- **Hands on Time:** ~20 minutes
- **Additional Materials:** Mueller Hinton plate, Etest strip, McFarland Standard/Turbidity meter, Tweezers/Forceps, alcohol pads cotton swab, tubed saline, control organisms

Modified Carbapenemase Inhibition Method (mCIM)

Basic Principle of mCIM

- 1.) Gather materials (swabs, reagents, tubes, plates).
- 2.) Prepare a suspension of the test organism.
- 3.) Add a meropenem disk to the suspension. Incubate for 4 hours, 35°C, ambient air.
- 4.) Prepare a 0.5 McFarland Solution of the *E. coli* carbapenem-susceptible strain (ATCC 25922).
- 5.) Inoculate plate and allow to dry.
- 6.) Remove the meropenem disk and place the in the middle of the prepared plate.
- 7.) Incubate at 35°C, ambient air, 18 hours to overnight.
- 8.) Inspect plate and interpret.



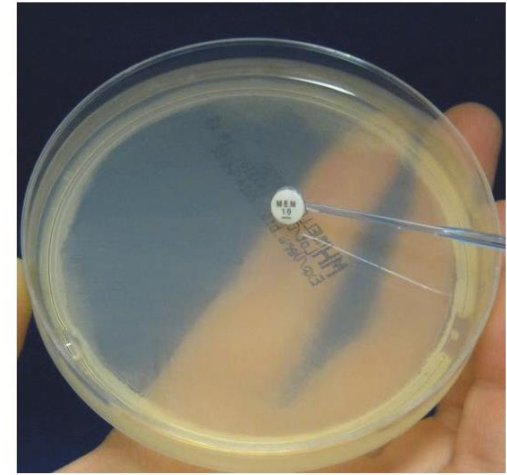
Suspend full loop
of bacteria in TSB



Add 10 μ g
meropenem disk



Incubate for 4
hours 35°C



Place on Mueller Hinton agar
inoculated with *E. coli* ATCC 25922



Incubate for 18-
24 hours 35°C



Positive (+)

Negative (-)

Read presence or absence of inhibition zone

Interpretation: Positive (6-15mm), Negative (≥ 19 mm), Indeterminate (16-18mm)

Testing Details – mCIM

- **Cost of Test:** ~\$1.00 (@ ISDH)
- **Kit Shelf Life:** ~2-3 months (Mueller-Hinton plate)
- **Time to Test:** 18-24 hours
- **Hands on Time:** ~20 minutes
- **Additional Materials:** Mueller Hinton plate, meropenem disk, Tweezers/Forceps, alcohol pad, McFarland standard/turbidity meter, cotton swabs, tubed saline, control organisms

NEXT UP: Break

THEN: Wet Workshop – MHT & mCIM