The Modified Carbapenem Inactivation Method (mCIM)

A test for the phenotypic assessment of carbapenemase activity in the *Enterobacteriaceae*.

Principal

The modified Carbapenem Inactivation Method (mCIM) is designed to rapidly detect carbapenemase production in isolates of *Enterobacteriaceae* (18-24 hours).

An isolate solution is briefly incubated with a carbapenem antibiotic disc, inducing carbapenemase production if present. The disk is then placed on top of a lawn of susceptible *E. coli* and incubated (18-24). Following incubation, presence/absence of a zone of inhibition is evaluated.

Carbapenemase production is detected by when the test isolate produces a zone of inhibition that measures 6-15mm OR if the isolate being tested produces several small distinct colonies of *E. coli* ATCC 25922 growing in the zone of inhibition around the disk within a 16-18 mm zone; carbapenemase production is not detected when a zone of inhibition that measures ≥19 mm; indeterminate carbapenemase production is detected with the test isolate produces a zone of inhibition that measures 16-18 mm.

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

Supply list

1. 1.7 mL tubes, sterile
2. Sterile cotton tipped swabs
3. 2 mL TSB tubes
4. 5 mL of 0.85% physiological saline or Mueller Hinton broth
5. Turbidity meter or prepared to a 0.5 McFarland Standard
6. Pipettor (100-1000 µL)
7. Sterile loop (10 µL)
8. Mueller Hinton agar plate
9. 10 µg meropenem disc
10. *E. coli* ATCC 25922: 18-24hr subculture
11. Test organisms: 18-24hr subculture on SB or MH Agar

Quality Control

Perform quality control testing with each run.
Positive *Klebsiella pneumoniae* ATCC BAA-1705
Negative *Klebsiella pneumoniae* ATCC BAA-1706

Testing

1. Add 1 µl loop-full of test organism to 2 ml of TSB.
   Note: Do not use growth from a medium containing a chromogenic substrate and carbapenem.
2. Vortex the organism-TSB suspension for 10-15 seconds after inoculation.
3. Add a meropenem disc to the suspension.
4. Incubate the suspension and disc at 35°C, for a minimum of 4 hours.
5. Shortly before the end of the 4 hour incubation, follow steps 6 and 7.
6. Prepare a 0.5 McFarland dilution of the *E.coli* ATCC 25922 in 5 mL of saline or broth.
   Streak a lawn of the suspension (*E.coli* ATCC 25922) to a Mueller Hinton agar plate.
7. Allow the plate to dry 3-5 minutes.
8. Place the incubated 10 µg meropenem disc on the prepared Mueller Hinton plate.
9. Let the inoculated plate incubate at 35°C for 18-24 hours.

Reading and interpretation of results

1. After incubation, examine the plate for the presence/absence of a zone of growth inhibition around the meropenem disc.
2. Measure the zone of inhibition next to the meropenem disc.
    a. If the zone size 6-15mm, the isolate is Positive for Carbapenemase Activity.
    b. If the zone size is 16-18mm, the isolate is Indeterminate for Carbapenemase Activity.
    c. If the zone size is ≥19 mm, the isolate is Negative for Carbapenemase Activity.
3. Record your results.
4. **Positive Control**, *Klebsiella pneumoniae* ATCC BAA-1705, shows growth of the *E.coli* ATCC 25922 next to or uninhibited by the meropenem antibiotic disc.
5. **Negative Control**, *Klebsiella pneumoniae* ATCC BAA 1706, shows a zone of growth inhibition around the meropenem antibiotic disc.

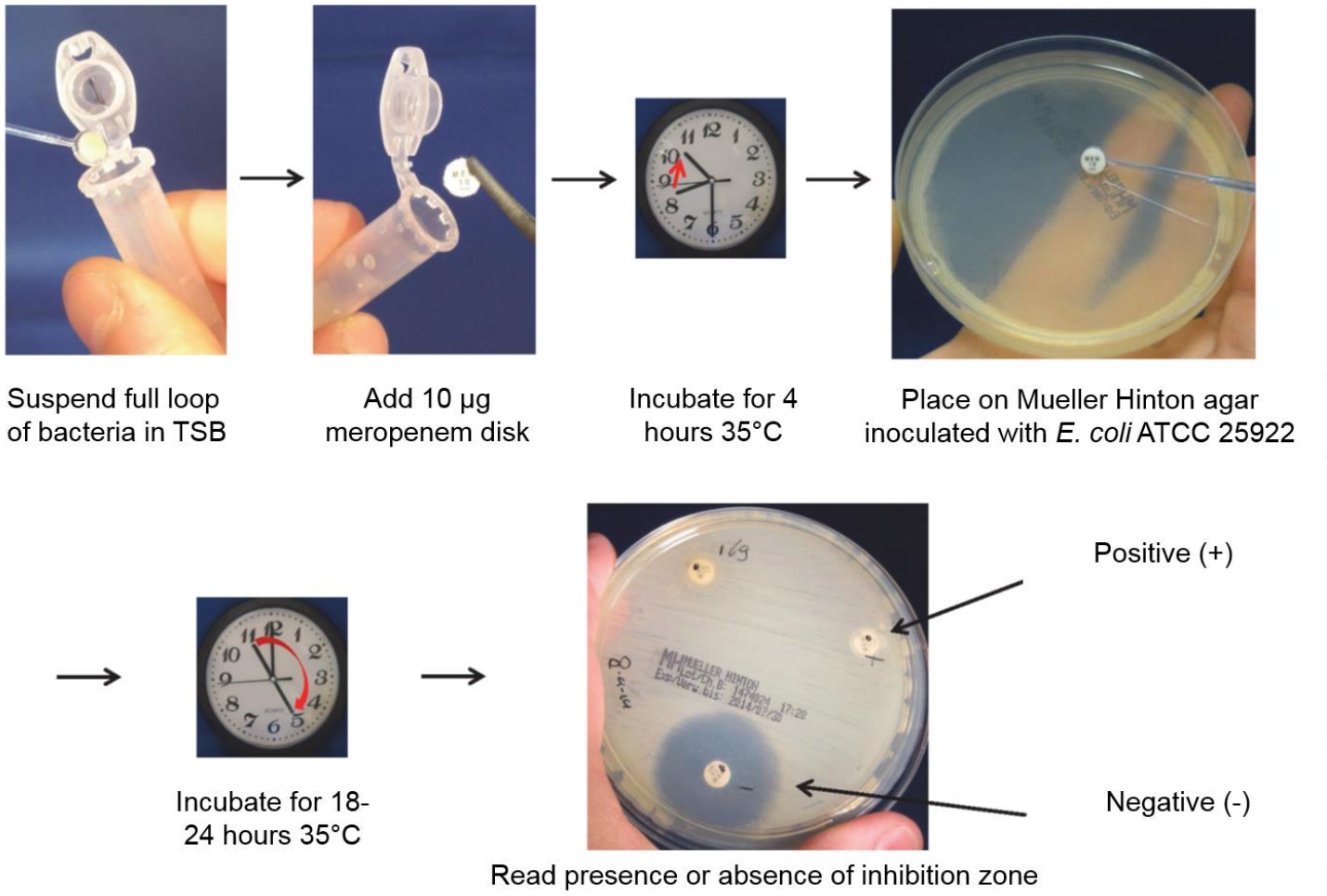

Fig 1. Schematic of the mCIM.

References

Kim van der Zwaluw et al, The Carbapenem Inactivation Method (CIM), a Simple and Low-Cost Alternative for the Carba NP Test to Assess Phenotypic Carbapenemase Activity in Gram-Negative Rods,
PLOS ONE, DOI: 10. 1371, March, 23, 2015.

CLSI M100-S27