Modified Hodge Test (MHT) for Carbapenemase Detection in *Enterobacteriaceae*

Principal

The Modified Hodge Test (MHT) detects carbapenemase production in isolates of Enterobacteriaceae [1,2]. In the United States, the most common carbapenemase found in Enterobacteriaceae is the *Klebsiella pneumoniae* carbapenemase (KPC). Other carbapenemases, like the metallo β lactamases (MBL) and the SME-1 in *Serratia marcescens,* can also produce a positive MHT, but are rare in the United States.

Carbapenemase production is detected by the MHT when the test isolate produces the enzyme, which allows growth of a carbapenem susceptible strain (E.coli ATCC 25922), towards a carbapenem disk. The result is a characteristic cloverleaf-like indentation. See Figure 1.

Supply list

1. Sterile cotton tipped swabs
2. 1 ml sterile pipette
3. 5 ml of 0.85% physiological saline or Mueller Hinton broth
4. Turbidity meter or prepared 0.5 McFarland Standard
5. Sterile loop (5 or 10 µl) or a sterile swab
6. Mueller Hinton agar plate
7. 10 µg meropenem susceptibility disk
8. E. coli ATCC 25922: 18-24hr subculture
9. Test organisms: 18-24hr subculture

Quality Control

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

Day 1 Testing

1. 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.
2. Allow to dry 3-5 minutes.
3. Place a 10 µg meropenem susceptibility disk in the center.
4. Using a 10 µl loop or sterile swab, pick up a visible inoculum from 3-5 colonies of the test organism.
5. In a straight line, streak test organism from the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate with one drug.
6. Incubate overnight at 35^{O}C in ambient air for 16-24 hours

Day 2 Reading and interpretation of results

1. After 16-24 hours of incubation, examine the plate for a clover leaf type indentation at the intersection of the test organism and the *E. coli* 25922 within the zone of inhibition of the carbapenem susceptibility disk.
2. **MHT Positive** has clover-leaf like indentation of the *E.coli* 25922 growing along the test organism growth streak within the disk diffusion zone.
3. **MHT Negative test** has no growth of the *E.coli* 25922 along the test organism growth streak within the disc diffusion.

Figure 1

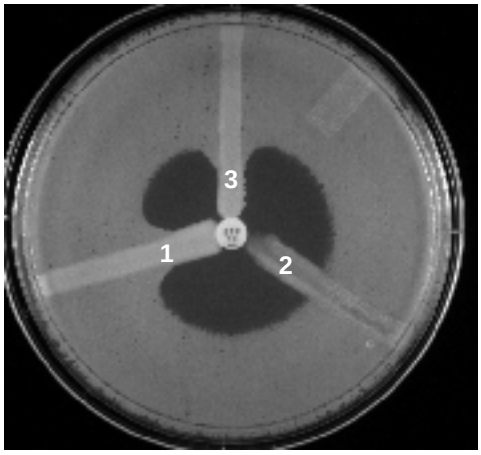

Figure 1. The MHT performed on a small MHA plate.
(1) *K. pneumoniae* ATCC BAA 1705, positive result
(2) *K. pneumoniae* ATCC BAA 1706, negative result;
and (3) a clinical isolate, positive result

References

1**. Anderson, K.,** Lonsway, D.R., Rasheed, J.K., Biddle, J, B. Jensen, B, McDougal, L.K., Carey R.B., Thompson, A. , Stocker, S., Limbago, B, J. B. Patel, J.B. **Evaluation of Methods to Identify the *Klebsiella pneumoniae* Carbapenemase in *Enterobacteriaceae***. JCM 45: 2723

2. **Lee, K., Y. Chong, H. B. Shin, Y. A. Kim, D. Yong, and J. H. Yum.** 2001. **Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species**. Clin. Microbiol. & Infect. **7:**88-91.