Modified Hodge Test for Carbapenemase Detection in *Enterobacteriaceae*

Background

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

Purpose

Carbapenemase production is detected by the MHT when the test isolate produces the enzyme and 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.

Reagents

1. 5 ml Mueller Hinton broth (MHB) or 0.85% physiological saline
2. Mueller Hinton agar (MHA)
3. 10 µg meropenem or ertapenem susceptibility disk
4. *E. coli* ATCC 25922: 18–24hr subculture

Equipment

1. Turbidity meter
2. $35^{O}C \pm 2^{O}C$ ambient air incubator

Supplies

1. Sterile cotton-tipped swabs
2. 1 ml sterile pipette
3. Sterile loop

Specimen

Test organisms: 18–24 hr subculture

Special safety precautions

Biosaftey Level 2

Quality control

Perform quality control of the carbapenem disks according to CLSI guidelines.
Perform quality control with each run.

- MHT Positive *Klebsiella pneumoniae* ATCC BAA-1705
- MHT Negative *Klebsiella pneumoniae* ATCC BAA-1706

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Procedure

Step 1	Prepare a 0.5 McFarland dilution of the *E.coli* ATCC 25922 in 5 ml of broth or saline.
Step 2	Dilute 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of MHB or saline.
Step 3	Streak a lawn of the 1:10 dilution of *E.coli* ATCC 25922 to a Mueller Hinton agar plate and allow to dry 3–5 minutes.
Step 4	Place a 10 µg meropenem or ertapenem susceptibility disk in the center of the test area.
Step 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.
Step 6	Incubate overnight at $35^{O}C \pm 2^{O}C$ in ambient air for 16–24 hours

Interpretation/Results

- 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.

- **MHT Positive** test has a clover leaf-like indentation of the *E.coli* 25922 growing along the test organism growth streak within the disk diffusion zone.

- **MHT Negative** test has no growth of the *E.coli* 25922 along the test organism growth streak within the disc diffusion.

See the CLSI guidelines (M100) for recommendations on detection of carbapenemase production in Enterobacteriaceae that test susceptible to carbapenem.

Expected values

A positive MHT indicates that this isolate is producing a carbapenemase.
A negative MHT indicates that this isolate is not producing a carbapenemase.

Method limitations

The class of carbapenemase can not be determined by the results of the MHT.
Some isolates show a slight indentation but do not produce carbapenmase.

Procedure notes

Up to four organisms can be tested on the same MHA plate with one drug. Two drugs with up to 4 organisms can be tested on a 150 mm Mueller Hinton agar plate.

Figure 1: *photo courtesy of CDC*

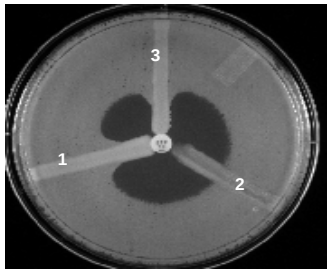

Figure 1. The MHT performed on a 100 mm MHA plate. (1) *K. pneumoniae* ATCC BAA 1705, positive result (2) *K. pneumoniae* ATCC BAA 1706, negative result; and (3) a clinical isolate, positive result

References

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