Ministry of Health

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Acknowledgment

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Acronyms:

BA	Blood agar
CRE	Carbapenem resistant enterobacterales
CPE	Carbapenemase producing enterobacterales
ATCC	American Type Culture Collection
eCIM	EDTA carbapenem inhibition method
EDTA	Ethylenediamine tetra-acetic acid
ID	Identification
QC	Quality Control
M	Molar
MDRO	Multidrug Resistant Organism
MHT	Modified Hodge test
ml	Milli liter
SOP	Standard operating procedure
mCIM	Modified carbapenem inhibition method
TSB	Tryptone soy broth
ul	Micro liter

1. Purpose

This document describes the procedure for screening and detection of acquired carbapenemases enzymes

2. Scope

This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. Definitions

3.1 Carbapenemase: carbapenem hydrolysing Beta-lactamase enzyme. Many of which are encoded on plasmids

3.2 Carbapenems: A Beta lactam antibiotics class that is active in treating aerobic and anerobic gram positive and gram-negative bacteria. Examples are Ertapenem, Imipenem and Meropenem

4. Procedure

4.1. Clinical background:

Carbapenem-resistant Enterobacteriaceae (CRE) has spread rapidly around the world in the past few years, posing great challenges to human health. The plasmid-mediated horizontal transmission of carbapenem-resistance genes is the main cause of the surge in the prevalence of CRE. Therefore, the timely and accurate detection of CRE, especially carbapenemase-producing Enterobacteriaceae, is very important for the clinical prevention and treatment of these infections. Acquired carbapenemases are diverse and include three of four Ambler's molecular classes of β-lactamases. For more detail on types of classes and activities of different carbapenemases , see table 1. Knowing the type of carbapenemases will affect the choice of antibiotics treatment. For example the antibiotic avibactam-ceftazidim is most effective against KPC isolates.

Enzyme type	Classification by ambler class	Activity spectrum	Organism(s)
KPC	A	All β-lactams	Enterobacterales; rare in P. aeruginosa
SME	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	S. marcescens
NMC–A IMI	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	Enterobacter species; rare in other Enterobacterales
GES	A	Depends on enzyme variant. Some are ESBLs, others e.g. GES-5 are carbapenemases	P. aeruginosa and Enterobacterales
FRI	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	Enterobacter species
IMP VIM NDM GIM, DIM, SIM, SPM1 (metallo-βlactamases)	B	All β-lactams except monobactams (aztreonam)	Pseudomonas species; Acinetobacter species; Enterobacterales
OXA	D	Carbapenems	A. baumannii; Enterobacterales and rare in P. aeruginosa

Table 1- Examples of carbapenemases classification, activity and organisms

4.2. Principle:

A variety of methods for the rapid detection of CRE phenotypes and genotypes have been developed for use in clinical microbiology laboratories. Major methods currently used for screening and conformations of carbapenemase production are based on phenotypic findings or molecular detections of the carbapenemase encoding gene. Combining two methods are can be considered to be implemented by each lab in order to increase sensitivity and specificity of detection

Methods	**Advantages**	**Disadvantages**
Modified Hodge test (MHT)	1. Detecting KPC 2. Simple and inexpensive	1. False-positive and false-negative 2. Insufficient for MBLs 3. Time consuming
Colorimetric assay (Carba-NP)	1. Detecting KPC and most MBLs 2. Type carbapenemases 3. Simple and inexpensive	1. Insufficient for OXA-48 2. Specific reagents 3. Various infecting factors
Modified carbapenem inactivation method (mCIM)	1. Detecting all carbapanemeses 2. Clear criteria of judgment 3. Simple and cost-effectiveness	1. Time consuming 2. Cannot distinguish between serine and metallo-carbapenemases
Spectrophotometric method	1. High sensitivity and specificity 2. Time saving 3. Simple and inexpensive	1. Specific instrument (spectrophotometer) 2. Various influencing factors 3. No standard equation and cut-off value 4. Small sample size
MALDI–TOF-based methods	1. Detecting KPC and NDM 2. Time saving 3. Easy to perform 4. Low measurement cost	1. Insufficient for OXA-48 2. No clear protocol and standard analysis 3. Expensive equipment
Molecular-based detection methods	1. Gold standards 2. Detecting all carbapanemeses genes 3. Type carbapenemase genes 4. Time saving	1. High technical requirements 2. Insufficient for expression of genes 3. High measurement cost

Table 2- The advantages and limitations of common detection methods

4.3. Pre – analytical stage:

4.3.1. Sample: fresh gram negative bacterial isolate with reduced susceptibility to carbapenem agent that is not known to be intrinsic.

4.3.2. Safety precaution:

- All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.

4.3.3. Quality control:

- Check the expiry dates of all media, reagents and stains before use.
- All media, reagents, kits, and stains **MUST** be quality controlled before use.
- Identification tests should be run with appropriate controls.
- Record the quality control results in the appropriate QC sheet.

4.4. Analytical stage:

4.4.1 Carbapenemase-producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems .Testing resistant or Intermediate to ertapenem is often the most sensitive indicator of carbapenemase production and usually test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, and ceftriaxone). However, some isolates that produce carbapenemases such as SME or IMI often test susceptible to these cephalosporins

4.4.2 Carbapenem inhibition method

4.4.2.1 Reagents and material:

- TSB (2 mL aliquots)
- Meropenem disks (10 μg)
- 1-μL and 10-μL inoculation loops
- Nutrient broth (eg, Mueller-Hinton, TSB) or normal saline (3.0–5.0 mL aliquots)
- MHA plates.
- Meropenem-susceptible indicator strain – *E. coli* (ATCC 25922)
- 0.5 M EDTA (only for eCIM)

4.4.2.2 Procedure (see annex, figure 2):

- **mCIM procedure** :

1. For each isolate to be tested, emulsify a 1-µL loopful of bacteria for Enterobacterales or 10-µL loopful of bacteria for *P. aeruginosa* from an overnight blood agar plate in 2 mL TSB.
2. Vortex for 10–15 seconds.
3. Add a 10-µg meropenem disk to each tube using sterile forceps or a single disk dispenser. Ensure the entire disk is immersed in the suspension.
4. Incubate at 35°C ± 2°C in ambient air for 4 hours ± 15 minutes.
5. Just before or immediately following completion of the TSB-meropenem disk suspension incubation, prepare a 0.5 McFarland suspension (using the colony suspension method) of E. coli ATCC 25922 in nutrient broth or saline.
6. Inoculate an MHA plate with E. coli ATCC 25922 as for the routine disk diffusion procedure making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 minutes. Allow the plates to dry for 3–10 minutes before adding the meropenem disks.
7. Remove the meropenem disk from each TSB-meropenem disk suspension using a 10-µL loop by placing the flat side of the loop against the flat edge of the disk and using surface tension to pull the disk out of the liquid. Carefully drag and press the loop along the inside edge of the tube to expel excess liquid from the disk. Continue using the loop to remove the disk from the tube and then place it on the MHA plate previously inoculated with the meropenem-susceptible E. coli ATCC 25922 indicator strain.
8. Invert and incubate the MHA plates at 35°C ± 2°C in ambient air for 18–24 hours.
9. Following incubation, measure the zones of inhibition as for the routine disk diffusion method.

- **eCIM procedure :**
    1. For each isolate, label a second 2-mL TSB tube for the eCIM test.
    2. Add 20 µL of the 0.5 M EDTA to the 2-mL TSB tube to obtain a final concentration of 5 M EDTA.
    3. Follow steps 1 through 9 above as for mCIM procedure.
    4. Process the mCIM and eCIM tubes in parallel.
    5. Place the meropenem disks from the mCIM and eCIM tubes on the same MHA plate inoculated with the meropenem-susceptible *E. coli* ATCC 25922 indicator strain.

4.4.2.3 Interpretation (see annex figures 3A, 3B, 4A, 4B, 4C, 4D):

- **mCIM :**
    - Carbapenemase positive:
        - Zone diameter of 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone
        - If the test isolate produces a carbapenemase, the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the meropenem-susceptible E. coli ATCC 25922.
    - Carbapenemase negative:
        - Zone diameter of ≥ 19 mm (clear zone)
        - If the test isolate does not produce carbapenemase, the meropenem in the disk will not be hydrolyzed and will inhibit growth of the meropenem-susceptible E. coli ATCC 25922.
    - Carbapenemase indeterminate:
        - Zone diameter of 16–18 mm
        - Zone diameter of ≥ 19 mm and the presence of pinpoint colonies within the zone
        - The presence or absence of a carbapenemase cannot be confirmed.
- **eCIM (Interpret only when mCIM test is positive):**

- Metallo-β-lactamase positive:
  - A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg, mCIM = 6 mm; eCIM = 15 mm; zone diameter difference = 9 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition.
  - If the test isolate produces a metallo-β-lactamase, the activity of the carbapenemase will be inhibited in the presence of EDTA such that the meropenem in the disk will not be hydrolyzed as efficiently as in the tube without EDTA. The result is inhibition of the meropenem-susceptible E. coli and an increase in the zone diameter for the eCIM zone diameter compared with the mCIM zone diameter.
- Metallo-β-lactamase negative:
  - A ≤ 4-mm increase in zone diameter for the eCIM vs zone diameter of mCIM (eg, mCIM = 6 mm; eCIM = 8 mm; zone diameter difference = 2 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition.
  - If the test isolate produces a serine carbapenemase, the activity of the carbapenemase will not be affected by the presence of EDTA and there will be no or marginal (≤ 4 mm) increase in zone diameter in the presence of EDTA compared with the mCIM zone diameter.

4.4.2.4 Reporting

Test	Results	Reporting
mCIM only	Negative	Carbapenemase not detected
	Positive	Carbapenemase detected
	Indeterminate	Testing inconclusive for the presence of carbapenemase
mCIM and eCIM combination	mCIM Negative	Carbapenemase not detected
	eCIM not set up	
	mCIM Postive	Serine carbapenemase detected
	eCIM negative	
	mCIM Positive	Metallo-β-lactamase detected
	eCIM Positive	
	mCIM Indeterminate	Testing inconclusive for the presence of carbapenemase
	eCIM not set up	

Notes

- If indeterminate results are obtained on repeat testing, consider performing a different phenotypic test, molecular test or sending isolate to CPHL for confirmation
- If both a serine carbapenemase and a metallo-β-lactamase are co-produced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur.
- mCIM only: For some tests, pinpoint colonies of the indicator organism (*E. coli* ATCC® 25922) may be observed within the zone of inhibition. If the colonies are present within a 6- to 18-mm zone of inhibition, the test should be considered carbapenemase positive. If colonies are present within a ≥ 19-mm zone, the test should be considered indeterminant.
- eCIM only: Ignore pinpoint colonies within any zone of inhibition. Interpret results strictly based on the difference in zone diameters between the mCIM and eCIM tests

Test positive and negative QC strains each day of testing [a]

QC Strain	Organism Characteristics	Expected Results
K. pneumoniae ATCC BAA-170	KPC positive Serine carbapenemase producer	mCIM positive eCIM negative
K. pneumoniae ATCC BAA-1706	Carbapenemase negative	mCIM negative
K. pneumoniae ATCC BAA-2146	NDM positive Metallo-β-lactamase producer	mCIM positive eCIM positive

[a] In addition, perform QC of meropenem disks and test media daily or weekly following the routine disk diffusion QC procedure

4.4.3 Modified Hodge Test (MHT)

4.4.3.1 Reagent and material

Reagents	Consumables/Supplies	Equipment
• Mueller Hinton agar (MHA) • 10 µg meropenem susceptibility disk • Distal water	• Sterile cotton-tipped swabs • 1 ml sterile pipette • Sterile loop	• Nephlometer/ MacFarlane standard turbidity meter device • Vortex • 35C ambient air incubator

4.4.3.2 Quality control strains

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

4.4.3.3 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 35OC ± 2OC in ambient air for 16–24 hours

4.4.3.4 Interpretation

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

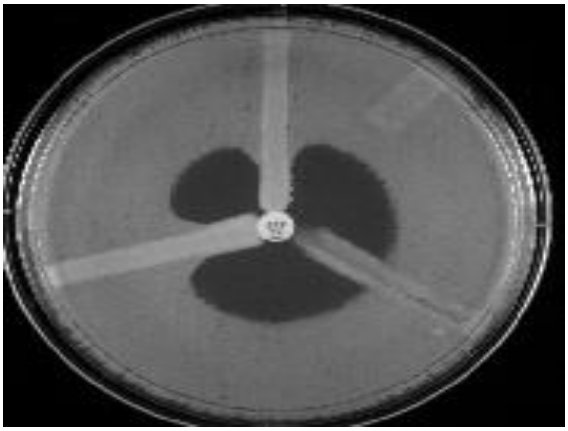

Figure 1 .The MHT performed on a 100 mm MHA plate. At 7 o'clock is *K. pneumoniae*ATCC BAA 1705, positive result . At 4 o'clock is *K. pneumoniae*ATCC BAA 1706, negative result; and at 12 o'clock is a clinical isolate, positive result

4.4.3.5 Reporting

MHT positive : carbapenemase production is detected

MHT negative : carbapenemase production not detected

Post – analytical stage:

5. Responsibilities

5.1. Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2. Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3. All lab staff:

- To adhere to the procedure.

- To document record and release results as recommended
- To report test failures or inciden

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 32nd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022.	Clinical and Laboratory Standards Institute	2022	122-146
UK Standards for Microbiology Investigations Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases)	NHS, UK	2022	1-32
Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae	Tsai et al. BMC Microbiology (2020) 20:315	2020	

8. Annexes:

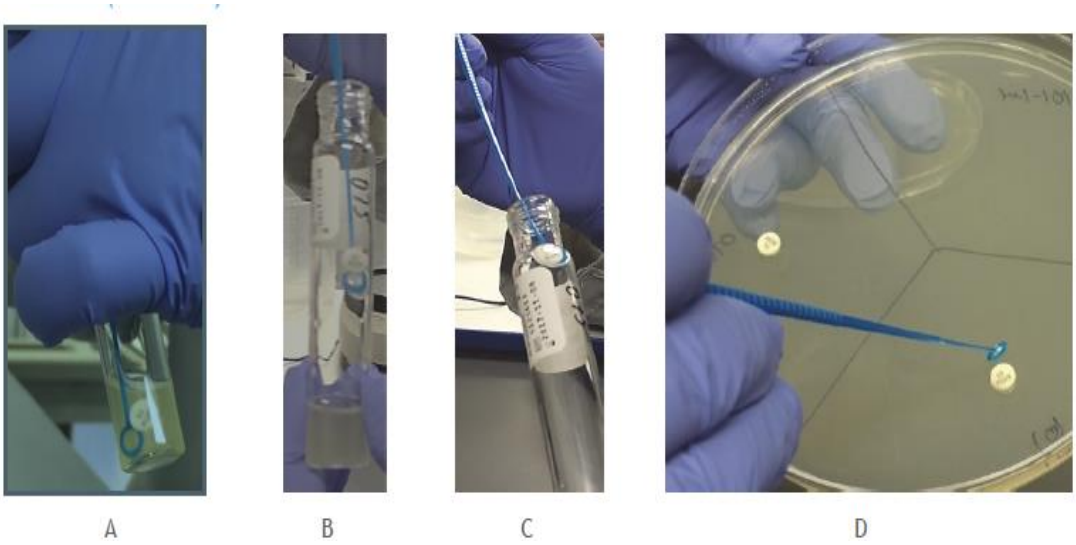

Figure 2. Procedure for Placing Meropenem Disks for the mCIM. Remove the meropenem disk with a 10-µL loop (A) and drag the loop against the inside edge of the tube to expel any excess liquid (B). Use the same loop to remove the disk from the tube (C) and place it on the MHA plate (D) previously inoculated with the meropenem-susceptible *E. coli* (ATCC 25922) indicator strain.

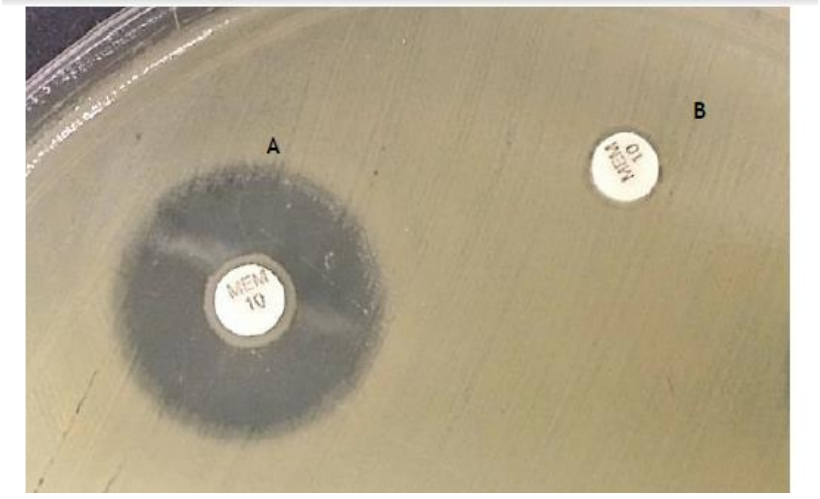

Figure 3 A mCIM Results for QC Strains: Negative Control *K. pneumoniae* ATCC®BAA-170 (A) and Positive Control *K. pneumoniae* ATCC BAA-1705 (B). NOTE: A narrow ring of growth around the meropenem disk as seen with the negative control (A) results from carryover of the test organism in the TSB and should be ignored.

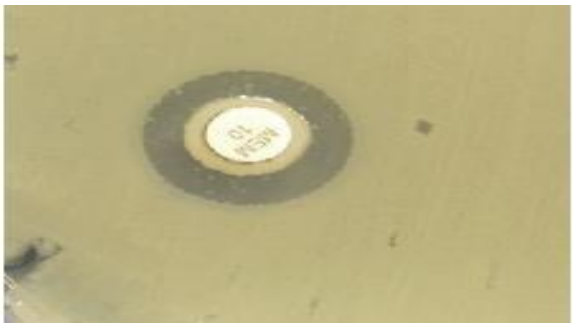

Figure 3 B. mCIM Test Interpretation
Result: positive mCIM
Report: carbapenemase detected
NOTE: A narrow ring of growth around the meropenem disk results from carryover of the test organism in the TSB and should be ignored

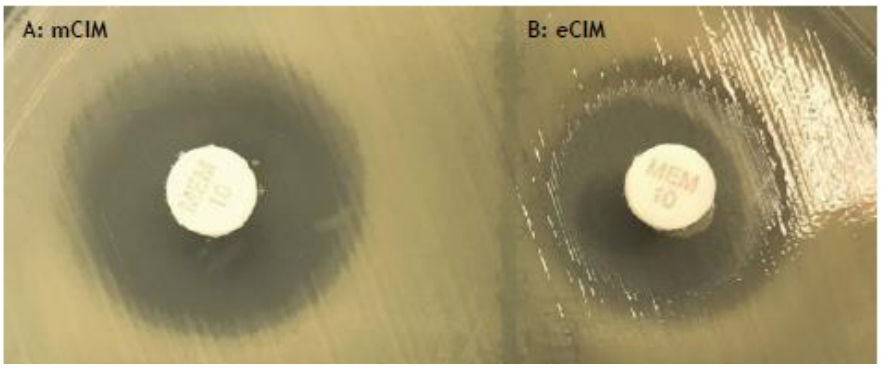

Figure 4 A. mCIM and eCIM Test Interpretation: Negative mCIM. "A" shows an mCIM negative result (zone diameter = 20 mm) and "B" shows an eCIM invalid result. Do not interpret the eCIM result when the mCIM is negative as the isolate is negative for carbapenemase production. Result: negative for carbapenemase production Report: carbapenemase not detected

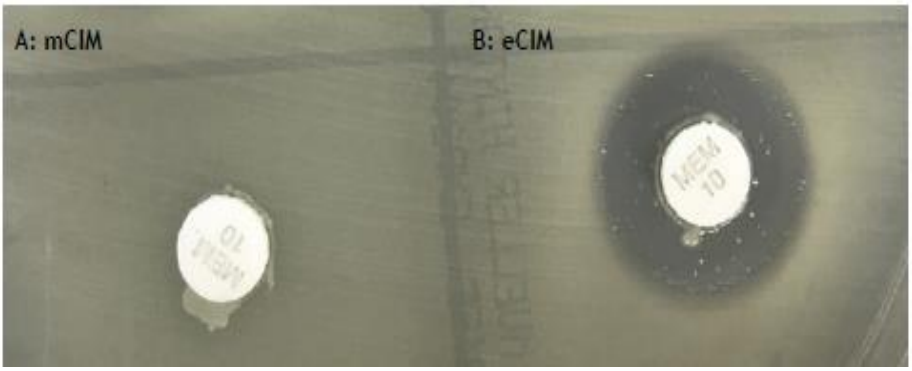

Figure 4 B. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter of 6 mm) and "B" shows an eCIM positive result (zone diameter = 15 mm with pinpoint colonies throughout the zone of inhibition). **NOTE:** The pinpoint colonies throughout the zone of inhibition are ignored when measuring the zone for the eCIM test. A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (15 mm − 6 mm = 9 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA.

Result: positive mCIM and eCIM

Report: metallo-β-lactamase detected

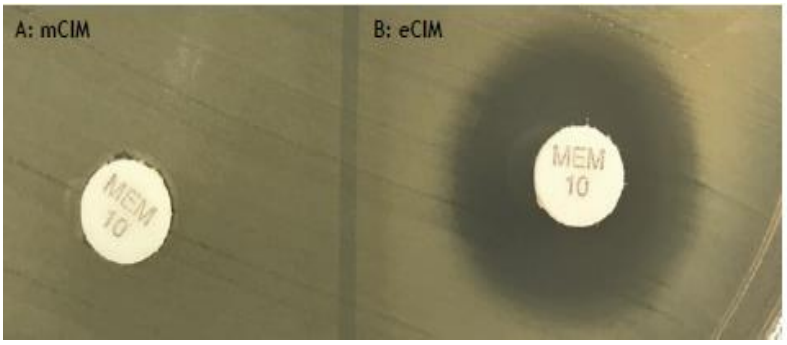

Figure 4 C. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM positive result (zone diameter = 19 mm). A ≥ 5-mm increase in zone diameter for eCIM vs diameter for mCIM zone (19 mm − 6 mm = 13 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA.

Result: positive mCIM and eCIM

Report: metallo-β-lactamase detected

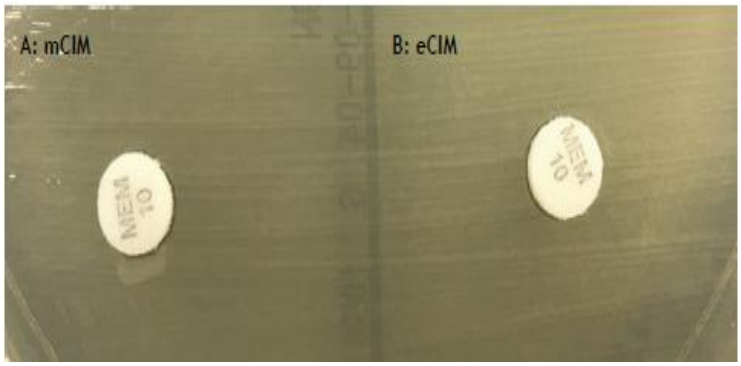

Figure 4 D. mCIM and eCIM Test Interpretation: Positive mCIM and Negative eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM negative result (zone diameter = 6 mm). Serine carbapenemases are not inhibited by EDTA and demonstrate a ≤ 4-mm increase in zone diameter for eCIM vs zone diameter for mCIM.
Result: positive mCIM and negative eCIM
Report: serine carbapenemase detected