PURPOSE: The MAST test is used to detect AmpC and/or Extended Spectrum Beta-Lactamase enzyme production in Gram-negative bacilli.

SAMPLE INFORMATION:

Type	Few, well isolated colonies

REAGENTS and/or MEDIA:

Type	MASTDISKS Combi AmpC and Extended Spectrum Beta-Lactamase (ESBL) Detection Disks: A = Cefpodoxime (10 ug) B = Cefpodoxime (10ug) + ESβL inhibitor C = Cefpodoxime (10ug) + AmpC inhibitor D = Cefpodoxime (10ug) + ESβL inhibitor + AmpC Inhibitor
Stability and Storage Requirements	• Store at 2°C to 8°C in the container provided until the expiry date shown on the pack label. • Allow to equilibrate to room temperature before opening.

SUPPLIES:

- Plastic Vitek tubes and caps
- 0.9% sterile saline
- Sterile swabs
- DensiCHEK Plus
- Mueller Hinton agar
- Forceps
- 35° ambient air incubator
- Small, metric ruler

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Quality control is performed weekly:
  - Positive: *Klebsiella pneumoniae* ATCC 700603, ESBL positive
  - Negative: *Escherichia coli* ATCC 25922, ESBL and AmpC negative
- A TQC order is automatically generated on Wednesdays to record the QC results.

PROCEDURE INSTRUCTIONS:

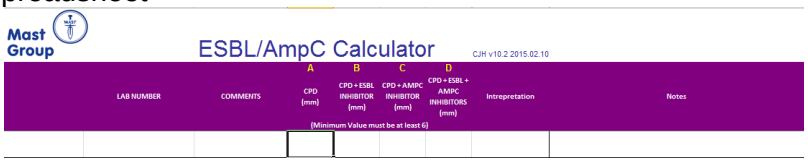
Step	Action
Performing the MAST test	
1	Remove the MAST disks from refrigerator for 1 hour and bring to room temperature before opening the container.
2	Remove Mueller Hinton agar from the refrigerator and bring to room temperature.
3	Dispense 3 mL of sterile 0.9% saline into a labelled plastic test tube. Pick several colonies from a fresh agar plate and prepare a suspension equivalent to a 0.5 McFarland standard.
4	Within 15 minutes of adjusting turbidity, dip a sterile cotton swab into the inoculum and rotate against the wall of the tube above the liquid to remove excess inoculum.
5	Swab the entire surface of the agar three times, rotating plate approximately 60° between streaking to ensure even distribution. To minimize aerosols, avoid hitting the sides of the plate. Finally, run swab around the edge of the agar to remove any excess moisture. Allow inoculated plate to stand for 3 to 15 minutes before applying disks.
6	Using sterile forceps, place one of each type of MAST disk onto the inoculated plate, ensuring sufficient space between the disks to allow formation of clearly defined zones of inhibition.
7	Invert the plate and incubate in the O_2 incubator for 18 to 24 hours.
8	After incubation, read plates only if lawn of growth is confluent.
9	Use a ruler held on the back of the plate to measure and record in mm the diameter of any zones of inhibition that are observed. Disks showing no zone of inhibition should be recorded as 6mm.

INTERPRETATION OF RESULTS:

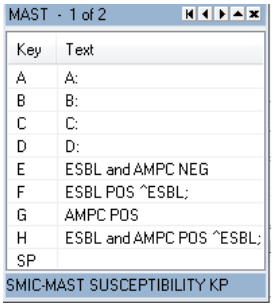
1. Manual Interpretation:

Step	Action	
1	**Step 1** - Compare the zone of inhibition of the Cefpodoxime disk **(A)** to the zones of inhibition of each the Cefpodoxime plus inhibitor disks **(B, C and D)**.	
	IF	**THEN**
	All zones are within 2 mm of each other	Organism is negative for both ESBL and AmpC activity
2	**Step 2** - Subtract **A** from **B** and then **C** from **D**.	
	IF	**THEN**
	Each of **B-A** and **D-C** is ≥ 5 mm **AND** the difference in zone diameter between disks **B** and **D** and **A** and **C** are ≤ 4 mm	The organism is demonstrating ESBL activity alone
3	**Step 3** - Subtract **B** from **D** and **A** from **C**	
	IF	**THEN**
	Each of **D-B** and **C-A** is ≥ 5 mm **AND** the difference in zone diameter between disks **A** and **B** and disks **C** and **D** are ≤ 4 mm	The organism is demonstrating AmpC activity alone
4	**Step 4** – Subtract **C** from **D**	
	IF	**THEN**
	D-C is ≥ 5 mm **AND** the difference in zone diameter between disks **A** and **B** is ≤ 4 mm	The organism is demonstrating ESBL and AmpC combined activity.

2. Template Interpretation:

Step	Action
1	Open up the ESBL calculator program: I:drive: Stanton Hospital Share → LAB → Microbiology → ESBL worksheet → ESBL Calculator Spreadsheet 
2	Input the zone sizes for the A, B, C and D disks.
3	The worksheet will calculate and interpret the results.

REPORTING RESULTS:

Step	Action
1	The MAST keypad allows the zone sizes for each disk to be recorded: 
2	Enter in all the zone sizes and select the correct interpretation from the keypad based on the results from the worksheet.
3	If the isolate is ESBL positive, a new isolate will be created by the LIS and called "ESBL confirmed". This isolate is for epidemiology reporting and should not appear on the final report. Change the isolate number from 2 to an A to prevent it from reporting.

LIMITATIONS/PRECAUTIONS:

1. To avoid potentially erroneous results, do not test cartridges from different batches together – batches should never be mixed.
2. Organisms producing a fully resistant profile i.e. no zone of inhibition on all disks, could indicate demonstration of an MβL or KPC carbapenemase production, which could also be masking concurrent ESBL or AmpC expression.

REFERENCES:

- MASTDISKS Combi AmpC and Extended Beta-Lactamase (ESBL) Detection Disks package insert, 02/18
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	4 APR 19	Initial Release	L. Steven