STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: MAST DISCS FOR ESBL	Revision Date: 20-April-2018	Issue Date: 20-April-2016
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PURPOSE:

Detection of plasmid mediated broad spectrum beta-lactamases (BSBL) including extended spectrum beta-lactamases (ESBL) and AmpC cephalosporinases (AmpC) is important for both therapeutic and epidemiological purposes.

REAGENT INFORMATION:

Type	4 cartridges, each cartridge containing 50 discs • Cartridge A: Cefpodoxime 10ug discs • Cartridge B: Cefpodoxime 10ug + ESBL inhibitor discs • Cartridge C: Cefpodoxime 10ug + AmpC inhibitor discs • Cartridge D: Cefpodoxime 10ug +ESBL inhibitor +AmpC inhibitor discs
Source	Alere Canada
Storage Requirements	Store at 2-8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening.

MATERIALS:

- Sterile swabs, forceps, applicator sticks
- Normal Saline (0.9% Sodium Chloride)
- DensiCHEK
- 35°C ambient air incubator
- Mueller Hinton Agar
- Small metric ruler

QUALITY CONTROL:

QC must be performed upon receipt of a new lot or shipment, and weekly.

- Must be run with both Positive and Negative controls
- A TQC order will be generated upon batch receiving the new lot/shipment and automatically generated every week as per weekly KB QC. To result the TQC order, log into TQC→Resulting Worklist → MICS – All Scheduled Micro → Run Worklist →MAST
- New lot/shipment QC should be done upon receipt to the lab.
- Weekly MAST QC should be done when performing Vitek weekly QC.

POSITVE CONTROL (AmpC pos): *Enterobacter cloacae ATCC 13406*

NEGATIVE CONTROL (ESBL and AmpC neg): *E.coli ATCC 25922***

**Deterioration/malfunction check: Compare all the zone diameters in the negative control (compare zone diameters of disks A, B, C and D). All diameters should be within 2mm of each other or less (ideally the zone diameters from all disks should be equal). Any greater differences between the disks imply malfunction or deterioration and the MAST disks should not be used.

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 where 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.

PROCEDURE INSTRUCTIONS:

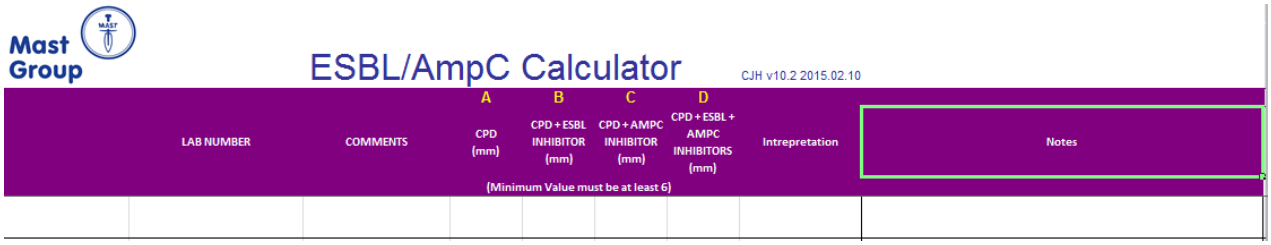
Step	Action
1	**LIS CODE: MAST** Using a pure, fresh culture of the test organism, prepare a suspension in saline equivalent to a 0.5 McFarland standard
2	Moisten a sterile swab with the suspension and swab the entire surface of MH agar 3 times, rotating plate approximately 60˚ between swabbing to ensure an even distribution of organism. Avoid hitting the sides of the plate to avoid aerosols. Run swab around the edge of the plate to remove any excess moisture. Wait for moisture to absorb into agar, approx 3 minutes. Do not wait more than 15 minutes to apply disks.
3	Using sterile forceps (may sterilize with an alcohol pad) place one of each type of MAST disc onto the inoculated media, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
4	Incubate the plate at 35-37°C for 18 to 24 hours.
5	Measure and record in mm the diameter of any zones of inhibition that are observed. Discs showing no zone of inhibition should be recorded at 6mm.

INTERPRETATIONS OF RESULTS:

1. Manual Interpretation

Step	Action	
1	Compare the zone of inhibition of the Cefpodoxime disc **A** to the zones on inhibition of each of the Cefpodoxime plus inhibitor discs **B, C,** and **D.**	
	IF	**THEN**
	All zones are within 2mm of each other	Organism is NEGATIVE for both ESBL and AmpC activity
2	Subtract **A** from **B ; C** from **D** ; **B** from **D**; **A** from **C**	
	IF	**THEN**
	Each of **B-A** and **D-C** >=5mm **AND** Each of **D-B** and **C-A** <5mm	The organism demonstrates ESBL activity alone.
	Each of **B-A** and **D-C** <5mm **AND** Each of **D-B** and **C-A** >=5mm	The organism demonstrates AmpC activity alone.
3	Subtract **C** from **D** and **A** from **B**	
	IF	**THEN**
	D-C >=5mm **BUT B-A** <5mm	The organism demonstrates ESBL and AmpC combined activity.

2. Template Interpretation

Step	Action
1	Open up the ESBL calculator program In the S: drive: LAB → Bacteriology → ESBL worksheet → ESBL calculator 
2	Input the following: • Zone interpretation for Disks A, B, C, D **NOTE:** for zone sizes where there is growth up to the disk input 6mm not 0mm
3	The system will calculate and interpret

ALTERNATIVELY A calculation program is available at www.mastgrp.com.

REPORTING RESULTS:

- In the plate log (Media field) add media “MAST”. A keypad will appear in which you can enter the zone sizes for A, B, C and D discs, and the interpretation.
- Go to “Isolates” and add, using A or B, not a number, the isolate “ESBL Confirmed”. **This will mark the isolate for epidemiology reporting.**
- From the AST Manual use the B-lactam charts to determine the comment to add to the isolate which is being reported. These are all in the comment keypad.
- Copy results to SOHS from all in-patients.

LIMITATIONS:

- Use discs only from the same batch/pack. Do not use cartridges from different batches together; batches should never be mixed.
- Occasionally a pattern will occur where the “A” disc has no zone while the “C” disc does. This is an indeterminate result and the organism needs to be sent to DynaLife for further testing.
- Organisms producing a fully resistant profile i.e. no zone of inhibition on all discs could indicate demonstration of KPC carbapenemase production, which could also be masking concurrent ESBL or AmpC expression.

RELATED DOCUMENTS:

- MIC51000: Kirby-Bauer

REFERENCES:

- Mast Group Ltd., MASTDISCS^{TM}ID test insert.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Oct12	Initial Release	S.Webber
1.1	31Dec13	Reporting procedure	S. Webber
1.2	15Apr14	Weekly QC positive control (*E.cloacae*)	Driedger (L)
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell
3.0	01Aug17	Update MH inoculation and limitations of procedure. Calculator moved and no longer saving patient information in spreadsheet.	L.Steven