THE UNIVERSITY OF SYDNEY

NeoCol: Standard Operating Procedure (SOP) for MDR Swab Processing V2.0

Purpose:

The purpose of this SOP is to describe the standard procedures involved in processing neonatal rectal, and maternal vagino-rectal swabs for the detection, identification, and antibiotic susceptibility testing of MDR bacteria.

Principal:

The rectal and vagino-rectal swabs taken are first screened for the presence of extended beta lactamase (ESBL)-producing and carbapenem-resistant Enterobacteriaceae (CRE) by inoculation onto two selective chromogenic growth media. Bacteria that grow on these plates after incubation are likely ESBL or CRE with their colour suggesting their species. To confirm the identity and antibiotic susceptibility profile of each colony, then are then tested with an automated analyser.

Responsibility:

This SOP applies to any laboratory staff who are processing swabs for the NeoCOL study. It is the responsibility of those users to always follow these guidelines when processing swabs for the study.

Safety Requirements:

Gloves and a laboratory gown should be always worn during sample processing. Standard hand hygiene practices should be followed before, and after handling of samples. Handle all specimens with care and treat them as potentially infectious material.

Materials:

- Sample for testing (swabs in amies transport medium)
- Non-Sterile gloves.
- x1 ESBL-detecting chromogenic agar (CHROMagar™ ESBL)
- x1 CRE-detecting chromogenic agar (CHROMagar™ KPC)
- Incubator
- MALDI-TOF automated identification system
- BD Phoenix Automated Identification and Susceptibility detection system
- Cartridge and associated reagents for MALDI-TOF (for isolate identification)
- Gram negative antibiotic susceptibility testing (AST) cartridge (Phoenix) and associated reagents

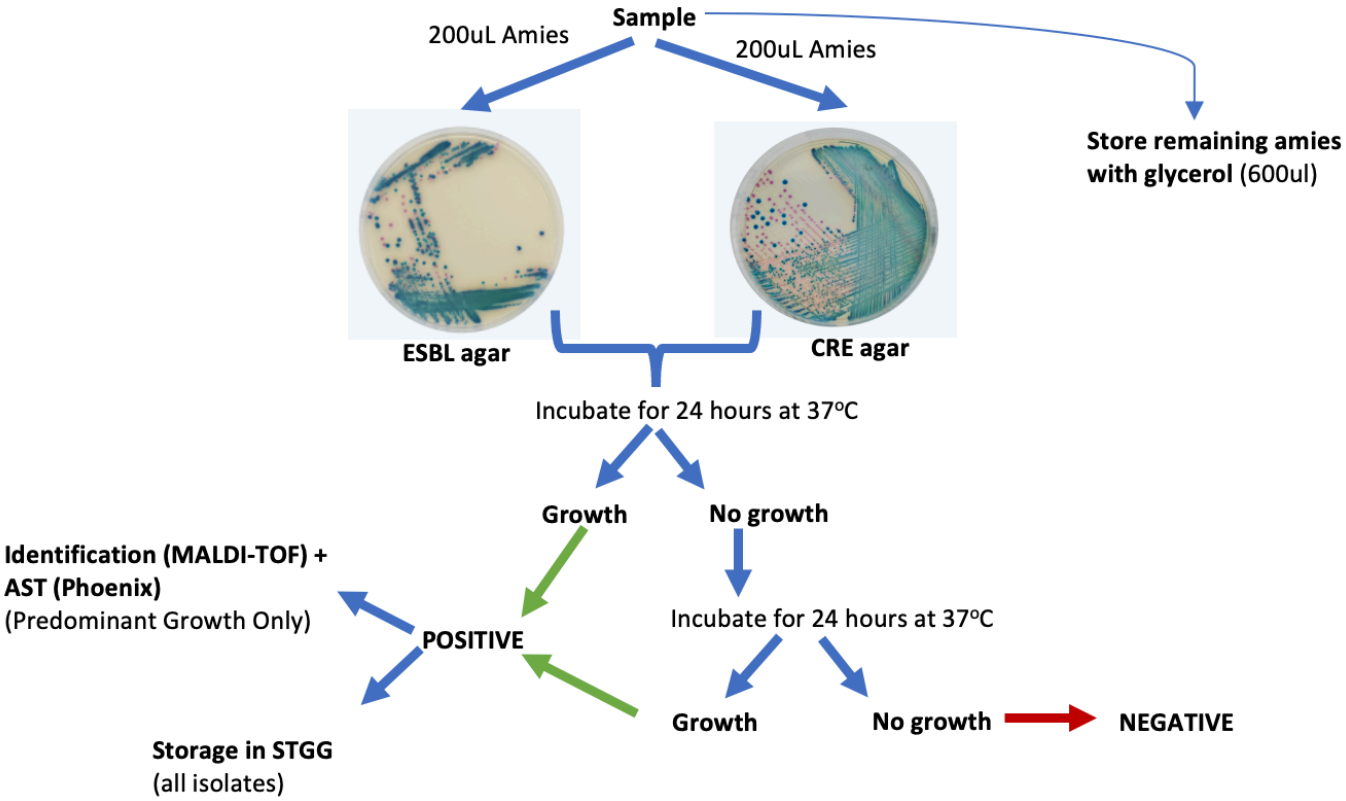
Procedure:

1. Check the label on the sample to ensure it is correct.
2. Check the expiry on the agar plate to ensure it has not expired.
3. Processing time:
    a. Vagino-rectal swabs for MDR bacteria should be processed *within 4 hours of collection*.
    b. If there are any unforeseen delays, samples may be stored at 4^{o}C for up to 24 hours
4. Screening & Isolation:
    a. Place the one ESBL and one CRE chromogenic agar plate on a clean work surface. Plate must be at room temperature prior to inoculation.
    b. Vortex the MDR sample containing amies medium and swab for 1 minute (to dislodge bacteria from swab into medium), then discharge the swab.
    c. Inoculate ~~200~~10uL of amies onto each of the ESBL and CRE agar plates and steak in a 4-quadrant streaking using a sterile inoculating loop.
    d. 100uL glycerol is added into cryotube and mixed with approximately 600 uL of collected specimen in amies media by vortex and stored in -80*C. Minimum final concentration of glycerol is 10%.
    e. Once sufficient liquid has been absorbed, streak the surface of the agar plate in a zig-zag motion. Replace the cover on the agar plate.
    f. Label the plate with the sample ID PLUS the time/ date of agar inoculation.
    g. Place the agar plate in an incubator at *37 degrees* for *24 hours*
    h. Record the sample into the 'Swab Result' Episode on RedCAP.
    i. After 24 hours, inspect the plate for the presence of any colonies.

i. If no colonies present → re-incubate for a further 24 hours. Record this into the RedCAP database under 'Swab result' Episode.
ii. If no growth after further 24 hours → the sample is *negative*. Record the result into the RedCAP database under 'Swab result' Episode.
iii. If colonies are present at 24 or 48 hours the sample is *positive* → remove plate and continue as below. Record the positive result, plate type (ESBL or CRE) and colour of the colony into the RedCAP database under the 'Swab Result' Episode.
iv. <u>All positive isolates</u> should be stored as per the NeoCOL GBS/MDR positive storage protocol (SOP07) in STGG medium.

5. ID & AST Testing:
   a. Perform ID and AST for <u>*the most predominant single unique colony from the ESBL and CRE plate*</u>. Other non-predominant colonies from each agar will be stored in STGG, and may be identified later.
6. ID testing:
   a. The Identification is done by MALDI-TOF by inoculating the overnight (no later than 24 hours) colony onto MALDI-TOF plate followed by air drying and matrix addition following manufacturer's instructions & local policy.
   b. Record the ID result into the 'Swab Result' Episode on RedCAP.
7. Susceptibility testing:
   a. Prepare and load the suspension sample from the colony into BD Phoenix cartridge as per manufacturer's instructions & local policy.
   b. Save the Phoenix AST result into the google drive folder.
8. Dispose of any waste as per local laboratory policies.

Appendix: Screening procedure for MDR Swabs



References

- CHROMagar ESBL information sheet. Online.
- CHROMagar KPC information sheet. Online.

Document History

Version	Author(s)	Approved by	Update Reason	Date	SOP No:
1.0	B. Dickson	P. Williams	New document	11JUL2023	NeoCOL_SOP05
2.0	B. Dickson	P. Williams	Update method/ flow chart	11APR2024	NeoCOL_SOP05

Site Training Record

Trainee Name	Read/Understand SOP (Tick)	Access to SOP (Tick)	Trainee Signature	Date	Trainer Initials