THE UNIVERSITY OF SYDNEY

NeoCol: Standard Operating Procedure (SOP) for MDR Swab Processing V3.0

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 (AST) 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. Chromogenic media detect specific microorganisms based on the colour change resulting from the interaction between the microorganism and the chromogenic substrate in the media. If the bacteria produce an ESBL enzyme (CHROMagar™ ESBL) or carbapenemase (CHROMagar™ KPC), this will hydrolyse the antibiotic substrate present in the agar and result in a colour change.

Bacteria that grow on these plates after incubation are likely ESBL or CRE with their colour suggesting their species. The identity of each isolate is then confirmed via MALDI-TOF based off the principle of mass spectrometry. Each isolates antibiotic susceptibility profile is then determined via an automated susceptibility analyser (Vitek 2 Compact) by a colorimetric assay. The system uses microbial growth or inhibition of growth as a measure of antibiotic susceptibility.

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) per 2 isolates
- x1 CRE-detecting chromogenic agar (CHROMagar™ KPC) per 2 isolates
- x1 Blood agar per isolate
- Incubator
- MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) automated identification system (Bruker)
- VITEK® 2 Compact (bioMerieux) Susceptibility detection system
- Cartridge and associated reagents for MALDI-TOF (for isolate identification)
- x1 Vitek AST-N415 Susceptibility card per isolate

Quality Control:

All chromogenic media should pass internal quality control and batch acceptance as per local laboratory protocol and manufacturer's instructions. At least one positive and one negative strain should be used for quality control testing to ensure functionality of media. To be completed weekly and for each new batch of media made.

CHROMagar ESBL QC testing:

Suitable ATCC Strains	Suitable for use if no ATCC available	Typical colony appearance
ESBL *E. coli* CIP 103982	Known *E. coli* ESBL + clinical isolate	Reddish, small colonies
ESBL *K. pneumoniae* ATCC® 700603	Known *K. pneumoniae* ESBL + clinical isolate	Metallic blue
E. faecalis ATCC® 29212	Known *E. faecalis* clinical isolate	Inhibited
P. aeruginosa ATCC® 10145	Known *P. aeruginosa* ESBL – clinical isolate	Inhibited

E. coli ATCC® 25922	Known *E. coli* ESBL – clinical isolate	Inhibited
C. albicans ATCC® 60193	Known *C. albicans* clinical isolate	Inhibited
S. aureus ATCC® 25923	Known *S. aureus* clinical isolate	Inhibited

CHROMagar KPC QC testing:

Suitable ATCC Strains	Suitable for use if no ATCC available	Typical colony appearance
E. coli IMP NCTC 13476	Known *E. coli* CRE + clinical isolate	Dark rose
K. pneumoniae ATCC® BAA 1705	Known *K. pneumoniae* CRE + clinical isolate	Steel blue
K. pneumoniae NCTC 13438	Known *K. pneumoniae* CRE + clinical isolate	Steel blue
E. faecalis ATCC® 29212	Known *E. faecalis* clinical isolate	Inhibited
K. pneumoniae ATCC® 13883	Known *K. pneumoniae* CRE - clinical isolate	Inhibited
C. albicans ATCC® 60193	Known *C. albicans* clinical isolate	Inhibited
S. aureus ATCC® 25923	Known *S. aureus* clinical isolate	Inhibited

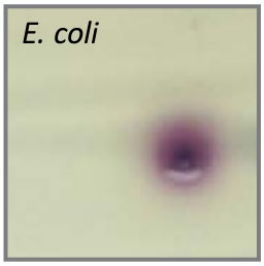
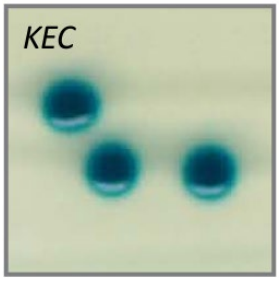
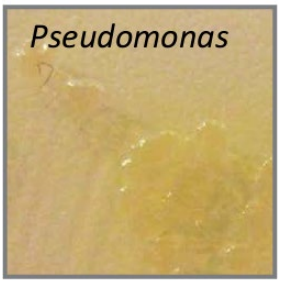
Vitek AST cards should pass weekly internal quality control and batch acceptance as per local laboratory protocol and manufacturer's instructions.

Procedure:

1. Check the label on the sample to ensure it is correct. (E.g. E020C07M = Hung Vuong Hospital, Participant 020, Child, Day 07, MDR swab)
2. Check the matching Laboratory Test Request Form for tests requested (note: for maternal culture samples, they will be labelled with a 'C' for culture, label associated plates with the sample ID and replace 'C' with 'M' for MDR culture. E.g. E020M00**C** will become E020M00**M**)
3. Check the expiry on the agar plate to ensure it has not expired.
4. Processing time:
    a. Samples will be stored at 4°C in the microbiology Department in Hung Vuong Hospital pending transport.
    b. These samples should be processed as soon as possible once transported to processing laboratory.
    c. Record arrival date and time in MDR Sample Processing Log.
    d. If they are not processed immediately, they must be stored at 4°C and processed when possible and within a **maximum** of 7 days post sample collection.
5. 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. Divide each plate in two by using a marker on the bottom side of the plate. This will allow two samples to be used per plate.
    c. Label each side of the plate with the sample ID PLUS the time/ date of agar inoculation.
    d. **For infant swabs:** Remove swab from transport container, taking care to not touch swab off surroundings. Inoculate top section of relevant half of ESBL agar first, then taking care to not touch swab off edge of the plate or surrounding area, repeat for CPE plate.
    e. **For maternal swabs:** Vortex the sample container, remove swab from transport container, taking care to not touch swab or surroundings. Inoculate 10µl amies liquid onto top section of relevant half of the agar, taking care to not touch loop on the edge of the plate, or surrounding area. Inoculate top section of relevant half of ESBL agar first, then taking care to not touch swab off edge of the plate or surrounding area, repeat for CPE plate.
    f. Carefully place swab back into transport container.
    g. Using a sterile 10 µl or 1 µl loop, streak the primary inoculum down the half of the agar plate in a zig-zag motion for the ESBL plate (See Appendix).
    h. Replace the plate with its lid.
    i. Using a new sterile 10 µl or 1 µl loop, repeat this process for the CRE plate. Place the agar plate in an incubator at *37 °C* aerobically for *24 hours.*

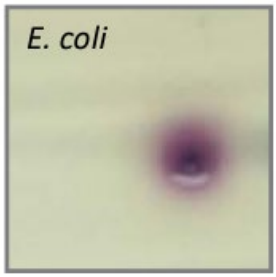
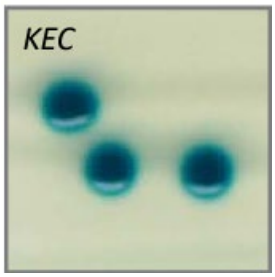
j. Remaining rectal swabs in amies should be transferred into STGG and vortexed for 20 seconds and stored in –80°C.
k. Note: For maternal culture, this sample will be used for culture of GBS and fungal plates also. Ensure all culture is complete prior to storing remaining amies in STGG.

6. Interpretation of ESBL results:

Microorganism	Typical colony appearance	Associated image
ESBL *E. coli*	Dark pink to reddish	
ESBL *Klebsiella, Enterobacter, Citrobacter*	Metallic blue (+/- reddish halo)	
ESBL *Proteus*	Brown halo	N/A
ESBL *Acinetobacter*	Cream	
ESBL *Pseudomonas*	Translucent, (+/- natural pigmentation cream to green)	
Stenotrophomonas	Colourless	N/A
Gram (+) strains	Inhibited	N/A

Non Resistant Other Gram (-) strains	Inhibited	N/A
Yeasts	Mostly inhibited	N/A

7. Interpretation of CPE results:

Microorganism	**Typical colony appearance**	**Associated image**
E. coli Carbapenem Resistant	Dark pink to reddish	
Klebsiella, Enterobacter, Citrobacter Carbapenem Resistant	Metallic blue (+/- reddish halo)	
Pseudomonas Carbapenem Resistant	Translucent cream to blue	
Acinetobacter Carbapenem Resistant	Cream, opaque	
Stenotrophomonas	Colourless	
Carbapenem Sensitive strains	Inhibited	
Gram (+) strains	Inhibited	
Yeasts	Mostly inhibited	

a. Record the sample into the 'Swab Result' Episode on RedCAP.
b. 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. All positive isolates should be stored as per the NeoCOL GBS/MDR positive storage protocol (SOP07) in STGG medium.

8. Preparation for identification & AST Testing:
    a. Using a 1 µl sterile loop, pick the *most predominant single unique colony from the ESBL plate* and sub-culture on to a MacConkey / blood agar plate.
    b. Using a 1 µl sterile loop, pick the *most predominant single unique colony from the CRE plate and* sub-culture on to a MacConkey / blood agar plate.
    c. Incubate the purity plate(s) aerobically at 37°C for 16-24 hours (overnight).
    d. The following day, ensure the plate is pure.
    e. This purity plate will be used for performing ID and AST. (i.e. performed only on the most predominant single unique colony from the ESBL and CRE plate)
    f. If there are other non-predominant colonies on the chromogenic plates, these should also be sub-cultured on to a MacConkey / blood agar plate.
    g. These will then be stored in STGG and may be identified later.

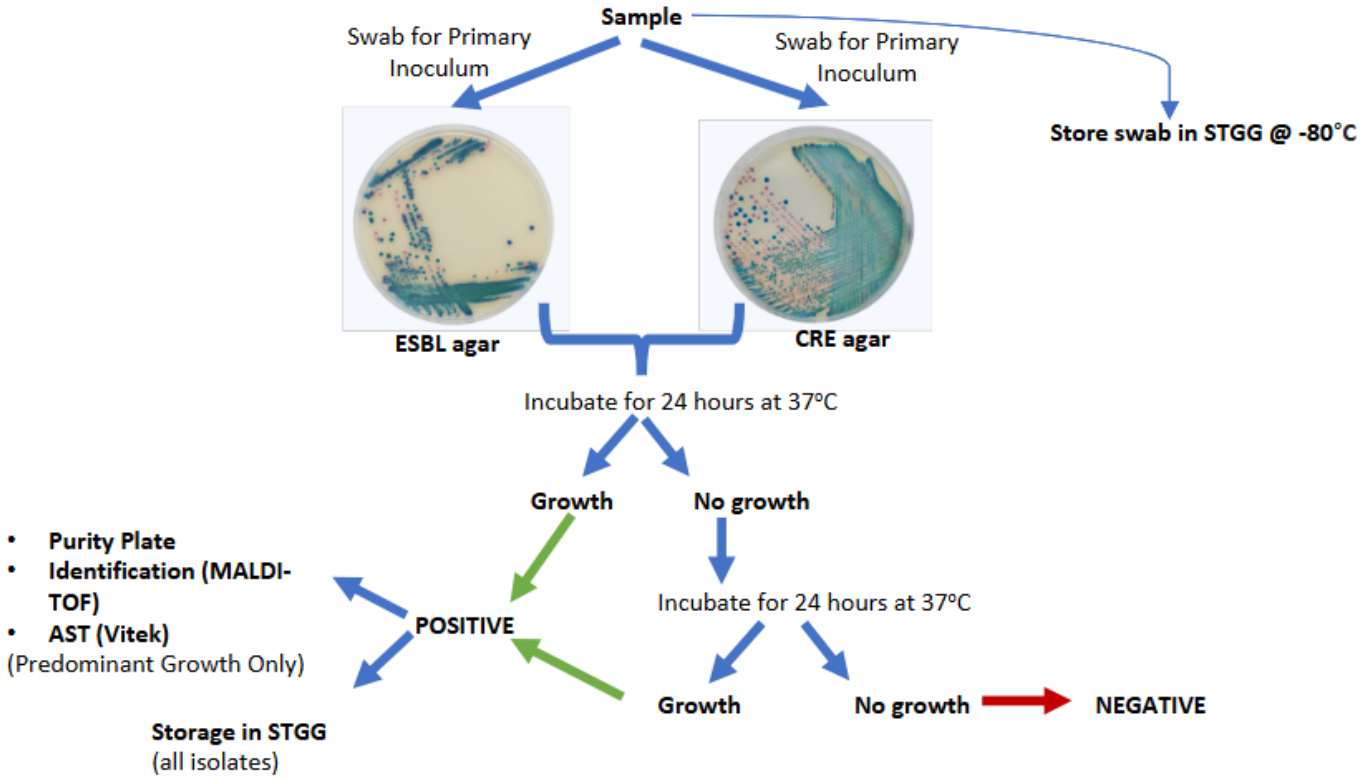
9. Species identification confirmation:
    a. The Identification is done by MALDI-TOF.
    b. Ensure purity plate is no older than 24 hours for optimal performance.
    c. Using an applicator stick / sterile 1 µl loop, smear colony onto MALDI-TOF slide.
    d. Allow sample to air dry and apply matrix as per manufacturer's instructions & local policy.
    e. Load prepared MALDI-TOF slide on to the MALDI-TOF as per manufacturer's instructions and local policy. Note: this step should be done in batches to carry out multiple IDs to ensure best use of resources. (N.B. sterile gloves should be worn for this step to prevent oils from hands transferring onto slide and potentially impacting analysis on analyser).
    f. Record the ID result into the 'Swab Result' Episode on RedCAP.

10. Susceptibility testing:
    a. Prepare inoculum using a 24-hour fresh culture. If there is insufficient growth to obtain a 0.5 McFarland suspension of the organism, sub-culture isolate onto the appropriate media for purity. Incubate overnight in 37°C and set up susceptibility tests the following day as outlined below.
    b. Label a pre-prepared 0.45% saline tube containing with isolate laboratory number.
    c. If using Densicheck or equivalent: The Densicheck must be calibrated prior to use as per manufacturer's instructions.
    d. Use the direct colony suspension method to make a suspension of the organism in 0.45% saline to the density of a McFarland 0.5 turbidity standard. Use several morphologically similar colonies (where possible) to avoid selecting an atypical variant. (NB. If caps not available to vortex sample, this process should be carried out in class II biosafety cabinet to prevent aerosols.)
    e. Adjust the density of the organism suspension to McFarland 0.5 by adding saline or more organism.
    f. A denser inoculum will result in increased MIC values and a decreased inoculum will have the opposite effect.
    g. The suspension should optimally be used within 15 min and always within 60 min of preparation.
    h. Label a MacConkey purity plate with the laboratory ID number.
    i. Load the suspension sample from the colony into the rack supplied with Vitek system, insert relevant AST card into the tube next to the isolate to be tested as per manufacturer's instructions & local policy.

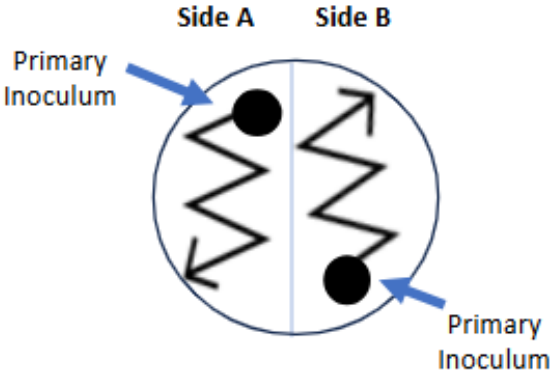
j. Using the Vitek workstation, scan the AST susceptibility card barcode and enter unique RedCap ID for isolate. If no barcode scanner available, manually enter unique RedCap ID for isolate.
k. Load onto Vitek system as per manufacturer's instructions & local policy.
l. Remove the rack containing the suspensions from the Vitek when it has completed its cycle.
m. Inoculate the labelled purity plate with the broken off plastic pipette tip remaining in the tube to the right of the relevant isolate. N.B. it is important to perform this step after the Vitek rack has completed its course in case any potential contamination has occurred inside the instrument.
n. Incubate purity plate at *37 °C* aerobically for *24 hours.* The AST results should be ready after 16-24 hours. Interpret results in accordance with CLSI guidelines on Vitek software and in accordance with BioArt rules built into Vitek system. N.B. Ensure the purity plate is pure to ensure reliability of Vitek AST results. If the purity plate is mixed, investigate the culture plate the suspension was prepared from for potential mixture.
o. This process must be repeated if purity plate is mixed.
p. Save the Vitek AST result into the google drive folder.

11. Record results in MDR Sample Processing Log.
12. Dispose of any waste as per local laboratory policies.

Appendix: Screening procedure for MDR Swabs



Appendix: Inoculation Method for Chromogenic Media



References

- CHROMagar™ ESBL information sheet. PDF.
- CHROMagar™ mSuperCARBA™ information sheet. PDF
- Vitek 2 Compact user manual. PDF.

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
3.0	R. Kelleghan	P. Williams	Adapted for NeoCOL 2.0	02FEB2025	NeoCOL_2.0_SOP05

Site Training Record

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