NeoCol: Standard Operating Procedure (SOP) for Fungal Swab Processing V1.0

Purpose:

The purpose of this SOP is to describe the standard procedures involved in processing neonatal and maternal rectal, axilla and groin swabs for the detection and identification of *Candida* species.

Principal:

The rectal, axilla, groin and vagino-rectal swabs taken are first screened for the presence of *Candida spp.* by inoculation onto a selective chromogenic growth media. One swab will be used for all these sites to give a pooled sample for identification of colonisation with *Candida spp*. Chromogenic media detect specific fungal pathogens based on colour change, resulting from the interaction between the microorganism and the chromogenic substrate in the media.

Fungal pathogens that grow on these plates after incubation are likely to be *Candida* spp*.,* with their colour suggesting their specific species. The identity of each isolate is subsequently confirmed via MALDI-TOF based on the principle of mass spectrometry.

Each isolate will be stored for future analysis for antimicrobial susceptibility testing.

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.
- Sterile plastic loops
- Sterile swabs
- x0.5 CHROMagar™ Candida Plus Agar per sample
- Parafilm / clingfilm
- x1 Sabouraud Dextrose agar per isolate
- Incubator
- MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) automated identification system (Bruker)
- MALDI-TOF matrix and slides

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.

CHROMagar™ Candida Plus Agar QC testing:

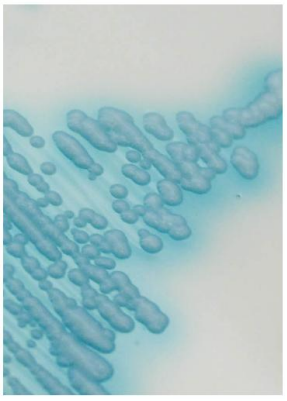
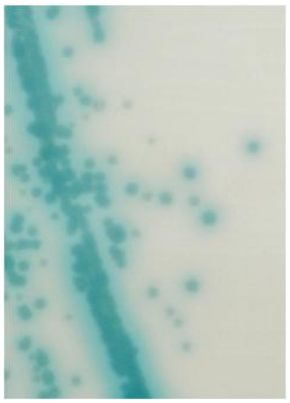
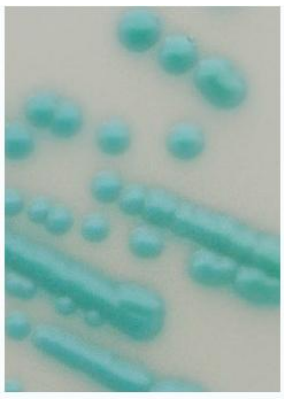
Suitable ATCC Strains	Suitable for use if no ATCC available	Typical colony appearance
C. albicans ATCC® 60193	Known *C. albicans* clinical isolate	Green-blue

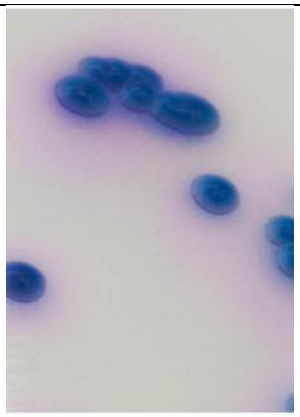
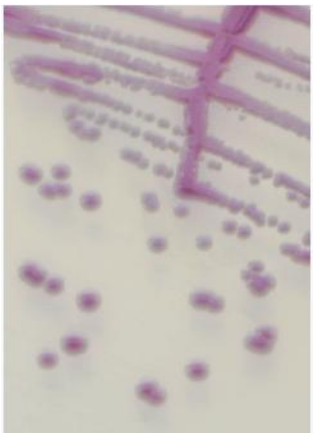
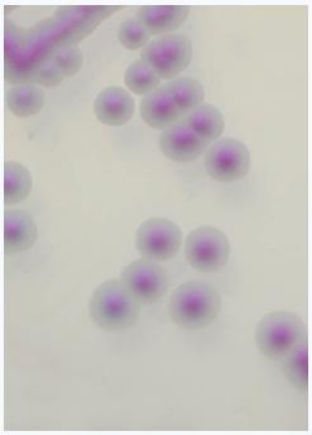
C. auris ATCC® MYA-5001	Known *C. albicans* clinical isolate	Light blue with blue halo, blue from the back side of the plate
C. tropicalis ATCC® 1369	Known *C. tropicalis* clinical isolate	Blue with pink halo
C. krusei ATCC® 14243	Known *C. krusei* clinical isolate	Pink and fuzzy
C. glabrata ATCC® 2001	Known *C. glabrata* clinical isolate	Pink
E. coli ATCC® 25922	Known *E. coli* clinical isolate	Inhibited

Procedure:

1. Check the label on the sample to ensure it is correct. (E.g. E020C07F = Hung Vuong Hospital, Participant 020, Child, Day 07, Fungal 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 'F' for fungal culture. E.g. E020M00**C** will become E020M00**F**)
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 Fungal 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 *Candida* 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 identification, PLUS the time/ date of agar inoculation.
    d. **For infant swabs:** Remove swab from transport container, taking care to not touch swab or surroundings. Inoculate top section of relevant half of the agar, taking care to not touch swab on the edge of the plate, or surrounding area.
    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.
    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 (See Appendix).
    h. Replace the plate with its lid.
    i. Remaining patient swabs in amies should be transferred into STGG and vortexed for 20 seconds and stored in –80°C.
    j. Note: For maternal culture this sample will be used for culture of MDR and GBS plates also. Ensure all culture is complete prior to storing remaining amies in STGG.
    k. Incubate plates at 35 - 37°C for 2-5 days. (Note: wrap plate in parafilm / clingfilm to prevent plates drying out if incubated for 5 days)

6. Interpretation of Candida results:

Microorganism	**Typical colony appearance**	**Associated image**
C. auris	Light blue with blue halo Blue from the back side	Front:  Back: 
C. albicans	Green-blue	
C. tropicalis	Blue with pink halo	

		
C. glabrata	Pink	
C. krusei	Pink and fuzzy	

C. parapsilosis	Pink/white (Note: this can often be mixed up with *C. auris*)	

a. Record the sample into the 'Swab Result' Episode on RedCAP.
b. At 48 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. At 72 hours, if there is no growth re-incubate plate for a further 48 hours (5 days incubation in total, checking daily after initial 48 hours incubation). Record this into the RedCAP database under 'Swab result' Episode.
    iii. At 5 days incubation, if no growth the sample is *negative*. Record the result into the RedCAP database under 'Swab result' Episode.
    iv. If colonies are present within 5 days, the sample is *positive*. Remove plate and continue as below. Record the positive result and colour of the colony into the RedCAP database under the 'Swab Result' Episode.
    v. <u>All positive isolates</u> should be stored as per the NeoCOL GBS/MDR positive storage protocol (SOP07) in STGG medium.

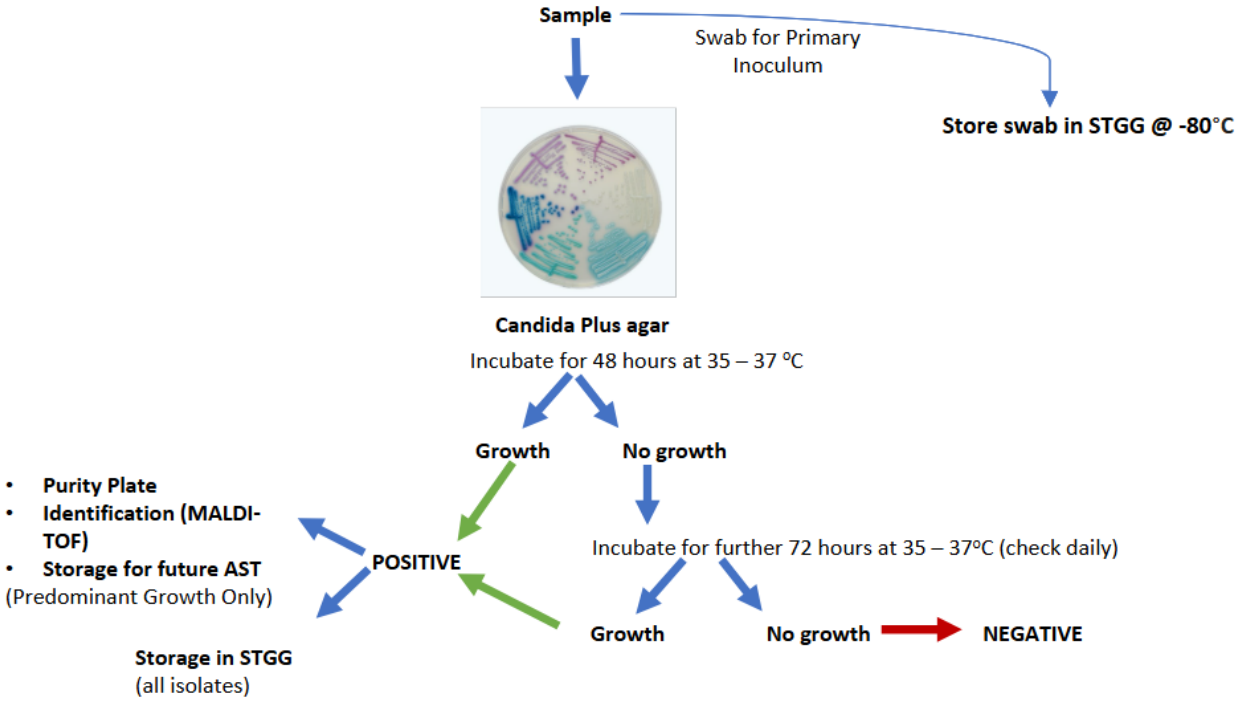
7. Preparation for identification & Storage:
    a. Using a 1 µl sterile loop, pick the <u>*most predominant single unique colony from the Candida plate*</u> and sub-culture on to a sabouraud dextrose agar plate.
    b. <u>Incubate the purity plate(s) aerobically at 35-37</u>°C for 16-24 hours (overnight).
    c. The following day, ensure the plate is pure.
    d. This purity plate will be used for performing ID and storage for AST. (i.e. performed only on the most predominant single unique colony from the *candida* plate)
    e. If there are other non-predominant colonies on the chromogenic plates, these should also be sub-cultured on to a sabouraud dextrose agar plate.
    f. These will then be stored in STGG and may be identified later.
8. Species identification confirmation:
    a. This secondary identification will be confirmed 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).

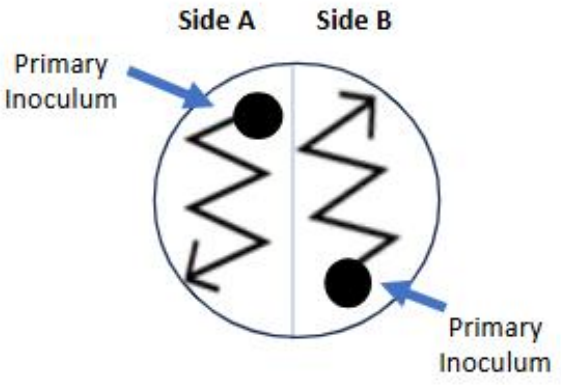
f. Record the ID result into the 'Swab Result' Episode on RedCAP.

9. Store isolate as per SOP07.
10. Record results in Fungal Sample Processing Log.
11. Dispose of any waste as per local laboratory policies.

Appendix: Screening procedure for Fungal Swabs



Appendix: Inoculation Method for Chromogenic Media



References

- CHROMagar™ Candida Plus Agar Information Sheet. PDF.
- Govender, N. P., Patel, J., Magobo, R. E., Naicker, S., Wadula, J., Whitelaw, A., Coovadia, Y., Kularatne, R., Govind, C., Lockhart, S. R., Zietsman, I. L., & TRAC-South Africa group (2016).

Emergence of azole-resistant Candida parapsilosis causing bloodstream infection: results from laboratory-based sentinel surveillance in South Africa. *The Journal of antimicrobial chemotherapy*, *71*(7), 1994–2004. https://doi.org/10.1093/jac/dkw091

Document History

Version	Author(s)	Approved by	Update Reason	Date	SOP No:
1.0	R. Kelleghan	P. Williams	New document	11MAR2025	NeoCOL_2.0_SOP09

Site Training Record

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