THE UNIVERSITY OF SYDNEY

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

Purpose:

The purpose of this SOP is to describe the standard procedures involved in processing maternal vagino-rectal swabs for the detection of Group B *Streptococcus* (GBS) bacteria.

Principal:

The vagino-rectal swabs taken are screened for the presence of GBS. First the swabs are inoculated into a selective growth broth and incubated which helps to improve the sensitivity of testing by removing other competing flora. Next the sample is inoculated onto a chromogenic agar (CHROMagar™ StrepB). Bacteria that grow on these plates are likely to be GBS. Colonies are then tested with the CAMP test to confirm that they are GBS by detecting the improved haemolysis of the bacteria in the presence of beta-haemolytic *S. aureus*. Each isolates antibiotic susceptibility profile and serotype will be analysed at a later date as per SOP10.

Responsibility:

This SOP applies to any laboratory staff who are processing GBS 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 worn at all times 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 (flocculated swab in amies medium)
- Sterile falcon tube (x1 per sample)
- Lim Broth (Todd-Hewitt broth with nalidixic acid (15 µg/mL) and gentamicin (8µg/mL) or colistin (10µg/mL)) (2mL per sample)
- 5% sheep blood agar plate (x2 per sample)
- CHROMagar™ StrepB (x0.5 per sample)
- Beta-lysin–producing strain of *S. aureus* ATCC 25923
- Non-Sterile gloves.
- Pipettes and Pipette Tips (20µL, 1000µL)
- Sterile inoculation loops (x3 per sample)
- Incubator
- Vortex

Quality Control

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™ StrepB QC testing:

Suitable ATCC Strains	Suitable for use if no ATCC available	Typical colony appearance
S. agalactiae ATCC® 12386	Known *S. agalactiae* clinical isolate	Pink / Mauve
S. agalactiae ATCC® 13813	Known *S. agalactiae* clinical isolate	Pink / Mauve
E. faecalis ATCC® 29212	Known *E. faecalis* clinical isolate	Blue
E. coli ATCC® 25922	Known *E. coli* clinical isolate	Inhibited

Procedure:

1. Check the label on the swab sample to ensure it is correct. (E.g. E020M00C = Hung Vuong Hospital, Participant 020, Mother, Day 00, Culture 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 'B' for GBS culture. E.g. E020M00**C** will become E020M00**B**)
3. Check the expiry on the agar plates to ensure they have 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 GBS Sample Processing Log.
    d. If they are not processed immediately, they must be stored at 4°C and processed as soon as possible 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. Enrichment:
    a. Label a sterile laboratory falcon tube with the sample ID PLUS the time/ date of inoculation.
    b. Add 2mL of Lim Broth to the falcon tube.
    c. Vortex the transport container containing the swab. Inoculate a 200µL of amies liquid into the Falcon tube containing Lim broth.
    d. Close the lid and incubate the broth at 35-37°C for 24 hours aerobically.
    e. Record the sample into the 'Swab Result' Episode on RedCAP.
    f. Remaining Vagino-rectal swabs in amies should be transferred into STGG and vortexed for 20 seconds and stored in -80°C
    g. Note: This sample will be used for culture of MDR and fungal plates also. Ensure all culture is complete prior to storing remaining amies in STGG.

6. Screening & Isolation:
    a. Divide the GBS chromogenic agar in two using a marker on the base of the plate in order to allow two samples to be used per one chromogenic plate.
    b. After 24 hours, inoculate 10µL of cultured broth onto the corresponding side of the chromogenic agar plate and streak down the plate in a zig-zag pattern using a sterile inoculating loop.
    c. Return the lid to the plate.
    d. Incubate the chromogenic agar plate for 24 hours at 35-37°C aerobically.

6. Interpretation of Results:

Microorganism	**Typical colony appearance**	**Associated image**
Group B *Streptococcus*	Pink / Mauve	

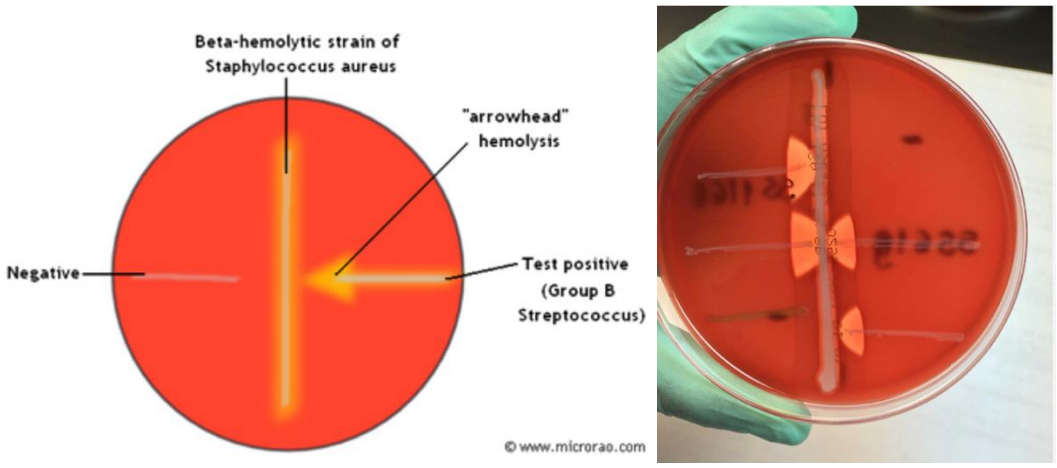
Enterococcus species	Blue	
Lactobacilli, Leuconostoc, Lactococci	Light pink Scanty growth to inhibited	N/A
Other microorganism	Blue, colourless or inhibited	N/A

e. After 24 hours, inspect the plate for the presence of any pink (GBS) colonies
   i. If no *pink* 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 (48 hours total) → the sample is *negative*. Record the result into the RedCAP database under 'Swab result' Episode.
   iii. If *any pink* colonies are present the sample is *positive* → remove plate and continue as below. Record the positive result into the RedCAP database under the 'Swab Result' Episode.

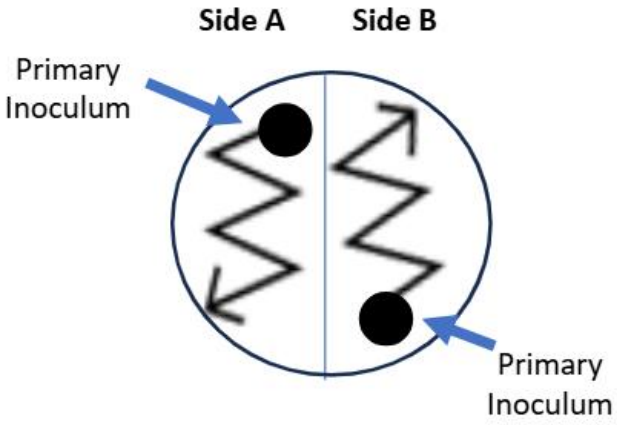
7. Confirmation:
   a. Suspected GBS from GBS Chromagar (pink colonies), is processed for subculture and CAMP test from same colony.
   b. The subculture is done by streaking the presumptive GBS *pink* colony onto 5% sheep blood agar followed by incubation at 35-37°C with 5% CO_2 for 24 hours. NB: The GBS colonies are grey to whitish grey surrounded by a weak zone of beta hemolysis. Non-hemolytic isolates may also be encountered.
   c. From the same colony, the CAMP test is performed as follows: streak a line of the *S. aureus* ATCC 25923 strain through the middle of the 5% sheep blood agar plate, and then a perpendicular line of the suspect GBS colony such that they meet in the middle (see Appendix below).
   d. Incubate at 35-37°C with 5% CO_2 for 24 hours. If no CO2 incubator available on site, incubate in glass bell jar / container with a candle and a capnophilic bacterial control strain (e.g. *Haemophilus influenzae* or *Neisseria gonorrhoeae)* on chocolate agar.
   e. Review for evidence of a positive CAMP reaction by the presence of a triangular zone 'arrow head' of enhanced beta-haemolysis in the diffusion zone of *S. aureus* beta-hemolysin/CAMP factor.
   f. Record the results of the CAMP test in the RedCAP database under the 'Swab Result' Episode.
   g. <u>All CAMP positive isolates</u> should be stored from the subculture plate as per the NeoCOL GBS/MDR positive storage protocol (SOP07).
   h. <u>Note:</u> 5% CO_2 is optimal for growth of GBS, however it will still grow in aerobic conditions. If only incubated in aerobic conditions due to no CO_2 conditions available, please be extra vigilant when reading plates and checking for purity with possible scanty growth of colonies.

8. Dispose of any waste as per local laboratory policies.

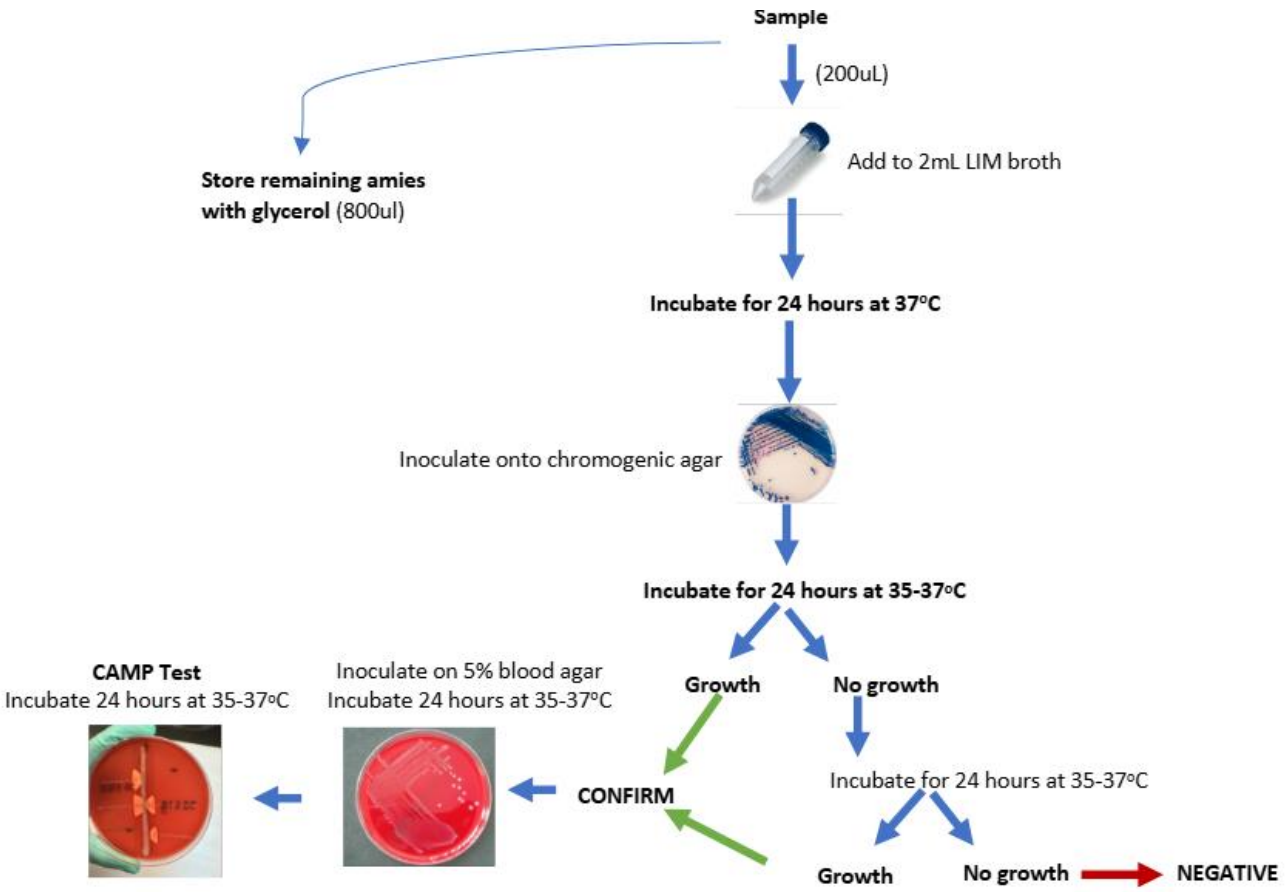
Appendix: CAMP Test using beta-haemolytic strain of S. aureus



Appendix: Inoculation Method for Chromogenic Media



Appendix: GBS laboratory processing flow chart



References

- Filkins L, Hauser J, Robinson-Dunn B, Tibbetts R, Boyanton B, Revell P. Guidelines for the detection and identification of group B streptococcus. Am Soc Microbiol.2020
- Safari D, Gultom SM, Tafroji W, Azzahidah A, Soesanti F, Khoeri MM, Prayitno A, Pimenta FC, da Gloria Carvalho M, Uiterwaal CS, Putri ND. Prevalence, serotype and antibiotic susceptibility of Group B Streptococcus isolated from pregnant women in Jakarta, Indonesia. Plos one. 2021 May 27;16(5):e0252328.
- de Melo SC, Gavena AA, Silva FT, Moreira RC, de Lima Scodro RB, Cardoso RF, Siqueira VL, de Pádua RA, Carvalho MD, Pelloso SM. Performance of Hitchens-Pike-Todd-Hewitt medium for group B streptococcus screening in pregnant women. Plos one. 2015 Apr 16;10(4):e0123988.
- CHROMagar™ StrepB 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_SOP06
2.0	B. Dickson	P. Williams	Update method/ flow chart	11APR2024	NeoCOL_SOP06
3.0	R. Kelleghan	P. Williams	Adapted for NeoCOL 2.0	07MAR2025	NeoCOL_2.0_SOP06

Site Training Record

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