

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

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. 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*.

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:

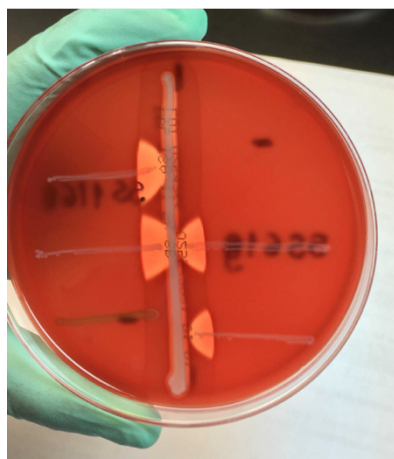
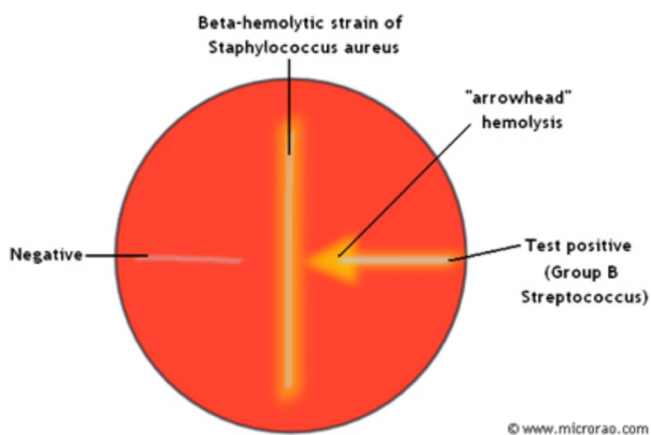
- Sample for testing (flocculated swab in amies medium)
- Sterile falcon tube (x1 per sample)
- Todd-Hewitt broth with nalidixic acid (15 µg/mL) and gentamicin (8µg/mL) or colistin (10µg/mL) (2mL per sample)
- 5% sheeps blood blood agar plate (x2 per sample)
- Chromogenic agar for the detection of GBS (haemolytic and non-haemolytic) (x1 per sample)
- Beta-lysin-producing strain of *S. aureus*
- Non-Sterile gloves.
- Pipettes and Pipette Tips (20µL, 1000µL)
- Sterile inoculator loops (x3 per sample)
- Incubator
- Vortex

Procedure:

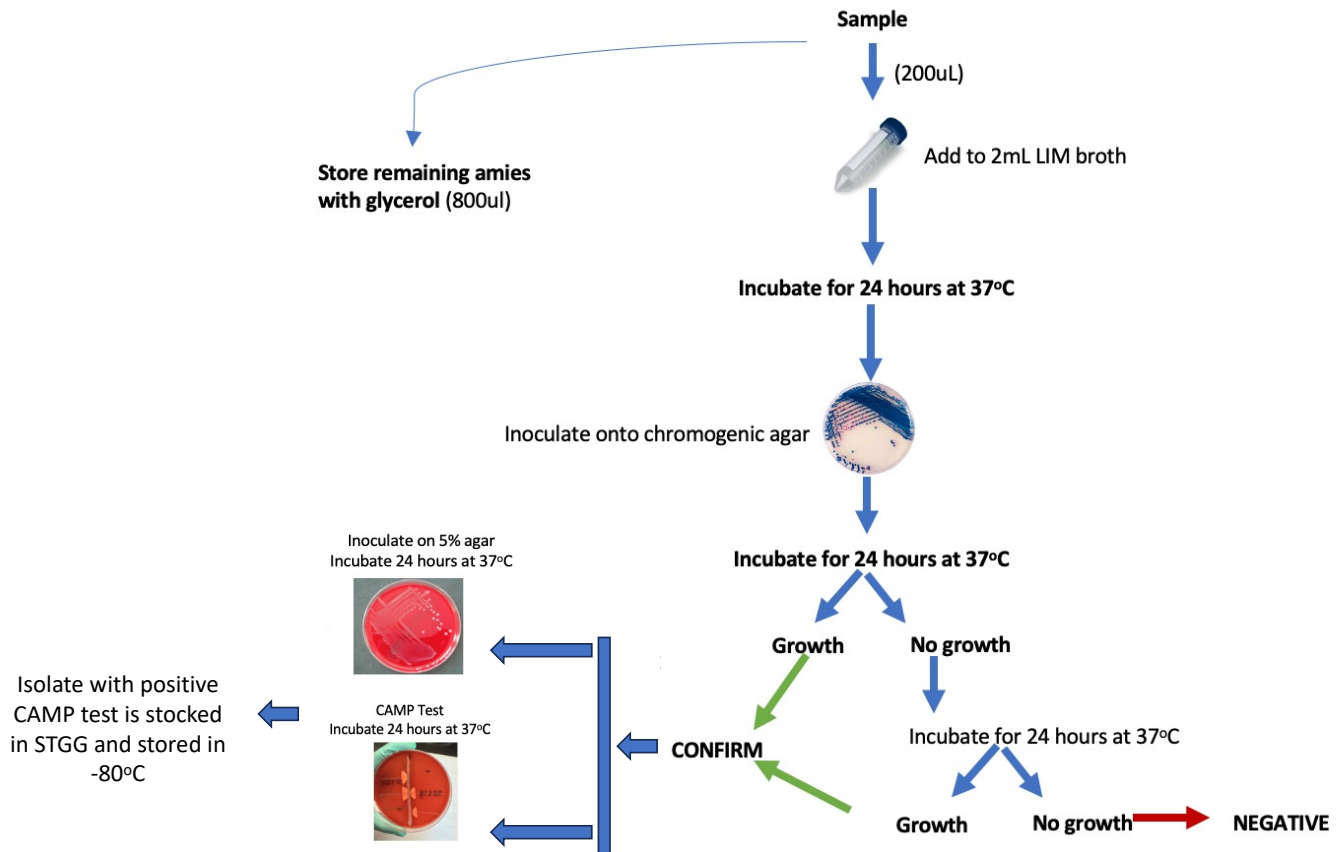
1. Check the label on the swab sample to ensure it is correct.
2. Check the expiry on the agar plates to ensure they have not expired.
3. Processing time:
 - a. Vagino-rectal swabs for GBS bacteria should be processed *within 4 hours of collection*.
 - b. If there are any unforeseen delays, samples may be stored at 4°C for up to 24 hours.
4. Enrichment:
 - a. Label a sterile laboratory falcon tube the sample ID PLUS the time/ date of inoculation.
 - b. Add 2mL of Todd-Hewitt broth to the falcon tube.
 - c. Vortex the GBS sample containing the amies medium and swab for 1 minute (to dislodge the bacteria into the amies).
 - d. Place 200uL of amies from the sample into the falcon tube with the Todd-Hewitt broth.
 - e. Close the lid and Incubate the broth at 37°C for 24 hours.
 - f. Record the sample into the 'Swab Result' Episode on RedCAP.
 - g. Add 100 glycerol into cryotube and Mix the remaining Vagino-rectal swabs in amies by vortex for 20 seconds and store in -80°C
5. Screening & Isolation:

- a. After 24 hours, inoculate a 10µL aliquot of cultured broth onto the chromogenic agar plate and streak in a 4-quadrant streaking using a sterile inoculating loop.
 - b. Incubate the chromogenic agar plate for 24 hours at 37°C.
 - c. 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.
6. 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 37°C with 5% CO₂ 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* 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 37°C with 5% CO₂ for 24 hours .
 - 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. Isolates with positive result of CAMP test are stocked into STGG from subculture plate. The stock isolates are then stored in -80°C
 - g. Record the results of the CAMP test in the RedCAP database under the 'Swab Result' Episode.
 - h. All positive isolates should be stored as per the NeoCOL GBS/MDR positive storage protocol (SOP07)
7. Dispose of any waste as per local laboratory policies.

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



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.

Document History

| Version | Author(s) | Approved by | Update Reason | Date | SOP No: |
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| 1.0 | B. Dickson | P. Williams | New document | 11JUL2023 | NeoCOL_SOP06 |
| 2.0 | B. Dickson | P. Williams | Update method/ flow chart | 11APR2024 | NeoCOL_SOP06 |
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Site Training Record

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