THE UNIVERSITY OF SYDNEY

NeoCol: Standard Operating Procedure (SOP) for Storage of Positive MDR & GBS Isolates v1.0

Purpose:

The purpose of this SOP is to describe the standard procedures involved in storage of positive Group B Streptooccus (GBS) and multidrug resistant (MDR) isolates identified during participant screening.

Principal:

Swabs collected from mothers and neonates will be screened for the presence of MDR bacteria and Group B Streptococcus (mothers only) with chromogenic agar. Each positive isolates will be transferred into a growth medium (STGG; skim milk-tryptone-glucose-glycerol broth) and stored in cryovials at -80^{o}C for potential further analyses. Each unique colony on an ESBL and MDR chromogenic agar, and each GBS isolate should be stored in a separate cryotube in STGG broth.

Responsibility:

This SOP applies to any clinical and laboratory staff who are involved in handling and processing MDR and GBS samples for NeoCOL study. It is the responsibility of those users to always follow these guidelines when handling and processing samples for the study.

Safety Requirements:

Gloves and a laboratory gown should be worn during sample handling. 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. Appropriate insulated gloves should be worn when using a -80^{o}C Freezer.

Materials:

- Sample: positive isolate from ESBL/CRE chromogenic agar, or GBS screening (after CAMP confirmation)
- Sterile inoculation loop (x1 per sample)
- Sterile cotton swab (x1 per sample)
- Blood agar (x1 per sample)
- Non-sterile gloves, freezer gloves, laboratory coat
- -80^{o}C freezer with a thermometer and temperature log.
- STGG broth (1mL per isolate)
    a) Reagents:
        - Skim Milk Power (Oxoid LP0031) (2g per 100mL)
        - Tryptone Soya Broth (Oxoid CM0129) (3g per 100mL)
        - Glucose (0.5g per 110mL)
        - Glycerol (10mL per 110mL)
        - Distilled Water
    b) Equipment
        - 500mL Duran bottle
        - Cryogenic tube (1x per isolate)
        - Stirring Heat plate
        - P1000 pipette tips
        - Autoclave
        - Autoclave tape

Procedure:

1. Prepare STGG broth (if not pre-made)
    a. Mix the following ingredients into a 500mL Duran bottle. For power ingredients, carefully weight them out using disposable weighing boats

No.	Ingredients	Amount	Unit
1	Skim milk powder (Oxoid LP0031)	2	G
2	Tryptone Soya Broth (Oxoid CM0129)	3	G
3	Glucose	0.5	G
4	Glycerol	10	mL
5	Distilled water	100	mL

    b. Place the bottle on the stirring hot plate set to 100^{o}C
    c. Heat and stire the loquid until the power has dissolved and the solution is clear
    d. Aliquot 1mL of the mixture into each cryotube and place in a box
    e. Place a strip of autoclave tape on the box.
    f. Loosen the screw-cap tops and autoclave at 121°C for 10 minutes.
    g. Tighten caps after autoclaving and allow to cool.
    h. When cool:
        i. Label with the medium, date and batch number if more than one bottle is prepared.
        ii. Perform QC and sterility testing.

2. Quality Assurance of STGG broth:
    a. Every batch of agar or broth must be quality controlled for both sterility and the ability to support growth of target organisms, and suppression of non-target organisms in certain cases.
    b. To perform QC testing for STGG media select two random cryotubes from each box from the batch.
        i. Tube 1:
            1. Vortex well.
            2. Plate 100 μl onto a blood agar plate and incubate overnight at 37 °C. There should be *NO* growth on the plate.
        ii. Tube 2:
            1. Vortex well.
            2. Inoculate with Streptococcus pneumoniae ATCC 49619 (or similar known bacterial isolate eg. *S. aureus* used for CAMP testing)
            3. Freeze at -80 °C for 48 h.
            4. Thaw out at room temperature and vortex well.
            5. Subculture 100 μl onto a blood agar plate and incubate overnight at 37 °C. After this there should be *good* growth on the plate.
    c. If the batch fails QC, all tubes should be discarded and a new batch prepared: consideration should be given to the source of failure (e.g. incorrect autoclave cycle, omission of supplement).
    d. STGG batches that pass should be stored in the prepared tubes at 2-8 °C for up to six months.
3. Storage of isolates:
    a. Each unique colony on an ESBL and MDR chromogenic agar, and each GBS isolate should be stored in a separate cryotube in STGG broth.
    b. Culture the organism on blood agar and incubate overnight at 37^{o}C. Note: Ensure that the culture is pure: if mixed, pick off a well isolated colony and re-culture, and confirm its identity before saving.
    c. Place the cryotube and plate containing the isolated colony in a class II biosafety cabinet.
    d. Using a sterile cotton swab, harvest the entire growth from the young culture plate and dispense into the labelled cryotube containing 1mL STGG medium. NB: This step must be performed in the class II biosafety cabinet.
    e. Place the tube into a cryobox and store at -80°C freezer.
4. Complete the NeoCOL Sample Storage Log.

References

- Nil

Document History

Version	Author(s)	Approved by	Update Reason	Date	SOP No:
1.0	B. Dickson	P. Williams	New document	03AUG2023	NeoCOL_SOP07

Site Training Record

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