

## NeoCol: Standard Operating Procedure (SOP) for Stool Sample Collection V3.0

### Purpose:

The purpose of this SOP is to describe the standard procedures involved in obtaining stool samples from mothers and neonates according to the NeoCOL study protocol.

### Principal:

All stools passed by the enrolled infant should be collected. Particular attention should be paid to ensuring study staff collect samples from:

- a) The first neonatal stool
- b) Occur within 2 hours of a planned rectal swab (before or after)

One stool sample to be collected from enrolled mothers if possible.

Stool samples provide a representative sample of the bacteria that colonise the lower gastrointestinal tract of mothers and neonates. The bacterial samples can be analysed to determine which species are present, and whether they contain resistance genes. Collecting stools at multiple time points will provide important information about the acquisition and persistence of resistant bacterial strains, and also enable evaluation of how changes in the infant microbiome may alter following antibiotic exposure, or prior to clinical changes

### Responsibility:

This SOP applies to any clinical staff who are collecting stool samples from mothers and neonates participating in the NeoCOL study. It is the responsibility of those users to follow these guidelines when collecting rectal swabs for the study.

### Safety Requirements:

Gloves should be worn during swab collection. Standard clinical hand hygiene practices should be followed before, and after sample collection. Handle all specimens with care and treat them as potentially infectious material.

### Materials:

- Non-Sterile gloves.
- x1 disposable sterile spatula or tongue depressor.
- x1 stool container
- Plastic zip lock bag for specimen transport

### Procedure:

1. Explain the procedure to the parent/guardian and obtain verbal consent (see NeoCOL\_2.0\_SOP11 for process for consent). Label each of the specimen containers according to the label coding system (see appendix 1).
2. Complete Sample Request form (see appendix 2).
3. Perform hand hygiene and put on gloves.
4. Open the stool container being careful not to touch the inside or rim.
5. Collect the stool sample with disposable sterile laboratory spatula. Collect sufficient sample to fill approximately 1/3 of the jar if possible. In neonates, it is best to collect an adequate amount of stool from different areas of the diaper to ensure a representative sample.
6. Securely close the lid of the container, and place into the plastic ziplock bag.
7. Dispose of any waste as per hospital policies.
8. Transport the stool sample to the laboratory within 30 minutes, via pneumatic tube systems within 30 minutes for storage in fridge (2–8°C) in hospital microbiology department pending transport to processing laboratory
9. Samples should be transported to processing laboratory on the same day of collection in order to be stored at -80°C for future microbiome analysis. This laboratory will be based in Ho Chi Minh and will be a different laboratory from where the swabs will be processed.
10. Document the samples in the NeoCOL Sample Collection Log.

### References

- Nil

## Appendix 1: Specimen Labelling Code

| Site |                     | Participant Number | Mother/Child |        | Timepoint (day)     | Sample Type |                                                                                                  | HMU Lab                                                                                                     |
|------|---------------------|--------------------|--------------|--------|---------------------|-------------|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| E    | Hung Vuong Hospital | 001 – 999          | M            | Mother | 00                  | C           | Culture:<br>-gBs swab (group B streptococcus)<br>-Mdr swab (multidrug resistant)<br>-Fungal swab | <b>C</b> changed to:<br><b>-B</b> for gBs plates<br><b>-M</b> for MDR plates<br><b>-F</b> for Fungal plates |
|      |                     |                    |              |        |                     | G           | Genetic swab                                                                                     |                                                                                                             |
|      |                     |                    |              |        |                     | S           | Stool                                                                                            |                                                                                                             |
|      |                     |                    | C            | Child  | 00, 03, 07, 14, DIS | M           | Mdr swab (multidrug resistant)                                                                   |                                                                                                             |
|      |                     |                    |              |        |                     | G           | Genetic swab                                                                                     |                                                                                                             |
|      |                     |                    |              |        |                     | S           | Stool                                                                                            |                                                                                                             |
|      |                     |                    |              |        |                     | F           | Fungal swab                                                                                      |                                                                                                             |
|      |                     |                    |              |        |                     |             |                                                                                                  |                                                                                                             |

E.g. **E001C03S**: Hung Vuong Hospital, Participant 001, Child, Day 003, Stool

## Appendix 2: Laboratory Test Request form for mother & child



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NeoCOL Study



Mother's participation code

E    M  
(e.g., E123M)

### LABORATORY TEST REQUEST FOR MOTHER

Sample collection time

- ☐ Day 0  
☐ Other day (specify) ...../...../2025

Fill in the Sample ID on the samples that have been taken.

Note: These samples ID must match the id affixed to the submitted test sample.

|                | MDR swab                                    | Genetic swab                                | GBS swab                                    | Fungal swab                                 | Stool                                       |
|----------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
|                | xxM                                         | xxG                                         | xxB                                         | xxF                                         | xxS                                         |
| Sample ID      | <input type="text"/> <input type="text"/> M | <input type="text"/> <input type="text"/> G | <input type="text"/> <input type="text"/> B | <input type="text"/> <input type="text"/> F | <input type="text"/> <input type="text"/> S |
| Notes (if any) |                                             |                                             |                                             |                                             |                                             |



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SYDNEY

NeoCOL Study



Child's participation code

E    C  
(e.g., E123C)

### LABORATORY TEST REQUEST FOR CHILD

Sample collection time

- ☐ Day 0      ☐ Day 21      ☐ Day 48  
☐ Day 3      ☐ Day 28      ☐ Day 55  
☐ Day 7      ☐ Day 35      ☐ Other day (specify) ...../...../2025  
☐ Day 14      ☐ Day 42      ☐ Discharge date (specify) ...../...../2025

Fill in the Sample ID on the samples that have been taken.

Note: These samples ID must match the id affixed to the transferred test sample.

|                | MDR swab<br>(Rectal swab)                   | Genetic swab<br>(Rectal swab)               | Fungal swab<br>(Oral - inguinal -<br>rectal samples) | Immunological sample<br>(Blood Sample)<br>(Collect only at birth, day 0,<br>at discharge, and during<br>infection) | Stool                                       |
|----------------|---------------------------------------------|---------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
|                | xxM                                         | xxG                                         | xxF                                                  | xxH                                                                                                                | xxS                                         |
| Sample ID      | <input type="text"/> <input type="text"/> M | <input type="text"/> <input type="text"/> G | <input type="text"/> <input type="text"/> F          | <input type="text"/> <input type="text"/> H                                                                        | <input type="text"/> <input type="text"/> S |
| Notes (if any) |                                             |                                             |                                                      |                                                                                                                    |                                             |

**Document History**

| Version | Author(s)    | Approved by | Update Reason                                       | Date      | SOP No:          |
|---------|--------------|-------------|-----------------------------------------------------|-----------|------------------|
| 1.0     | B. Dickson   | P. Williams | New document                                        | 11JUL2023 | NeoCOL_SOP03     |
| 2.0     | R. Kelleghan | P. Williams | Adapted for NeoCOL 2.0                              | 7MAR2025  | NeoCOL_2.0_SOP03 |
| 3.0     | R. Kelleghan | P. Williams | Updated specimen labelling and storage requirements | 04JUN2025 | NeoCOL_2.0_SOP03 |
|         |              |             |                                                     |           |                  |

**Site Training Record**

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