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1. Introduction to the PRECISE Network

The PRECISE Network is being implemented in three countries in Sub-Saharan Africa: The Gambia, Kenya, and Mozambique.

The PRECISE Network has the following objectives:

1. To develop a unique cohort of pregnancies affected by placental disease and assess the prevalence of these disorders in women in three sub-Saharan African countries
2. To develop a cohort of non-pregnant women of reproductive age for comparison
3. To investigate social/cultural and health system barriers to access, effective management and appropriate care pathways
4. To investigate the potential for introducing novel methods for the diagnosis and management of placental disorders in sub-Saharan Africa
5. To identify biological markers that predict stillbirth, preterm birth, preeclampsia, intra-uterine growth restriction and other adverse maternal and neonatal outcomes
6. To facilitate future research by establishing in-country biorepositories of high quality biological samples and associated phenotypic data.

This document presents the standard operating procedures (SOPs) to guide the harmonised implementation of the PRECISE Network biorepositories across all three countries.

2. Purpose and Scope of the Standard Operating Procedure

2.1. PURPOSE for the SOP

This Standard Operating Procedure (SOP) describes the timing and the procedures for the collection, processing and storage of biological samples (venous and umbilical cord blood, placenta and umbilical cord tissues, urine, vaginal swabs and neonatal stool) from women and their newborn babies. Blood, urine and vaginal swab samples will be collected from the mother at specified times (see Table 2.1) during the pregnancy, at birth and at the postpartum visit; placental tissue and membranes, and cord blood samples will be collected at the time of the birth; or if cord blood was not collected, blood will be collected from the newborn before discharge or at the postpartum visit through a routine heel prick.
2.2. SCOPE of the SOP

This SOP covers the collection, processing, temporary and long-term storage of blood, placenta and umbilical cord tissues, urine, and vaginal swabs. It does not cover how to draw blood or how to conduct any assays performed with any of the samples after processing.

<table>
<thead>
<tr>
<th>Samples Collected</th>
<th>Enrolment (Non-Pregnant)</th>
<th>Booking (Pregnant)</th>
<th>Antenatal Visit (28^+2-36^+6)</th>
<th>Intrapartum</th>
<th>Delivery</th>
<th>Within 48 hours of delivery</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>16 ml</td>
<td>16 ml</td>
<td>16 ml</td>
<td></td>
<td>16 ml</td>
<td>16 ml</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>20 ml</td>
<td>20 ml</td>
<td>20 ml</td>
<td></td>
<td>20 ml</td>
<td>20 ml</td>
<td></td>
</tr>
<tr>
<td>Vaginal swabs</td>
<td>4 swabs</td>
<td>4 swabs</td>
<td>4 swabs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cord blood</td>
<td></td>
<td></td>
<td></td>
<td>16 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental tissue &amp; cord</td>
<td></td>
<td></td>
<td></td>
<td>13 small pieces, 1 membrane roll</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn blood</td>
<td></td>
<td></td>
<td></td>
<td>*2-3 drops from heel prick</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal stool</td>
<td></td>
<td></td>
<td></td>
<td>Sample/swab</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Heel prick will ONLY be done if cord blood is not collected at delivery.

Table 2.1  Overview of type of biological samples & the collection schedule

<table>
<thead>
<tr>
<th>Time of sample collection</th>
<th>Type of biological sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Booking</td>
<td>Maternal blood, maternal urine, maternal mid-vaginal swabs</td>
</tr>
<tr>
<td>Follow up (Between 28(^{+2}) weeks – 36(^{+6}) weeks’ gestation)</td>
<td>Maternal blood, maternal urine</td>
</tr>
<tr>
<td>Intrapartum (at the onset of labour)</td>
<td>Maternal mid-vaginal swab</td>
</tr>
<tr>
<td>Delivery</td>
<td>Umbilical cord blood, placenta and cord tissue, placental membranes</td>
</tr>
<tr>
<td>Within 48 hours post-partum</td>
<td>Newborn heel prick only if cord blood is not collected, maternal blood and maternal urine.</td>
</tr>
<tr>
<td>Post-partum visit (between 6 weeks and 6 months after delivery)</td>
<td>Neonatal stool swab/sample, maternal blood and urine.</td>
</tr>
</tbody>
</table>
2.3. ROLES AND RESPONSIBILITIES
The site co-ordinator (or his/her designee) is responsible for the implementation of SOP documentation at the participating sites.

The site co-ordinator is responsible for ensuring that all appropriate personnel are trained on this SOP.

All health care providers and technicians who implement this SOP at study sites are responsible for reading and understanding this SOP.

2.4. SAFETY PROCEDURES

- Risks
  - Biofluid exposure

- Required Safety Equipment
  - Lab coats
  - Closed toe shoes
  - Face shield/safety goggles (recommended)
  - Gloves

All research personnel and technicians are expected to be trained and follow universal safety precautions when handling biological or hazardous materials and when performing any of the procedures described in this SOP. This training is available on The Global Health Network.

The standard SOP approach assumes that all biological material is infective and potentially carrying HIV virus or other virulent factors. Therefore, all possible precautions are to be adhered to – both those outlined in this SOP and those imposed by the affiliated institution.

In the event of an outbreak in the surrounding area of the study site of a highly-contagious infectious disease like Ebola or a similar airborne or highly virulent pathogen, PRECISE Project Management will initiate an immediate suspension of sampling at the site. This situation is to be reported to the Project Management team as soon as it arises.

2.5. HARMONISED approach
The PRECISE SOPs are harmonised with other, similar large pregnancy cohort studies such as AMANHI, INTERBIO-21°, GPPS and HelTI as well as other publicly available SOPs. This way, samples from any of these studies can be pooled and analysed in future collaborative projects, increasing the power to detect underlying causes of poor pregnancy outcomes in LMICs.

3. Maternal Blood (pregnant and non-pregnant women)

**Time of collection**

1. Non-pregnant women – at time of enrollment
2. Pregnant women at booking - anytime ≤ 28° weeks’ gestation
3. Pregnant women at antenatal visit between 28°1 - 36°6 weeks’ gestation
4. Pregnant women after delivery - up to 48 hours after delivery
5. Pregnant women at post-partum follow-up visit
3.1. MATERIALS NEEDED

The equipment and designated containers are listed below:

Table 3.1 Overview of the maternal venous blood sample collection and processing

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Processing and aliquot assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Vacutainer EDTA 7 ml tube</td>
<td>EDTA tube</td>
</tr>
<tr>
<td></td>
<td>a) <strong>approximately 3 drops</strong> (the amount that will cover the active zone of the card) of whole blood applied to each of the sample zones on the blood spot card (<strong>only at booking sample collection for pregnant and non-pregnant women</strong>)</td>
</tr>
<tr>
<td></td>
<td>b) <strong>1 x 500 μl of whole blood</strong>, to be stored in a 0.7 ml FluidX tube,</td>
</tr>
<tr>
<td></td>
<td>c) <strong>6 x 500 μl plasma aliquots</strong> stored in 0.7 ml FluidX tube, to be closed by orange caps</td>
</tr>
<tr>
<td></td>
<td>d) <strong>1 x buffy coat</strong>, to be stored in a single 0.7 ml FluidX tube, to be closed by a white cap</td>
</tr>
<tr>
<td></td>
<td>Remaining sediment (centrifuged erythrocytes) is discarded</td>
</tr>
<tr>
<td>BD Vacutainer Serum Tube 6 ml</td>
<td>Serum tube (clotting for 30 minutes, then centrifugation)</td>
</tr>
<tr>
<td></td>
<td>a) <strong>6 x 500 μl serum aliquots</strong>, stored in 0.7 FluidX tube, to be closed by blue screw caps</td>
</tr>
<tr>
<td></td>
<td>Remaining sediment (centrifuged erythrocytes) is discarded</td>
</tr>
</tbody>
</table>

3.1.1. Kit Contents

a. **1 blood spot card** (**only at booking visit**) (Fisher 09.923.344)

b. **BD Vacutainer Safety-Lok** blood collection set 21G x 3/4” (Fisher Scientific 02-683-20)

c. **14 FluidX microtubes 0.7 ml** (Brooks 66-0700-00)

d. **6 external thread caps, orange** (68-53100-Z6N)

e. **1 external thread cap, red** (68-53100-Z2N)

f. **1 external thread cap, white** (68-53100-Z1N)

g. **6 external thread caps, blue** (68-53100-Z4N)
3.1.2. Other equipment and supplies

a. BD Vacutainer Serum Tube 6 ml (Fisher Scientific 02-683-94)
b. BD Vacutainer EDTA 7 ml tube (Fisher Scientific 02-685-2B)
c. Extra Safety-Lok set (Fisher Scientific 02-683-20)
d. Tourniquet
e. Silica gel
f. Antiseptic wipes
g. Gauze sponges
h. Adhesive bandages
i. Sharps container
j. Gloves
k. Pipette and 5 filter tips
l. Transfer pipette
m. Racks for FluidX tubes (Brooks Life Science Cat. # 66-51004)

3.2. MATERNAL BLOOD SAMPLE COLLECTION (PREGNANT AND NON-PREGNANT WOMEN)

Blood should be collected by a trained phlebotomist, midwife or research technician. This SOP does not detail the entire collection process.

1. Attach a unique participant ID label to each of the collection tubes and to the label on the kit bag.

2. A certified phlebotomy technician should draw blood into each of the vacutainer tubes using the blood collection kit provided. The order of the draw is: 6 ml serum tube (red top) then 7ml EDTA tube (purple top).

3. The serum tube should be inverted gently 5 times slowly (not shaken) to ensure that the reagents mix with the blood sample.

4. The EDTA tube should be inverted gently 8 to 10 times slowly (not shaken) to ensure that the reagents mix with the blood sample.

5. Record the time of sample collection, either by writing this on the kit bag or entering this into the database.

6. Set the timer for 30 minutes as soon as the blood draw has been completed as a reminder to centrifuge the tubes and place the serum tube in the rack to clot at room temperature.

7. Place the EDTA tube in a fridge/cool box until processing.

8. Once the serum tube has been sitting at room temperature for 30 minutes, if it is not going to be processed immediately, store it in a fridge/cool box until it is processed.
3.3. PROCESSING EDTA TUBE (PURPLE TOP)

3.3.1 Blood spot card - for 1st PRECISE visit only for pregnant and non-pregnant women

1. Flip over the card cover to expose the active zone (circle), without touching the active zone on the paper.

2. Remove the cap from the EDTA collection tube (purple top) and, using a pipette, withdraw 0.125 ml of blood. Carefully place drops of blood onto a pre-labelled zone, so the blood fills the designated circle (ideally just reaching the outer circular line).

3. Allow sufficient quantity of blood to soak through to completely fill the pre-printed active zone on the filter paper. Allow blood to dry in a clean place for 2-6 hours.

4. Once dried, flip the cover back over, align with the previous cards and store in the original cardboard box. Place the entire box in the sealable plastic bag and add silica gel pillows for storage.

5. Indicate on the box which sample ID range numbers are stored inside.

3.3.2 Whole blood

6. From the same EDTA tube (purple top), using a pipette, aliquot 500 μl of whole blood into the pre-barcoded FluidX tube with a red cap.

3.3.3 Plasma and buffy coat

7. Ensure the cap is securely fastened on the EDTA tube and place the tube in the centrifuge. Centrifuge at 4°C for 15 minutes at 2000 g.

8. Record the start time of centrifugation in the database.

9. After centrifugation, three layers should be visible as the blood separates. Take care when removing tube from centrifuge so not to disturb layers.

   a. **Top golden layer is plasma**, usually semi-transparent in nature.

      o If it is pink or red, then it is haemolysed and this should be noted.

      o If it is opaque or white in colour, it is lipaemic and this should be noted.

      o Occasionally there is a lipid layer on the plasma surface. Avoid inclusion of the lipid layer at the top of the plasma when taking aliquots.

   b. **Middle white layer is the buffy coat** made up of white blood cells. Avoid disturbing this layer when taking plasma aliquots.

   c. **Bottom dark red layer is the red blood cells**. It is not collected and should be discarded according to the on-site laboratory SOPs.

10. Using a pipette, transfer as many 500 μl aliquots of plasma as possible into the pre-barcoded FluidX tubes from the collection kit. Be careful not to disturb the buffy coat.

11. If the last aliquot is not a full 500 μl, collect as much plasma as you can without disturbing the buffy coat and record the volume of plasma collect in the database.
12. If there is any plasma remaining once the 6 aliquots have been prepared, any leftover plasma should be added to the final aliquot. The final volume of the 6th aliquot must be recorded in the database.

13. Fasten the orange caps onto the FluidX tubes.

14. Using a sterile transfer pipette take the buffy coat layer. Aspirate slowly and carefully, using a circular motion, to pull all the visible buffy coat material. Some contamination of red blood cells with the underlying layer is expected. Store in the pre-barcoded FluidX tube and close with the white cap.

15. Check that all aliquot tube caps are secure and that each tube has a sample ID label.

3.4 PROCESSING SERUM TUBE (RED TOP)

1. The serum tube must be left for the blood to clot for 30 minutes after collection. As close to 30 minutes after collection as possible, the sample should be processed.

2. Ensure the cap is securely fastened on the collection tube and place the serum tube in the centrifuge. Centrifuge at 4°C for 15 minutes at 2000 g.

3. Record the start time of centrifugation in the database.

4. After centrifugation, two layers will be visible, serum and sediment (erythrocytes). Normally the serum will be golden in colour and semi-transparent.
   a. If it is pink or red, then it is haemolysed and this should be noted.
   b. If it is opaque or white in colour, it is lipaemic and this should be noted.
   c. Avoid inclusion of the lipid layer at the top of the serum when taking aliquots.

5. Using a pipette, transfer as many 500 μl aliquots of serum as possible into the pre-barcoded FluidX tubes from the collection kit with blue caps.

6. If the last aliquot is not a full 500 μl, collect as much serum as you can and record the sample volume in the database.

7. If there is any serum remaining after the 6 aliquots have been prepared, any remaining serum should also be transferred to the 6th serum aliquot and the volume should be recorded in the database.

8. Fasten the blue caps onto the FluidX tubes and check that they are secure and that each tube has a sample ID label.
Converting RCF (g) to RPM:

3.5 STORAGE OF MATERNAL BLOOD SAMPLES

1. Once the aliquots have been prepared, the sample IDs, time of freezing and storage location should be recorded.

2. Place the samples in the -80°C freezer or liquid nitrogen (LN₂) storage container (where the LN₂ is in a gas phase) for freezing and storage. Do not flash freeze these samples or place them in liquid nitrogen, this is unnecessary for these sample types. The storage of these samples is site specific so refer to local SOPs for further sample storage instructions.

4. Maternal Urine (all samples)

Time of collection

1. Non-pregnant women – at time of enrollment
2. Pregnant women at booking - anytime ≤ 28\textsuperscript{th} weeks’ gestation
3. Pregnant women at antenatal visit between 28\textsuperscript{th}-36\textsuperscript{th} weeks’ gestation
4. Pregnant women after delivery - up to 48 hours after delivery
5. Pregnant women at post-partum follow-up visit

4.1. MATERIALS NEEDED

The equipment and designated containers are listed below.

Table 4.1 Overview of the maternal urine sample collection and processing
<table>
<thead>
<tr>
<th>Processing and aliquot assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 3 x 0.5 ml of urine to be stored in 0.7ml FluidX tubes closed with a yellow screw cap</td>
</tr>
<tr>
<td>b. 1 x 0.5 ml of urine sediment to be stored in 0.7ml FluidX tube closed with a green screw cap</td>
</tr>
</tbody>
</table>

4.1.1. Kit Contents

a. 4 FluidX microtubes (Brooks 66-0700-00)
b. 3 external thread caps, yellow (68-53100-Z3N)
c. 1 external thread cap, green (68-53100-Z6N)

4.1.2. Other equipment and supplies

a. 1 x 90 ml urine collection and transport container
b. 1 x 5 ml transfer pipette
c. 1 x 15 ml conical centrifuge tube (Fisher 62.554.205)

4.2. URINE SAMPLE COLLECTION

4.2.1. For collection at facility/lab – preferred option

1. Provide woman with the participant ID-labelled urine collection cup.

2. Ask the woman to provide a sample using the following instructions:
   - Remove the lid from the urine collection cup.
   - Begin to urinate into the toilet.
   - Pass the collection container into the urine stream, collecting at least 30 to 59 ml into the container.
   - Finish urinating into the toilet.
   - Screw the lid on the container and wipe any excess urine from the outside.
   - Wash hands
   - Return the container to the PRECISE lab technician for processing.

3. Collect the urine cup, ensure the lid is screwed on tightly. Record the time of sample collection, either write this on the kit bag or scan the participant ID into the database to record the sample collection time.

4. Store the urine in a fridge/cool box until it is processed.
4.2.2. When the woman cannot produce any urine at the study facility

Urine collection by the woman at home WILL NOT BE DONE in this study. Where for any reason, other than pathological, a woman is not able to provide urine during the visit, the study teams should take the following actions:

1. Explain to the woman that the urine sample collection is an important part of the study and that the study team will provide them bottled water to drink to help them fill their bladder and to be able to pass urine.

2. Explain that they may be asked to stay at the facility or return to the facility (if their homes are in close proximity to the facility) within 30 minutes or whenever they feel the urge to void.

3. Impress upon them to stay at the facility and explain that there may be instances where when they are at home and feel the urge to pass urine, they may not be able to hold till they return to the facility and the opportunity might be missed.

4. If for some reason they are unable to wait at the facility, study team should proceed with the blood sampling and retain the remaining kit separately (to avoid label confusion). THIS IS TO BE PERFORMED ONLY EXCEPTIONALLY, as it can lead to substantial labelling and misidentification problems.

4.3. URINE SAMPLE PROCESSING

Specimen processing should be completed and aliquots frozen within 2 hours of collecting urine from participants.

1. Using a single-use 5 ml transfer pipette, aliquot 0.5 ml of urine from the collection container into each of 3 pre-barcoded FluidX yellow-capped tubes.

2. To prepare the sediment for collection, using the single use 5 ml transfer pipette to transfer 14 ml of urine into the 15 ml conical (Falcon) tube.

3. Record the time of centrifugation in the database and centrifuge the sample at 4°C for 10 minutes at 2000 g.

4. After centrifugation, decant and discard the supernatant without disturbing the sediment in the conical tube.

5. Collect 0.5 ml of the sediment in the pre-barcoded FluidX green capped tube.

6. Check each of the aliquot caps are secure and each aliquot has a specimen ID label.

4.4. STORAGE OF MATERNAL URINE SAMPLES

1. Once the aliquots have been prepared, the sample IDs, time of freezing and storage location should be recorded in the database.

2. Place all the urine samples in the -80°C freezer or LN₂ storage container (where the LN₂ is in a gas phase) for freezing and storage. Do not flash freeze the samples or place them in liquid nitrogen, this is unnecessary for these sample types. The storage of these samples is site specific so refer to local SOPs for further sample storage instructions.
5. Umbilical cord blood

Time of collection

1. At the time of delivery – cord blood must be collected within 30 minutes from the delivery

Table 5.1 Overview of the cord blood sample collection & processing (similar to maternal blood)

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Processing and aliquot assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Vacutainer EDTA 7 ml tube</td>
<td><strong>EDTA tube</strong></td>
</tr>
<tr>
<td></td>
<td>a) approximately 3 drops (the amount that will cover the active zone of the card) of whole blood applied to each of the sample zones on the blood spot card</td>
</tr>
<tr>
<td></td>
<td>b) 1 x 500 μl of whole blood, to be stored in a 0.7 ml FluidX tube, to be closed by a red cap</td>
</tr>
<tr>
<td></td>
<td>c) 6 x 500 μl plasma aliquots stored in 0.7 ml FluidX tube, to be closed by orange screw caps</td>
</tr>
<tr>
<td></td>
<td>d) 1 x 500 μl buffy coat, to be stored in a single 0.7 ml FluidX tube, to be closed by a white cap</td>
</tr>
<tr>
<td></td>
<td>Remaining sediment (centrifuged erythrocytes) is discarded</td>
</tr>
<tr>
<td>BD Vacutainer Serum Tube 6 ml</td>
<td><strong>Serum tube</strong> (clotting for 30 minutes, then centrifugation)</td>
</tr>
<tr>
<td></td>
<td>a) 6 x 500 μl serum aliquots, stored in 0.7 FluidX tube, to be closed by blue caps.</td>
</tr>
<tr>
<td></td>
<td>Remaining sediment (centrifuged erythrocytes) is discarded</td>
</tr>
</tbody>
</table>

5.1. MATERIALS NEEDED
The equipment and designated containers are listed below:

5.1.1. Kit Contents

a. 1 blood spot card (Fisher 09.923.344)

b. BD Vacutainer Safety-Lok blood collection set 21G x 3/4” (Fisher Scientific 02-664-3)

c. 14 FluidX microtubes 0.7 ml (Brooks 66-0700-00)

d. 6 external thread caps, orange (68-53100-Z6N)

e. 1 external thread cap, red (68-53100-Z2N)
f. **1 external thread cap, white** (68-53100-Z1N)

g. **6 external thread caps, blue** (68-53100-Z4N)

### 5.1.2. Other equipment and supplies

| a. BD Vacutainer Serum Tube 6 ml (Fisher Scientific 02-683-94) |
| b. BD Vacutainer EDTA 7 ml tube (Fisher Scientific 02-685-2B) |
| c. Extra Safety-Lok set (Fisher Scientific 02-664-3) |
| d. Silica gel |
| e. Gauze sponges |
| f. Sharps container |
| g. Gloves |
| h. Pipette and filter tips |
| i. Transfer pipette |
| j. Racks for FluidX tubes (Fisher Scientific 66-51004) |

### 5.2. CORD BLOOD SAMPLE COLLECTION

Cord blood collection must be completed at the latest within 30 minutes after delivery of the placenta to avoid clotting of blood. Cord blood should be collected by a trained midwife or research technician. In case of time delays proceed to SOP section 5.6 (delayed cord blood collection).

If the delivery takes place at home, the placenta should be transported to the laboratory for processing as soon as possible.

1. Immediately after delivery of the placenta, place two clamps on the cord, one as close to placenta as possible, and the other at the end of the cord. **Please note: Early clamping and cutting of the cord (within 60 seconds) of the baby’s birth is not necessary. If you are practising delayed cord clamping, please continue to do so.**

2. Record the time of placenta delivery.

3. Wear personal protective equipment including eye protection and two sets of gloves. Have extra gauze available as the cord blood can be under high pressure.

4. Identify the fetal vein and using clean dry gauze wipe excess blood and fluids from a small 2-3 cm area of the fetal vein for a clear puncture.

5. Insert the needle into the vein, following the direction of the vein. Avoid puncturing all the way through the vein.

6. Collect 6 ml of blood into the serum tube (red cap) and then collect 7 ml of blood into the EDTA tube (purple cap).

7. The serum tube (red cap) should be inverted gently 5 times slowly (not shaken) to ensure that the reagents mix with the blood sample.

8. The EDTA tube (purple cap) should be inverted gently 8 to 10 times slowly (not shaken) to ensure that the reagents mix with the blood sample.
9. Remove collection needle from cord vein and dispose of in sharps container.

10. Set timer for 30 minutes as soon as the blood draw has been completed as a reminder to centrifuge both tubes.

11. Record the time of sample collection, either write this on the kit bag or scan the participant ID into the database to record the sample collection time.

12. Attach a participant ID label to each of the collection tubes and to the kit label.

13. Transfer the samples to the laboratory for processing.

14. Place the serum tube in the rack to clot at room temperature.

15. Place the EDTA tube in a fridge/cool box until processing.

16. Once the serum tube has been sitting at room temperature for 30 minutes, if it is not going to be processed immediately, store it in a fridge/cool box until it is processed.

5.3. PROCESSING EDTA TUBE (PURPLE TOP)

5.3.1 Blood spot card

1. Flip over the card cover to expose the active zone (circle), but not touching the active zone on the paper.

2. Remove the cap from the EDTA tube (purple top) and using a pipette, withdraw 0.125 ml of blood. Carefully place drops of blood onto a pre-labelled zone, so the blood fills the designated circle (ideally just reaching the outer circular line).

3. Allow sufficient quantity of blood to soak through to completely fill the pre-printed active zone on the filter paper. Do not layer successive drops of blood or apply blood more than once in the same collection circle. Allow blood to dry in a clean place for 2-6 hours.

4. Once dried, flip the cover back over, align with the previous cards and store in the original cardboard box. Place the entire box in the sealable plastic bag and add silica gel pillows for storage.

5. Indicate on the box which sample ID range numbers are stored inside.

5.3.2 Whole blood

1. From the same EDTA tube (purple top), using a pipette, aliquot 500 μl of whole blood into the pre-barcoded FluidX tube with red cap.

5.3.3 Plasma and buffy coat

1. Ensure the cap is securely fastened on the EDTA tube and place the tube in the centrifuge. Centrifuge at 4°C for 15 minutes at 2000 g.
2. Record the start time of centrifugation in the database.
3. After centrifugation, 3 layers should be visible as the blood separates.
   a. **Top golden layer is plasma**, usually semi-transparent in nature.
      - If it is pink or red, then it is haemolysed and this should be recorded.
      - If it is opaque or white in colour, it is lipaemic and this should be recorded.
      - Occasionally there is a lipid layer on the plasma surface. **Avoid** inclusion of the lipid layer at the top of the plasma when taking aliquots.
   b. **Middle white layer is the buffy coat** made up of white blood cells. Avoid disturbing this layer when taking plasma aliquots.
   c. **Bottom dark red layer is the red blood cells**. It is not collected and should be discarded according to laboratory SOPs.
4. Using a pipette, transfer as many 500 μl aliquots of plasma as possible into the pre-barcoded FluidX tubes from the collection kit. Be careful not to disturb the buffy coat.
5. If the last aliquot is not a full 500 μl, collect as much plasma as you can without disturbing the buffy coat and record the plasma volume in the database.
6. If there is any plasma remaining once the 6 aliquots have been prepared, any leftover plasma should be added to the final aliquot. The final volume of the 6th aliquot must be recorded in the database.
7. Fasten the orange caps onto the FluidX tubes.
8. Using a sterile transfer pipette take the buffy coat layer. Aspirate slowly and carefully, using a circular motion, to pull all the visible buffy coat material. Some contamination of red blood cells with the underlying layer is expected. Store in the pre-barcoded FluidX tube and close with the white cap.
9. Check that all aliquot tube caps are secure and that each tube has a sample ID label.

**5.4. PROCESSING OF SERUM TUBE (RED TOP)**

1. The serum tube must be left for the blood to clot for 30 minutes after collection. As close to 30 minutes after collection as possible, the sample should be processed.
2. Ensure the cap is securely fastened on the collection tube and place the serum tube in the centrifuge. Centrifuge at 4°C for 15 minutes at 2000 g.
3. Record the start time of centrifugation in the database.
4. After centrifugation, two layers will be visible, serum and sediment (erythrocytes). Normally the serum will be golden in colour and semi-transparent.
   - If it is pink or red, then it is haemolysed, and this should be recorded.
   - If it is opaque or white in colour, it is lipaemic and this should be recorded.
   - **Avoid** inclusion of the lipid layer at the top of the serum when taking aliquots.
5. Using a pipette, transfer as many 500 μl aliquots of serum as possible into the pre-barcoded FluidX tubes from the collection kit with blue caps.
6. If the last aliquot is not a full 500 μl, collect as much serum as you can and record the sample volume in the database.
7. If there is any serum remaining after the 6 aliquots have been prepared, any remaining serum should also be transferred to the 6th serum aliquot and the volume should be recorded in the database.

8. Fasten the blue caps onto the FluidX tubes and check that they are secure and that each tube has a sample ID label.

5.5. DELAYED CORD BLOOD SAMPLE COLLECTION

5.5.1. Overview
This section of the protocol is only to be used in cases when the blood in cord is starting to clot and normal cord blood collection is not possible (usually in cases where the placenta is received at the site for the processing **more than 30 mins after delivery**). It is important to note that the blood may not be fully clotted unless around 3-6 hours post-delivery. This protocol, therefore, will be used to collect both semi-clotted blood and actual clots that will be used for fetal DNA extraction. The importance of this section of the protocol is to collect as much of the fetal blood and clots without contamination by maternal tissue (please note that even minimal contamination with maternal tissue may affect the results).

5.5.2. Kit Contents
The equipment and designated containers are listed below.

| a. 1 x 5ml tube (Sarstedt 60.542.024) |

5.5.3. Other equipment and supplies

| a. Steel dissecting tray |
| b. Scissors/ Scalpels (optional) |
| c. Forceps |
| d. Kidney tray *(for cord blood clots only)* |
| e. Gauze sponges |
| f. Sharps container |
| g. Gloves |

5.5.4. Sample collection

1. Place placenta into a tray, cord facing up; extend the cord next to placenta; identify the section of the cord that seems to contain the clot (thicker and possibly darker in colour than the remaining parts).

2. Clean the cord surface with gauze to reduce the contamination.

3. Place the section of the cord where you are collecting potential semi-clotted blood or clots in a clean petri dish. If this process is taking place within 3 hours of the placenta delivery, try to collect semi-viscous blood sample into an EDTA tube and proceed in line with section 5.3. However it is important you collect blood in the EDTA vacutainer if you only have a small volume of blood. If more than three hours have passed from the delivery of placenta or the blood is already clotted, proceed to step 4. If, however you have collected blood in an EDTA tube, you do not have to proceed in collecting clots.
4. Use the scissors to cut the cord in several smaller parts of about 1-1.5 cm in length, and gently expose the clotted material outwards.

5. If any semi-clotted blood is available ensure that this is collected in the petri dish or a similar temporary container, making sure that risk of sample contamination with maternal tissue is reduced to a minimum.

6. Use the forceps to collect one or more pieces of clots and place them in a pre-barcoded white-cap freezing 5 ml tube (no reagents); try to fill the tube as much as possible, again trying not to contaminate the sample with maternal blood or tissue. Also transfer the semi-clotted blood into the tube.

5.6. STORAGE OF CORD BLOOD SAMPLES

1. Once the aliquots have been prepared, the sample IDs, time of freezing and storage location should be recorded in the database.

2. Place the samples in the -80°C freezer or LN$_2$ storage container (where the LN$_2$ is in a gas phase) for freezing and storage. Do not flash freeze these samples or place them in liquid nitrogen, this is unnecessary for these sample types. The storage of these samples is site specific so refer to local SOPs for further sample storage instructions.

6. Placenta and umbilical cord tissue

**Time of collection**

| At the time of delivery – all cord and placenta tissue samples must be processed within 30 minutes from the delivery |

**Key points:**

- **a.** Cord blood collection should occur before tissue collection.

- **b.** All placental tissue collected for preservation **MUST be collected and processed <30 minutes** after the woman delivers the placenta. If placenta arrives later than **30 minutes after the delivery of the placenta**, proceed with the same sampling scheme, **BUT NOTE THE TIME OF SAMPLING.**

- **c.** Placenta and cord should be fresh and **MUST NOT HAVE BEEN TREATED WITH ANY PRESERVATIVES OR REAGENTS** (e.g., formalin or ethanol) before tissue collection.

6.1. MATERIALS NEEDED

The equipment and designated containers are listed below:

**6.1.1. Kit Contents**

- **a.** 1 x 8 mm biopsy punch (Fisher 12-460-413)

- **b.** 3 x 5 ml tubes for flash frozen samples (Sarstedt 62.558.201)
c. One specimen cup for formalin samples (75.562.105)
d. 4 x 8 ml tubes for storage in ethanol (Sarstedt 60.542.024)

6.1.2. Other Apparatus and Supplies Needed on Site

| a. Sterile disposable scalpel (Fisher SCA-310-150A) |
| b. Additional 8mm biopsy punches (Fisher 12-460-413) |
| c. Sterile forceps, straight, fine point, 150mm (Fisher DKC-370-070F) |
| d. Scissors |
| e. Extra-long histology forceps (Fisher 10316C) |
| f. Large stainless steel dissecting tray (Fisher 8019) |
| g. Plastic cutting board |
| h. Camera |
| i. Laboratory scales |
| j. Metric rulers |
| k. Gloves |
| l. Plastic Apron |
| m. Eye protection (goggles, panel, face shield or an extractor) |
| n. Formalin Solution: 10% normal buffered formalin. Store at room temperature (15-30°C) until used. Discard if visible signs of contamination develop, such as colour change or cloudy appearance. |
| o. Ethanol Solution: 70% histology grade ethanol solution |
| p. Sterile phosphate buffered saline (PBS) buffer |

6.2. PREPERATORY ACTIONS AND PHOTOS

Two people should be prepared and ready for placenta sample collection and processing.

6.2.1. Setting up the bench

1. Set up the bench with all required equipment, taking care with the sharp instruments.

2. Place all the 5 ml tubes for flash freezing on the rack.

3. Put 40 ml formalin into the collection cup and 5 ml into the placental tissue formalin tubes for histological samples.

Take the photos first, before taking placental samples

6.2.2. Required Equipment

| a. Participant ID Labels |
| b. Two metric rulers |
| c. Digital Camera |
| d. Gauze |
6.2.3. Responsibilities of the 2nd person supporting in the sample collection

a. One of the two people involved in the sample collection as mentioned above will be mainly responsible for the picture taking. This person should handle the camera whilst the other person positions the placenta for the picture taking.

b. Once the photographs are taken, the person handling the camera for the photographs will then assist in the sample collection especially with the washing of the samples from the punches for processing for storage.

6.2.4. Photographs and Dimensions

1. Excess blood should be wiped off the placenta with sterile gauze and the placenta should then be placed on the white plastic dissecting board. The umbilical cord, including separated sections of cord, should be laid flat in the field of view of the photo. Position the membranes so they do not obscure the placenta.

2. Make sure the ID label is clearly visible in ALL photos for identification.

3. No flash should be used, use low light setting on the camera

4. Photo 1: Is a picture of the whole placenta and umbilical cord with the “Fetal Side Up” (the side to which the cord attaches).
   a. Make sure the unique Participant ID label is visible in the photo.
   b. Place metric rulers at right angles next to the placenta and make sure they are visible in the photo for reference.

5. Photo 2: Is a picture of the whole placenta and umbilical cord with the “Maternal Side Up” (the rough side). The point where the umbilical cord comes out of the placenta should be face down against the absorbent pad.
   a. Make sure the unique Participant ID label is visible in the photo.
   b. Place metric rulers next to tissue and make sure they are visible in the photo.

6. Photos should be uploaded and saved to a computer. The file should be named in the format ‘Unique Participant Number-photo type-date’ e.g. 258-105891-maternal-31012018.
6.3. PLACENTAL SAMPLE COLLECTION AND PROCESSING

6.3.1. Tissue processing

The first step is to relocate the placental membranes to the side, gently rolling them on to avoid tissue destruction, since samples of this are to be taken later. Next, the cord insertion should be oriented in the top middle portion, to be avoided while working on the maternal placenta side.

After relocating of the membrane, the initial step of the sampling protocol is to define three regions of placenta, A, B and C. All three sites need to have a good central and peripheral sampling tissue sections, that need to be macroscopically free from apparent lesions, large blood clots or areas that do not look normal, and should not be close to the cord insertion (approximately 3 cm from the placental edges or the cord insertion site), where possible.

6.3.2. Full thickness biopsy punches

1. Clean the sites A, B and C with a dry gauze to remove any blood.

2. Use an 8 mm punch to collect two full thickness vertical samples at site A, one more centrally, the other more on the periphery (closer the placental edge) but both more than 3 cm from the insertion point or the edge of the placenta (where possible).

3. Rinse in 1 X sterile phosphate buffered saline (PBS) to remove excess blood and/or clots, and blot briefly.

4. Repeat the same process in sites B and C to collect full thickness samples, one central sample and one peripheral sample.

5. Place the three central samples (one from sites A, B and C) in a pre-barcoded tube placing the samples on the edge on the tube. This process will allow the samples to be taken from the tube one a time during the analysis stage.

6. Place the three peripheral samples (one from site A, B and C) in a pre-barcoded tube placing the samples on the edge on the tube. This process will again allow the samples to be taken from the tube one a time during the analysis stage.

7. Scan the sample ID and record the storage location of the sample in the database.

8. Using extra-long forceps, carefully lower the capped tube into liquid nitrogen making sure only the bottom of the tube is submerged, you do not want any liquid nitrogen getting in to the tube. Once the sample has frozen, remove the tube from the liquid nitrogen.

9. Place the samples in the -80°C freezer or LN₂ storage container for storage. The storage of these samples is site specific so refer to local SOPs for further sample storage instructions.
6.3.3. Tissue block

1. Select the largest of three sites (site C)

2. In the area next to where you took the central punch, select an area to collect the tissue section which will be 1 cm x 0.3 cm x full thickness. Carefully dissect out this area using a scalpel or scissors ensuring all layers present. Try to retain as much tissue structure as possible by minimising any manipulation of the sample. Place the sample in an 8ml tube filled with formalin labelled with ‘Central sample’.

3. Repeat the process above collecting a full thickness sample in the area next to where you took the peripheral punch. Place the sample in an 8ml tube with formalin labelled with ‘Peripheral sample’.

6.3.4. Umbilical Cord Tissue Sample Collection and Processing

1. The umbilical cord should be sampled from the fetal end of the cord remaining on the placenta after delivery. This is the end furthest from the placenta.

2. Avoid sections of the umbilical cord that were previously clamped or sustained needle punctures during the cord blood collection.

3. Avoiding the clamp site, cut the cord at base where it is attached to the placenta disc.

4. **Cut four 0.5 cm segments:** Using forceps, securely hold the distal end of the umbilical cord. Using scissors or a sharp scalpel, cut four 0.5cm segments of the cord.

5. Rinse in 1 X PBS to remove excess blood and/or clots, and blot briefly. Put all 4 sections in one 5 ml tube making sure the samples are seperated and placed against the edge of the tube.

6. Record the sample ID’s and storage location in the database, this will register the time of sample freezing.

7. Immediately, using extra-long forceps, carefully lower the capped tube into liquid nitrogen making sure only the bottom of the tube is submerged, you do not want any liquid nitrogen getting into the tube. Once the sample has frozen, remove the tube from the liquid nitrogen.

8. Place the samples in the -80º freezer or LN$_2$ storage container for storage. The storage of these samples is site specific so refer to local SOPs for further sample storage instructions.
9. **Cut 1.0 cm segment for formalin**: Dissect another segment, this time approximately 1cm in length from the same part of the cord. Place this sample in the large container with formalin.

10. Record the time the sample was placed in formalin in the database.

### 6.3.5 Placental Membrane Sample Collection and Processing

1. Locate the placental membranes with the maternal side up. Make sure that the area selected has both layers of membranes. Orient the placenta so that the disc lies flat and the membranes are spread out as much as possible without damaging them. The point of membrane rupture is easier to see from the maternal surface (see picture with arrow).

2. Using a scalpel, cut a 1 cm wide strip of membrane from the rupture site to the edge of the placental disc (margin).

3. Detach the membrane strip from the placental disc with a small amount of placental tissue on the edge, to allow membrane rolling around a short wooden stick. Place the membrane roll in the large container with formalin with the cord tissue.

4. Attach a participant ID label to the formalin containers.

### 6.4. SAMPLES IN FORMALIN

1. Place a participant ID label on the containers and store at 4°C for 48-72 hours.

2. After 48-72 hours, carefully decant the formalin into a formalin waste container then add 40 ml (or enough to cover the samples) of 70% ethanol to the large container and 6 ml to each small tube. Shake the containers for 1 minute and decant the ethanol. Repeat this process twice.

3. Transfer the samples from the large formalin pot into pre-barcoded 8 ml specimen tubes from the collection kit. Add 4 ml of 70% ethanol or enough to ensure they are covered. Store at 4°C. For the two punches already in 8 ml tubes, add ethanol to these tubes to ensure the sample is fully submerged and store at 4°C.

4. Scan the sample IDs and enter the storage locations of the samples into the database.

5. Arrange for these samples to be embedded in paraffin according to local SOPs.

### 6.5. TRIMMED PLACENTA WEIGHT MEASUREMENT

1. The placenta must be trimmed prior to weighing. Cut off the umbilical cord at the point that it attaches to the placental disc and trim off all the membranes.

2. The placenta weight should be taken at room temperature. Use a metric scale to record the weight of the tissue. Make sure to “tare” (zero) the scale or subtract the weight of the empty container holding the tissue when weighing all tissue.
3. Place the placenta on the scales and record the weight in the database.

4. Discard the placenta samples in a biohazard waste container or return to the family, depending on your local conditions and the study logistics possibilities.

7. Mid-vaginal swab

Time of collection

| 1. Non-pregnant women – at enrollment       | 2. Pregnant women during the booking visit | 3. Pregnant women intrapartum - after arrival at facility, before delivery |

7.1. MATERIALS NEEDED

The equipment and designated containers are listed below.

Table 7.1 Overview of the vaginal swab processing

<table>
<thead>
<tr>
<th>Processing and aliquot assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Biochemistry swabs – the Dacron swabs to be collecting in a 2ml FluidX tube closed with a clear/natural cap but stored in 0.7 ml FluidX tube closed by a blue cap.</td>
</tr>
<tr>
<td>b) Microbiome swabs – the FLOQswabs to be stored in 2 ml FluidX tube closed by an orange cap.</td>
</tr>
</tbody>
</table>

7.1.1. Kit Contents

<table>
<thead>
<tr>
<th>a. Sterile Dacron swabs (TSC SLS, SWA2046)</th>
<th>b. Sterile FLOQswabs (Copan Thermo Fisher Scientific 493CE02)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c. 7 FluidX microtubes 0.7 ml (Brooks 66-0700-00)</td>
<td>d. 7 external thread caps, light blue</td>
</tr>
<tr>
<td>e. 2 Fluid X tubes, 1.8ml (Brooks 64-7506)</td>
<td>f. 2 external thread caps, orange (Brooks 65-7552)</td>
</tr>
</tbody>
</table>

7.1.2. Other equipment and supplies

<table>
<thead>
<tr>
<th>a. Mini Complete Protease inhibitor tablet (Roche Diagnostics 11697498001)</th>
<th>b. Sterile PBS (Sigma D8662)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c. Sterilised TE buffer (x1 molecular biology grade; Promega V6232)</td>
<td>d. Transfer pipettes (1.5 ml sterile; VWR 612-0965)</td>
</tr>
<tr>
<td>e. Sterile 30 ml universal tubes (Elkay 500-1000-301)</td>
<td>f. FluidX tubes 1.8 ml with light green caps (68-53100-Z8N)</td>
</tr>
<tr>
<td>g. Clean scissors (clean with ethanol or cleaning fluid between samples to prevent contamination)</td>
<td></td>
</tr>
</tbody>
</table>
7.2 SAMPLE PREPARATION

7.2.1 Preparation of protease inhibitor solution for biochemistry:

1. Where possible in Class 2 hood, add 50 ml of sterile PBS + 1 Mini Complete protease inhibitor tablet to a 50 ml sterile falcon tube. Vortex and allow to sit at room temperature for about 20 min for tablet to dissolve. Vortex again.

2. Where possible in the class 2 hood, remove the lids from the 1.8 ml FluidX tube with clear/natural caps and aliquot 750 µl of the PBS+protease inhibitor into each tube. Replace caps.

3. Place the tubes in a freezer for storage until use. They can be stored for a maximum of 6 months.

7.2.2 Preparation of TE buffer in tubes for microbiome swabs:

1. Prior to sample collection, ensure the two 1.8 ml tubes with orange caps from the kit have 1 ml of TE buffer aliquoted into them in the laboratory. These should then be immediately transferred to the clinic for sample collection. If the tubes are not used, the whole kit should be stored in the fridge until a kit is required.

7.3. VAGINAL SWAB SAMPLE COLLECTION

1. Before samples are collected, collect a sample collection kit ensuring you have two tubes with orange caps with TE buffer (TE buffer stored in the fridge will need to be aliquoted in to the tubes in the kit prior to sample collection) and two tubes with a natural/clear cap containing PBS+protease inhibitor (stored in the freezer, thawed before adding swab).

2. The samples in the natural/clear capped tubes must be kept on ice or in a fridge once removed from the freezer.

3. Participants will be asked to lie on a couch, having removed their underwear, with a sheet over their waist to maintain dignity. The participant will be asked to bend their legs with their feet together moved close to their bottom; knees moved outwards.

BIOCHEMISTRY SWABS

4. The research nurse/midwife wearing gloves will part the labia and gently insert the two Dacron swabs into the mid-vagina (approximately 4-5 cm) and rotate gently 360° for 15-20 seconds. The exposure is needed for the vaginal fluid to enter the swab stick. Failure to retain the stick for this duration might result in insufficient vaginal fluid collection, making the sample useless.

5. After removal of swabs from vagina, take the two pre-made tubes containing the PBS+protease inhibitor (natural/clear cap). Carefully open each of the two tubes and cut off the Dacron swab tips using scissors, placing one in each tube. Make sure that the tip is covered by the fluid.

MICROBIOME SWABS

6. Using the FLOQswabs, repeat the collection process as above, ensuring the swabs are gently rotated 360° in the mid-vagina for 15-20 seconds to collect sufficient sample.

7. For the FLOQswabs, place one swab into a 1.8 ml FluidX tubes containing TE buffer with orange cap. Rotate against the side of the tube for 60 seconds to remove the bacteria. The swab should then be removed from the tube and discarded.
8. Repeat this process with the second FLOQswab in the second FluidX tube with orange cap.

9. Keep tubes on ice for transportation to the laboratory and kept in the fridge/cool box on arrival in the lab until the samples are processed. Please note that the sample must be processed within an hour of collection.

10. Record time of sample collection on the kit bag.

7.4. SWAB SAMPLE PROCESSING

1. Record the time samples arrived in the lab in the database

2. The orange capped tubes with the microbiome FLOQswab sample do not need any processing. Record the details in the database and store.

3. To process the samples taken with Dacron swabs for biochemistry, remove the two swab tips from the tubes with natural/clear caps and place into two clean tubes. Spin the tubes (that originally contained the swab) containing PBS + protease inhibitor and the clean tubes containing the tips for 10 minutes at 4°C at 2500 g.

4. Remove the tips and pool all of the PBS and extra residual sample into one tube and spin the tube again for 10 mins at 4°C at 2500 g.

5. Using a pipette, transfer as many 200 μl aliquots of the resultant supernatant as possible into FluidX tubes with light blue caps for storage. If the final sample is less than 200 μl, record the volume in the database.

6. Enter data in the database.

7. Place the samples in the -80°C freezer or LN₂ storage container (where the LN₂ is in a gas phase) for freezing and storage. Do not flash freeze these samples or place them in liquid nitrogen, this is unnecessary for these sample types. The storage of these samples is site specific so refer to local SOPs for further sample storage instructions.

8. NEWBORN STOOL SWAB

Time of collection

1. Six weeks to six months after delivery

8.1 MATERIALS NEEDED

The equipment and designated containers are listed below.

Table 8.1 Overview of the stool sample processing

<table>
<thead>
<tr>
<th>Processing and aliquot assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 1 x stool swabs to be stored in lysis shield tube with green cap</td>
</tr>
</tbody>
</table>
8.2 NEWBORN STOOL SWAB SAMPLE COLLECTION

1. Using the sterile swab provided to swab the baby’s nappy to collect a small amount of stool. Roll the cotton swab in the stool to coat the tip.

2. Place the swab (covered in stool) into a green capped tube and snap off the swab head at the break point leaving the tip of the swab in the tube.

3. Close the tube tightly and shake for 30 seconds.

4. Repeat this process for the second swab placing the sample in the second green capped tube.

5. Record the time of sample collection and transport to the lab for processing.

8.3 NEWBORN STOOL SAMPLE PROCESSING

1. Place the samples in the -80°C freezer or LN\textsubscript{2} storage container (where the LN\textsubscript{2} is in a gas phase) for freezing and storage. Do not flash freeze these samples or place them in liquid nitrogen, this is unnecessary for these sample types. The storage of these samples is site specific so refer to local SOPs for further sample storage instructions.

9. Heel prick

**Time of collection**

*During the post-natal visit, in cases where no cord blood sample was collected*

9.1. MATERIALS NEEDED

The equipment and designated containers are listed below.

9.1.1. Kit Contents

| a | 1 Lancet (Sarstedt 85.1019) |
| b | 1 Blood spot card (Fisher 09.923.344) |

9.1.2. Other equipment and supplies

| a | Cotton wool or gauze |
| b | Gloves |
| c | Disinfectant (alcohol) |

9.2. HEEL PRICK SAMPLE COLLECTION

*Flip over the card cover to expose the active zone (circle), but not touching the active zones on the paper.*

1. Clean the selected area of skin with a skin disinfectant and allow to dry for 30 seconds. Warming up of the foot facilitates dilatation of blood vessels and ensures faster and easier protocol completion

2. Position the foot with the puncture site downwards. Take care to keep away from bony prominences. Press the loaded lancing device against the skin and push the white plunger.
3. While holding the foot correctly, apply and release pressure to allow a drop of blood to form. Allow a large drop of blood to collect.

4. Once a drop of blood has formed, lightly touch the drop to the pre-printed circle on the blood spot card, allowing it to soak onto the circle. Allow sufficient quantity of blood to soak through to completely fill the pre-printed active zone on the filter paper. Do not layer successive drops of blood or apply blood more than once in the same collection circle.

5. Record the sample collection time in the database.

9.3 SAMPLE PROCESSING

1. Once the sample has arrived in the laboratory, allow blood to dry in a clean place for 2-6 hours.

2. Once dried, flip the cover back over, align with the previous cards and store in the original cardboard box. Place the entire box in the sealable plastic bag and add silica gel pillows for storage.

3. Record the sample ID and the samples storage location in the database.
## 10. Colour coding scheme for the FluidX tubes

<table>
<thead>
<tr>
<th>Volume</th>
<th>Description</th>
<th>Cap Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7 ml</td>
<td>whole blood tube (mother, cord blood)</td>
<td>red</td>
</tr>
<tr>
<td>0.7 ml</td>
<td>plasma tube (mother, cord blood)</td>
<td>orange</td>
</tr>
<tr>
<td>0.7 ml</td>
<td>buffy coat tube (mother, cord blood)</td>
<td>white</td>
</tr>
<tr>
<td>0.7 ml</td>
<td>serum tube (mother, cord blood)</td>
<td>blue*</td>
</tr>
<tr>
<td>0.7 ml</td>
<td>urine tube (mother)</td>
<td>yellow</td>
</tr>
<tr>
<td>0.7 ml</td>
<td>urine sediment tube (mother)</td>
<td>green</td>
</tr>
<tr>
<td>0.7 ml</td>
<td>vaginal swab – biochemistry tube (mother)</td>
<td>light blue</td>
</tr>
<tr>
<td>1.8 ml</td>
<td>vaginal swab – microbiome tube (mother)</td>
<td>orange</td>
</tr>
</tbody>
</table>