Tuesday, March 20, 2024

PARAM SAMPLE COLLECTION AND STORAGE:

Sample collection summary:

- For hospital-based recruitments – Sample collection to be taken at one time point (date of first visit, i.e., baseline), after assessments and neuroimaging (especially blood).
- For community-based recruitments – Following recruitment and neuropsychological assessments sample collection to be taken at a specific date and time at the clinical / primary care facility.
- [NB: Additional follow up sampling may be considered for a subset of individuals at a later stage]
- Kindly maintain an excel sheet of all the bio-samples collected and mention the date and time of collection on each sample.
- **Collect samples as early in the day as possible.**
- Pregnant women can be recruited between 12weeks to 36weeks of their pregnancy. Before delivery, the biosamples can be collected at any point of their pregnancy. Ensure that the woman who is being recruited consents to giving bio-samples and has done all her scans at the same hospital where the delivery will take place and consents for sharing scans too as they will be important to fill in the USG form given on RedCap.

Type of samples to be collected:

Sample	***Pregnant Mothers 12/36 weeks***	***Mother at/ around delivery***	***6- 72months baby***	***6years- 30 years***
Blood	Blood	Cord Blood for baby DNA	DBS	Blood
Nail	Nail		Nail	Nail
Placenta		Placenta		
Saliva	Saliva		Saliva	Saliva
Urine	Urine	If possible	Urine	Urine
Stool			Stool	Stool
Hair			Hair	Hair
Breast Milk		To be collected without colostrum, before discharge from the hospital, if possible.		

Sample for DNA/RNA and cytokines:

Dried Blood spots for Children (6 months to 5years):

Uses: A review(Maslagova et al 2020)

Polychlorinated biphenyls and organochlorine pesticides were measured using high-resolution gas chromatography mass spectrometry (GC-HRMS) in DBS samples from newborns (Ma et al 2013). Cytokines can be measured from DBS eluates by multiplex assays(Krakowiak et al 2017). Small amounts of RNA and DNA eluted can be used for particular assays. Metabolites and lipids can be measured by tandem mass spectroscopy.

Materials required:

Gloves, DBS card, Lancet (2mm), skin disinfectant, gauze or cotton wool, pen, sharps container.

Process:

- Choose the area to be pricked and ask mother to warm this area by gently rubbing it with her hands. a) infants 6wks-4mo: heel, b) infants 4mo-10mo: big toe, c) infants>10mo or >10kg: finger.
- Wash hands and wear gloves. Position the baby with the foot or hand down, clean the spot to be pricked with skin disinfectant, allow to dry for 30 seconds.
- Gently squeeze and release the area to be pricked until it is ready to be bled, prick the infant in the selected spot with a 2mm lancet.

- Wipe away the first spot of blood, allow a large spot of blood to collect.
- Touch the filter paper gently against the large drop and allow it to completely fill the circle. Collect at least 3 full circles.

- Clean the area with gauze and apply gentle pressure to stop bleeding. Ensure the wound is clean and bleeding has stopped for at least five minutes. Recheck the wound before the baby leaves your care.
- Fix the barcode label on the card and complete the documentation on the card.
- Allow the blood on the card to dry completely at room temperature. After 1-3 hours carefully place the card into a ziplock cover and seal properly.
- These covers can be placed in a box before storage in a freezer at -80 degrees for long term storage. Short term storage can be in the refrigerator or -20 degrees. Label boxes with site names, date and content along the RedCap ID before shipping, with serial numbers of DBS inside and the name of the centre.

Refs:

Ma, W.L.; Yun, S.; Bell, E.M.; Druschel, C.M.; Caggana, M.; Aldous, K.M.; Buck Louis, G.M.; Kannan, K. Temporal trends of polybrominated diphenyl ethers (PBDEs) in the blood of newborns from New York State during 1997 through 2011: Analysis of dried blood spots from the newborn screening program. Environ. Sci. Technol. 2013, 16, 47, 8015–8021.

Malsagova K, Kopylov A, Stepanov A, Butkova T, Izotov A, Kaysheva A. Dried Blood Spot in Laboratory: Directions and Prospects. Diagnostics (Basel). 2020 Apr 23;10(4):248. doi: 10.3390/diagnostics10040248. PMID: 32340321; PMCID: PMC7235996.

Saliva collection from babies and young children (Children 6 months - 3years old)

Uses: For detection of Alpha-Amylase, C-Reactive Protein , Cortisol , Cotinine, IgG/IgM, Interleukin-1 Beta, Interleukin-6, Osteocalcin, Secretory Immunoglobulin A, Testosterone, DNA Analysis, Uric Acid

Precautions for Saliva collection:

- Avoiding foods with high sugar or acidity, or high caffeine content, immediately before sample collection, since they may compromise the assay by lowering saliva pH and increasing bacterial growth.
- Documenting consumption of alcohol, caffeine, nicotine, and prescription/over-the-counter medications within the prior 12 hours.
- Documenting vigorous physical activity and the presence of oral diseases or injury.
- Not eating a major meal within 60 minutes of sample collection.
- Rinsing mouth with water to remove food residue and waiting at least 10 minutes after rinsing to avoid sample dilution before collecting saliva.

Materials required :

Swab Method: Supplier - Salimetrics , USA

SalivaBio's Children's Swab(SCS) (Children 6 months - 6 years old) : 5001.06

SalivaBio Infant Swab Device(SIS): 5001.08 (Thinner and smaller for smaller mouths)

Swab Storage Tube(SST) : 5001.05

The Children's Swab system performs safely and effectively and features a 125mm swab which is long enough to hold one end firmly while placing the other end in subject's mouth (thus eliminating any choking hazard). The Children's Swab's thin

diameter (8mm) suits smaller mouths, and the durable polymer withstands chewing. Each swab comes individually wrapped to minimize the possibility of environmental contaminants, causes no change in sample pH, and has verified recoveries.

The volume of sample recovered is typically in the range of 200-1000 µL.

Process:

- Open the protective package and remove the SIS/SCS. Do not use swab if cuts or tears are present.
- Securely hold one end of the SIS/SCS device and place the other end under the subject's tongue (when possible). With small children, it may only be possible to collect pooling saliva (at the corners of the mouth or under the tongue).
- Collect for a full 60-90 seconds by resting the swab inside the mouth, or collect in intervals by re-introducing the swab into the mouth as needed, **until the lower third of the swab is saturated** (some participants may require longer than 90 seconds of total collection time).

Immediately after collection, use one of the following procedures for storing the sample:

A. If storing the swab in a Swab Storage Tube for centrifugation in lab

- Remove cap and insert the saturated end of the swab into the swab basket of the swab storage tube (SST).
- Fold over the dry end of the swab into the swab basket as well.

- Recap SST tightly. Note: Do not throw away any parts of the tube assembly. Label the exterior of the SST as shown with an identifying, bar-coded, cryo-label.

B. If processing samples in-house prior to freezing, centrifuge the storage tube for 15 minutes at 1500 g to extract the saliva. You may discard swab basket and swab after this. Keep SST in upright position. Recap tube and proceed with freezing.

Immediately after collection, freeze samples at or below -20°C. If freezing is not possible, refrigerate immediately at 4°C and maintain at this temperature for no longer than necessary (ideally less than 2 hours) before freezing at -20°C (temperature of a regular household freezer) or below. You may store saliva samples at -20°C (or below) in the swab or in the Swab Storage Tube for up to 4 months. • Extract and transfer saliva samples to screw-cap cryovials and store at -80°C for long-term storage periods >4 months. • Freeze-thaw cycles should be minimized for some analytes. Determine storage conditions prior to sample collection. •

https://salimetrics.com/collection-method/infant-swab-device/

https://salimetrics.com/saliva-collection-handbook/

Saliva collection from Children of 4years and above till the age of 28 years

Uses: For detection of Alpha-Amylase C-Reactive Protein, Cortisol, Cotinine, IgG/IgM, Interleukin-1 Beta, Interleukin-6, Osteocalcin, Secretory Immunoglobulin A, Testosterone, DNA Analysis, Uric Acid

Precautions for Saliva collection:

- Avoiding foods with high sugar or acidity, or high caffeine content, immediately before sample collection, since they may compromise the assay by lowering saliva pH and increasing bacterial growth.
- Documenting consumption of alcohol, caffeine, nicotine, and prescription/over-the-counter medications within the prior 12 hours.
- Documenting vigorous physical activity and the presence of oral diseases or injury.

- Not eating a major meal within 60 minutes of sample collection.
- Rinsing mouth with water to remove food residue and waiting at least 10 minutes after rinsing to avoid sample dilution before collecting saliva.

Materials required:

Process:

- Let the individual rinse mouth with clean water, with no trace of chewing gum, candy, flavoured drinks or food particles.
- Attach the funnel to the saliva collection tube and ask the participant to use the funnel to spit into the tube. Ask the Participant to accumulate saliva in their mouth and try to fill the tube uptil 5ml.
- Once the collection is done, the funnel can be discarded and the collection tube can be tightly capped.
- Once the sample has been collected, ensure that the sample is properly coded and labelled along with the time and date of collection, and send it for immediate processing.

Blood collection from participants who consent:

Potential Uses: Samples for DNA, RNA, Serum for Cytokines, pesticides, heavy metal, biochemistry (Glucose, TSH, HDL-cholesterol, triglycerides, SGOT, SGPT, albumin), hemogram and blood grouping. Plan to test serum for hs-CRP, DHA, IL-6, IL-1β

Requirements :

10ml K2EDTA tubes (lavender top) BD Cat No. 367525

6ml K2EDTA tubes (lavender top) BD Cat No 367863

3.5ml Vacutainer SST Gel Tubes (yellow top) BD Cat No. 367956

Vacutainer Flash Back Needles, 22G X1" BD Cat No. 365076

3ml EDTA and 3.5ml gel tubes for hemogram and clinical biochemistry for participant give back (optional)

Bar code labels of PARAM ID and hospital ID

RNAlater

Cryovials for storing samples after centrifugation

Cryoboxes for storing cryovials

Micropipette with sterile 1 ml tips (cut and uncut)

Lab centrifuge to spin the vacutainers

Refrigerator/ freezer for long term storage

Process:

Due to differences, such as age between the participants, the maximum amount of blood that can be collected from each subject could vary.

Order of collection will be as below:

Older Participants(~ 15ml)

Vacutainer	Blood	Purpose	Process		Storage
10 ml EDTA tube	8-10ml	Plasma buffy coat for RNA buffy coat for DNA RBC pellet	Centrifuge	2 vials 200ul buffy coat + 600ul RNA later ~800ul buffy coat for DNA 1ml	-80C -80C -80C -80C

3.5 ml gel tube	2.5ml	serum	Centrifuge	2 vials	-80
3ml EDTA tube	1ml	Hematology		Send to clinical lab for haematology with hospital ID labels	RT
3.5ml gel tube	2.5ml	Biochemistry		Send to lab for biochemistry with hospital ID labels	RT

Younger Participants (~ 10ml)

Vacutainer	Blood	Purpose	Process		Storage
6 ml EDTA tube	~5ml	Plasma buffy coat for DNA RBC pellet	Centrifuge	2 vials ~800ul buffy coat for DNA 1ml	-80C -80C -80C -80C
3.5 ml gel tube	2.5ml	serum	Centrifuge	2 vials	-80C
3ml EDTA tube	1ml	Hematology		Send to clinical lab for haematology with hospital ID labels	RT
3.5ml gel tube	2.5ml	Biochemistry		Send to lab for biochemistry with hospital ID labels	RT

A. <u>Processing of EDTA tubes (10ml tubes):</u>

- Blood sample collected in 10ml/6ml EDTA tubes to be placed on ice / until processed(maximum 4hours)
- Label the cryo vials of recruited subject with barcode labels.
- Centrifuge blood tubes at 4°C, 2000 g for 10minutes
- Use 1 ml pipette to transfer the plasma. Approximately equal amounts in two 2ml freezer vials. Do not disturb the buffy coat layer of white cells when you do this.

- To collect the buffy coat first gently re-suspend creamish coloured buffy coat layer into the remaining plasma, using the 1ml cut tips by pipetting up and down gently.
- 200ul buffy coat + 600ul RNA later (stored for RNA) in a 2ml cryo vial labelled RNA on the PARAM id label.
- Remaining buffy coat (~800ul) is stored separately in another cryovial for DNA isolation.
- Takeout 1 ml RBC from the bottom of EDTA tube, transfer to cryo vials labelled as R.

A. Processing of EDTA tubes (6 ml tubes):

- Blood sample collected in 10ml/6ml EDTA tubes to be placed on ice.
- Label the cryo vials recruited subject with barcode labels.
- Centrifuge blood tubes at 4°C, 2000g for 10minutes
- Use 1 ml pipette to transfer the plasma. Approximately equal amounts in two 2ml freezer vials. Do not disturb the buffy coat layer of white cells when you do this.
- To collect the buffy coat first gently re-suspend creamish coloured buffy coat layer into the remaining plasma, using the 1ml cut tips by pipetting up and down gently.
- Buffy coat (~800ul) is stored separately in another cryovial for DNA isolation.
- Takeout 1 ml RBC from the bottom of EDTA tube transfer to cryo vials labelled as R.

- *10ml blood tubes may yield more plasma. Use multiple freezer vials. Avoid putting more than 1ml in one freezer vial.

B. Processing of gel tubes

- Allow tubes to stand for 30 minutes. Centrifuge at 4500 rpm for 5 minutes.

- The ~ 1.5ml serum from these tubes to be collected and stored in 2 freeze vials (~ 800 ul) at -80 C.

- EDTA tubes for routine analysis and serum gel tubes to be labelled with hospital ID and sent to the appropriate labs ASAP for analysis.

Collection of Babies blood from umbilical cord

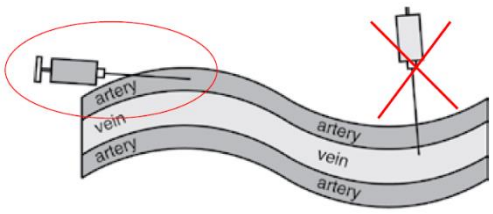
Use: DNA Isolation as it is the only source of blood from the baby

Measure the total length of the umbilical cord, using a disposable measuring tape. Cord blood which is the blood left in the umbilical cord and placenta after a baby is born is an ideal sample for collection of adequate blood sample from the new born.

At delivery the cord should be cut and clamped as normal, a section of cord should then be isolated between two clamps, as soon as possible. The blood sample should be taken between these two clamps.

Angle the needle of the syringe so it is 45 degrees from the vessel to be sampled. 2-10 ml Blood is drawn from the cord and transferred to a 10 ml K2EDTA tube which can be processed as mentioned earlier to get plasma, buffy coat for RNA and DNA as well as RBCs.

A mix of both venous and arterial cord blood could be used. In case the cord blood gets clotted, we will use the same for DNA Isolation. Hence please collect in purple capped EDTA. For DNA isolation, venous/arterial cord blood is acceptable or even a mix of both.



Cord Blood Gas Analysis

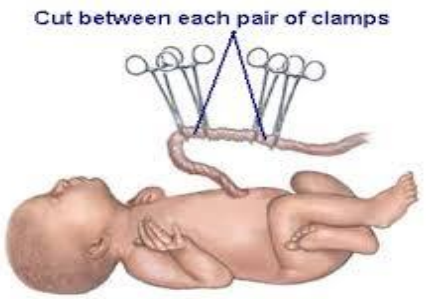
Equipment Required

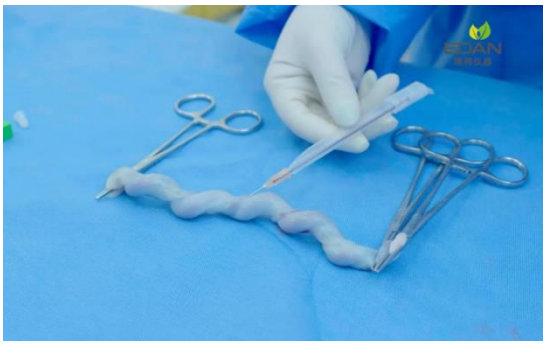
- two 2ml heparinized syringe
- Disposable measuring tape

Use: Arterial Venous pH difference

Procedure:

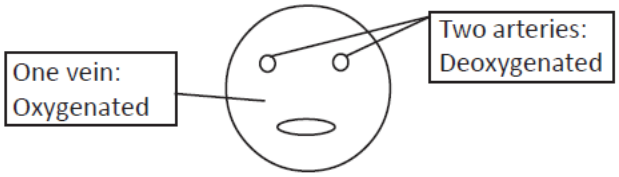
To prepare the Cord, first measure the total cord length with a measuring tape in cms. At delivery the cord should be cut and clamped as soon as possible. Double clamp, a 20-cm long segment of cord, the blood sample should be taken between these two clamps.





Both a **venous** and **arterial** sample should be obtained.

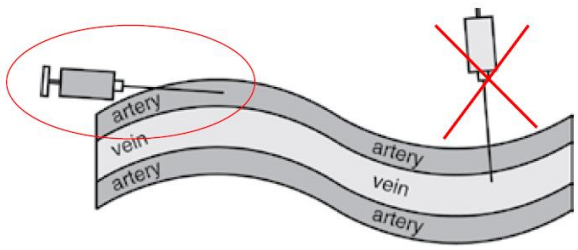
There are two smaller arteries taking deoxygenated blood from the baby to the placenta and a larger thin-walled vein bringing oxygenated blood from the placenta to the fetus.



Identify the smaller umbilical artery from the larger vein. Use a 2ml syringe and draw up a small quantity of heparin 1000iu per ml. Withdraw the plunger fully and allow the heparin to run down the sides of the syringe. Push the plunger back in place expelling the majority of the heparin. Leaving too much heparin will make your sample acidotic. Not putting heparin into the syringe will cause your sample to clot. Alternatively use a pre-heparinised syringe.

Sample umbilical artery first at 45 degree. Correct positioning of the needle in the vessel will help to prevent inadvertent passage of the needle through one vessel and into another.

In the two syringes the venous sample will be redder/brighter than the deoxygenated arterial samples.
Fill the syringe if possible and expel any air, this will give a more accurate pH value
The syringe should be capped off to air. Do not contaminate the sample with air, remove air bubbles, label both samples correctly transport and take the sample to the blood gas analyser immediately.



The sooner after the delivery the sample is taken and analysed the more accurate the results. A sample can be left at room temperature either in the cord or in a prepared syringe for **30 minutes**. If there must be a longer delay the placenta or sample should be placed in a fridge (4 degree).

Arterial Venous pH difference if gas analysis is done

The mean difference between the venous and arterial pH is 0.08. To ensure that both vessels have been sampled the difference needs to be at least 0.03 units.

If the two results are very similar (less than 0.03 unit difference) a second set of samples should be obtained taking care to sample both the artery and vein.

Urine samples

Uses: for neurotoxin metabolites estimation (i.e., Hydrocarbons, Cotinine, Arsenic, Fluoride, pesticides)

Materials needed :

Wide mouth urine collection container, plastic droppers for transferring urine from collection container to tubes, 15 ml falcon tubes appropriately labelled, Cardboard box to store falcon tubes in freezer. Powder-free gloves, Refrigerator (2–8ºC) or ice until sample can be frozen.

Process:

Urine: 20 ml mid-stream urine in clean, disposable, capped containers

Urine sample should be collected after blood collection

Inform the subjects to clean themselves with water and wipe themselves dry with a tissue paper, before they start mid-stream urine sample collection. Ensure that the subject understands the term "mid-stream" urine.

Ensure sample pots are labelled correctly. Provide patient with one sample pot and ask them to collect 20 ml mid-stream urine in clean, disposable, capped container.

Using a plastic disposable dropper, ~ 10 ml urine will be distributed in 2 falcon tubes appropriately labelled with participant ID labels. Tubes will be stored in freezer in appropriate stands/ boxes to enable cataloguing of samples. Samples will be shipped in a cold chain.

The time elapsed between the taking of the urine sample and sample processing and freezing must be recorded for all samples.

Hair Sample collection (Provided by Dr Ravish, NIMHANS)

Use: Hair cortisol analysis is considered a promising biomarker for assessing HPA axis activity and chronic stress, as it offers several advantages over other measures of cortisol, such as saliva or blood tests. Hair cortisol levels can be affected by factors such as hair washing, dyeing, and the use of certain hair products. Since hair grows approximately 1 cm per month, it is claimed that 3 cm of hair would reflect cortisol levels to which the individual was exposed in the last 3 months. The reported hair cortisol concentration reference interval in healthy individuals with low levels of stress was 40–128 pg/mg hair (P2.5-P97.5). Hair cortisol concentrations in stressed individuals were higher than in the group with low levels of stress 250 (182–520) pg/mg hair. (Gonzalez et al 2019). ELISA methods are available to measure this.

Requirements:

- Clean and sharp scissors
- Ziplock cover labelled
- Post-It Papers.

Process:

- The entire length of hair to be sampled (up to a pencil-width in diameter) to be secured pre- and post-intervention. Hair should be cut as close to the scalp as possible (care taken not to nick the skin) with clean scissors. Note: The standard sampling area is the posterior vertex region of the skull (Sauvé et al., 2007). The process usually includes the collection of a hair sample from the posterior vertex of the scalp, close to the scalp, and the analysis of a specific length of the hair, typically 3 cm,
- The posterior end of the hair sample should be marked with tape and stored in zip-lock cover at room temperature. till further use.

- The sample can be wrapped in Post-It paper and placed in a ziplock bag on which the participant ID will be stuck. And write ‘R’ for the root side and ‘T’ for the tail side of the hair on the post-it paper
- These ziplocks can then be kept in a labelled box of appropriate size prior to shipment at room temperature. Hair cortisol is stable at room temperature indefinitely.

Ref: Gonzalez, D., Jacobsen, D., Ibar, C. *et al.* Hair Cortisol Measurement by an Automated Method. *Sci Rep* **9**, 8213 (2019). https://doi.org/10.1038/s41598-019-44693-3

Nail clippings:

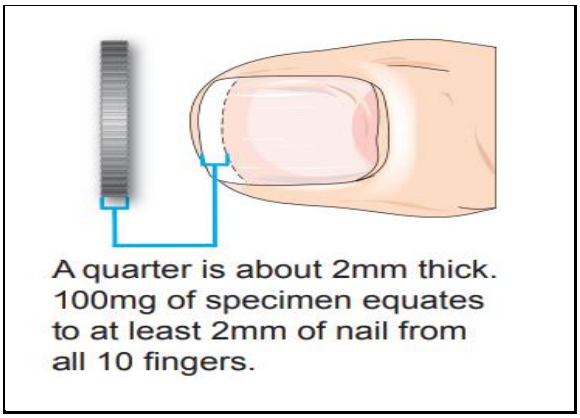
Use: Nails have been used for cytokine analysis specially in diseases like psoriasis. They can also be used for assessments of trace elements or even as a source of DNA(not ideal)

Materials required:

- Metal nail clipper,
- Non-ethanol based alcohol pad
- Disposable gloves
- Collection Paper/A4 Sheet
- Zip lock bags
- Hand-wash facility.

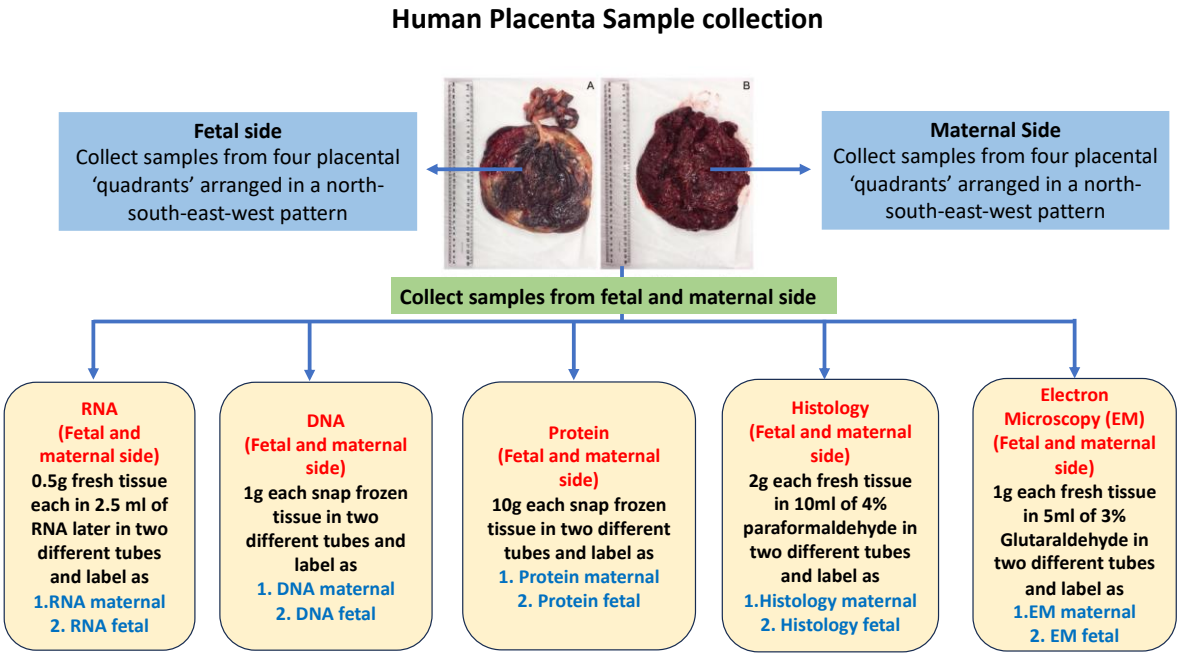
Process:

- Prior to each collection, wipe the clippers with a non-ethanol based alcohol pad.
- Have the donor wash their hands with soap and water prior to specimen collection. Remove dirt from the nails.
- The nails should look like natural nails and must not have an unusual appearance. Nails must be clear of any substances including but not limited to: cosmetic treatments (e.g. polish, artificial acrylic, gel or silk overlay), non-cosmetic substances (e.g. dirt, substance residue oils, stains, inks or dyes, etc.). When removing fingernail polish prior to collection, a non-ethanol based polish remover such as isopropyl alcohol or acetone should be used. If the nail does not look like natural nail or has an unusual appearance for any reason, do not collect the nail.
- Have the donor clip their nails as close to the nail bed as comfortable. It is recommended to clip over a new, plain sheet of paper to capture the clippings.
- Do not mix fingernail and toenail specimens. Do not collect toenails if the client is diabetic or suffers from peripheral artery disease.
- To ensure there is enough specimen to complete the assay it is recommended that 100mg of specimen is submitted for all tests below 10-panel.



Nail clippings can be stored in smaller zip-lock bags labelled with the participant ID. These samples should be stored at -80 C.

Human placental tissue collection.



Use: RNA, DNA and cytokines

Materials:

- Phosphate buffered saline (PBS) pH 7.2
- RNA later (Cat no: R0901) or (Cat no: AM7021)

Equipment

- Biosafety cabinet.
- Personal protective equipment.
- Balance/scale capable of measuring between 100 g to 1 kg to one decimal place.
- Gauze swabs; optional.
- Ruler or similar equipment to use as scale bar, camera.
- Dishes such as deep petri dish for the rinsing of placental samples.
- Dissection equipment, e.g. scissors, forceps, biopsy punch, scalps.
- Tubes and sample pots, and labels for tubes or pen to write labels.

Process:

This procedure describes the collection of Placental sample collection (for RNA Note 1, and DNA isolation) All procedures should be carried out as quickly as possible to minimise degradation. Typically, sample processing should be completed within 30 minutes of birth, if not possible it should be done within 45minutes.

1. Prior to sampling, set up Health and Safety, Ethical Clearance, and the Recording of Sample and Patient Details.

2. Set up a sample processing station in the biosafety cabinet.

3. Photograph the placenta from the chorionic (fetal) and basal (maternal) aspects against a scale bar (Figure 1).

4. Weigh the placenta to the nearest decimal place after cutting the cord. Measure the length of the placenta

5. Place the placenta in the processing station with the basal plate uppermost (Figure1B).

6. Obstetricians team will collect placental tissues within 45 minutes after delivery

7. After washing the placenta with normal saline, 4 chunks of an intact placental lobule free of maternal decidua, calcification, and fascia to be extracted vertically from the full-thickness placentas in the position of 5-cm periumbilical and cut longitudinally into tissues smaller than ≤ 0.5 cm in size (each piece to contain both maternal and fetal sides). Rinse the 4 chunks of tissue in PBS one last time before cutting into smaller pieces.

8. One chunk of the placental tissue sample can be used for DNA isolation and stored in 2ml freeze vials after being cut into bits smaller than ≤ 0.5 cm in size. The additional 3 chunks after being cut into bits smaller than ≤ 0.5 cm in size will then be submerged in 5 volumes of RNAlater solution (e.g., a 0.5 g sample requires about 2.5 mL of RNAlater solution) and saved at 4°C overnight and later stored at − 80 °C.

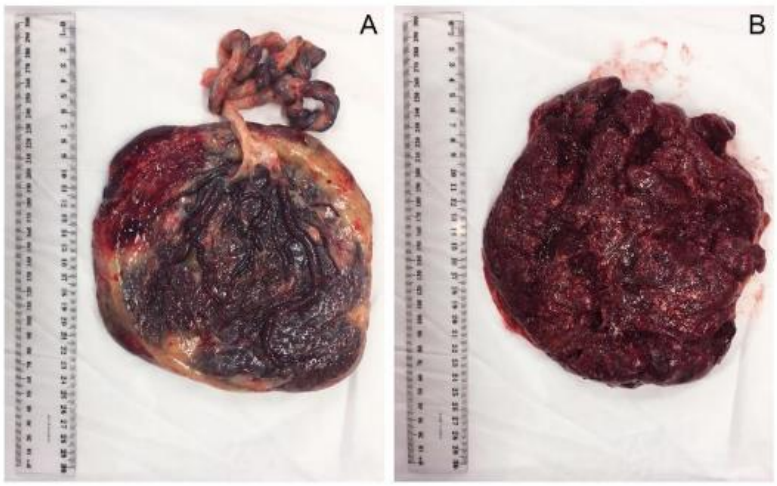


Figure 1. Photographs of a placenta from the (A) chorionic or fetal aspect and (B) basal or maternal aspect. A ruler is included for scale.

Note 1: Identifying random sampling sites: Sampling sites can be selected from four placental 'quadrants' arranged in a north-south-east-west pattern. Alternatively, use the cord insertion as a guide: Mid-way between where the cord was inserted, and the edge of the placenta take samples from each of the four quadrants of the placenta.

References

1. G. J. Burton, N. J. Sebire, L. Myatt, D. Tannetta, Y. L. Wang, Y. Sadovsky, A. C. Staff, C. W. Redman, Optimising sample collection for placental research. Placenta 35, 9-22 (2014).

2. Zhou, J., Tong, J., Ru, X. et al. Placental inflammatory cytokines mRNA expression and preschool children's cognitive performance: a birth cohort study in China. BMC Med 21, 449 (2023). https://doi.org/10.1186/s12916-023-03173-2

Breast Milk post-delivery:

Uses: To estimate lipid and protein content

Materials Required:

- 50ml sterile Wide mouth container.
- 2ml aliquots

Process: 10ml of milk can be collected (if possible before mother and baby leave the facility) and stored frozen in 2ml aliquots until analysis.
Note: Ensure that the sample is collected after the baby has been fed so as to collect the Hind milk.

- Breastmilk sample will need to be expressed and dropped off on the same day. Material required would be 1 x 50ml sterile Wide mouth urine collection container.
- Breast milk may be expressed manually or using a breast pump. It is important to note on the request form which way the sample was collected. On the breast milk pot, write the date of the breast milk sample collection.
- The milk sample should be expressed directly into the sterile breast milk pot supplied, regardless of the method used to collect the sample. Another vessel, bottle or container should not be used for collecting the milk sample.
- Avoid using creams, ointments and soaps on the breasts/nipples prior to collecting milk samples.
- If creams/ ointments have been used please wash the breasts with soap and then thoroughly rinse with water prior to the collection.
- It is recommended that sample collection be at or close to a regular feeding time, usually at least two hours after the previous feed.

- Ensure the entire breast milk sample is collected at one time and is not spread over several feeds.
- Wash breasts and hands with warm water to remove any traces of dirt or debris. Dry breasts and hand well.
- Sit or stand in a comfortable position.
- Remove lid of the pot and express 10ml into the sterile breast milk pot. To prevent contamination of the breast milk do not to touch the inner surface of the container or the lid.
- If expressing manually, the sterile pot can sit under the breast and collect the milk sample. Encourage the let-down reflex by expressing in a quiet, relaxing area. Gently massage your breasts by stroking towards the nipple and gently rolling your nipple between your finger and thumb. Looking at your baby, or thinking about your baby, may also help with the let-down reflex.
- Position the finger and thumb about 2 to 3 centimetres behind the tip of the nipple. Press the finger and thumb together towards the chest, without sliding your fingers on your skin, and gently squeeze.

- If using a breast pump, set up the pump next to a comfortable chair and ensure sterile milk pot is within arm's reach. Instead of attaching your baby's bottle to the pump, the sterile pot will sit under the pump and collect the milk.
- Fill in the date and time of the sample collection on the label. Place the lid on the pot and seal tightly to prevent leakage. Place the pot in the biohazard bag provided.

  For instructions on how to manually express milk, please visit https://www.healthywa.wa.gov.au/Articles/A_E/Expressing-and-storing-breast-milk

Collection of Fecal Samples :

Use: Estimation of Gut biome and possibly microplastics

Requirements: Feces collection tube with stabiliser (DNA/RNA Shield™ reagent, ZymoResearch R1101)

Fecal Catcher if Western toilet is to be used.

Exclusion criteria : Persons who have diarrhoea or digestive problems or on antibiotic treatment for any infection.

Process:

- DNA/RNA Shield™ reagent pre-filled into vials ensures sample stability during transport/storage at ambient temperatures without refrigeration or specialized equipment. Specimen collected can be frozen (-20/-80°C) for prolonged periods.
- Prepare and collect fecal specimen using preferred fecal specimen collection set/kit. Note: Method of collecting the fecal sample must prevent feces from falling into toilet water to avoid sample contamination.
- Unscrew the collection tube cap and use the spoon to scoop one spoonful of feces (approximately 1 gram or 1 mL in volume) from a sample.
- Place the sample in the collection tube.
- Tighten the cap and shake to mix the contents thoroughly (invert 10 times) to create a suspension. Note: Some fecal material may be difficult to re-suspend. As long as the material is submerged, the sample is stabilized. foaming/frothing during shaking is normal.
- Dispose of unused fecal material and thoroughly wash hands according to your institution's guidelines.

XXXXXXXXXX

NOTE TO ALL

- ***Kindly ensure each site prints its own set of barcodes and labels and has a unique hospital Id for each sample.***
- ***Kindly refer to the picture given below when shipping all samples to NIMHANS.***

TO

DR MEERA PURUSHOTTAM

MOLECULAR GENETICS LABORATORY

NEUROBIOLOGY RESEARCH CENTRE

NIMHANS, OPPOSITE NIMHANS LIBRARY

BANGALORE 560029

SITE NAME & CODE
SAMPLE NAME
SAMPLE QUANTITY
SERIAL NUMBERS
TIME PERIOD

Example

NIMHANS, BANGALORE (16)
SALIVA SAMPLES
100 SAMPLES
160001 – 160100
01-01-2024 to 29-02-2024