Standard Operating Procedures for Biological Sample Collection and Storage (c-VEDA)

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September 13, 2016

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September 13, 2016

1. Sample collection

1.1. 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]

Samples to be obtained:

- Blood (all subjects; for DNA, RNA and Lead estimation)
  - EDTA tubes for plasma and buffy coat [for DNA] (to be aliquoted and frozen)
  - EDTA tube for routine hematology (fresh, not to be frozen)
  - Tempus tubes for RNA (to be frozen)
- Saliva (for DNA): Buccal swabs in tubes only for these subjects from whom sufficient amount (i.e., at least 10 ml) of blood cannot be obtained
- Urine (all subjects) for neurotoxin metabolites estimation (i.e., Hydrocarbons, Cotinine, Arsenic, Fluoride)

Amount and type of samples collected:

- **Urine:** 2 x 25 ml urine container from all subjects

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

  Note: the EMLA (lidocaine/prilocaine) anesthetic cream can be used when obtaining blood samples from kids.

  Below are indications of how to split down sample collection according to amount of blood that can be obtained:

  Target = collect 25 ml blood (for subjects over 10y old)

  Order of blood collection: first tubes for DNA, then for RNA and finally for hematology

  - **25 ml of blood to be collected in the following order:**
    - 1) 20 ml blood in 2 x 10 ml EDTA tubes for plasma and buffy coat [for DNA]
    - 2) 1 x 3ml blood in Tempus tube [for RNA]
    - 3) 1 x 2 ml blood in 4 ml EDTA tube [for routine hematology]

  - **If only 15 ml of blood can be collected, then:**
    - 1) 1 x 10 ml blood in 10 ml EDTA tube for plasma and buffy coat [for DNA]
    - 2) 1 x 3 ml blood in Tempus tube [for RNA]
    - 3) 1 x 2 ml in 4 ml EDTA tube [for routine hematology]
  - **Otherwise, if quantity of blood that can be collected is less than 10 ml (e.g.,**

children under 10 years old)

- 1) 1 x 6 ml blood in 6 ml EDTA tube [for DNA]
- 2) 1 x 3 ml blood in Tempus tubes [for RNA]
- + Saliva (buccal swabs for DNA)
- *And offered to either parent* as incentive in some sites**:** 1 x 2 ml blood (from parent) in 4 ml EDTA tube [for routine hematology]

1.2. Materials

- Blood collection kit (per patient)
- 1 x Vacutainer needle holder
- 1 x Latex gloves
- 1 x Tourniquet
- Alcohol wipes
- Cotton wool
- Small plasters
- 1 x Sharps bin for used needle or needle/holder combination

Sample tubes for blood collection

- 10 ml EDTA tubes (BD Vacutainer EDTA tube, lavender lid, Catalogue #367525)
- 4 ml EDTA tubes (BD Vacutainer EDTA tube, lavender lid, Catalogue #367861)
- 6 ml EDTA tube (BD Vacutainer EDTA tube, lavender lid, Catalogue #367863)
- 3 ml Tempus tubes (Thermofisher, 4342792)

Sample tubes for saliva collection

- Buccal swabs in tubes (Copa Flock Technologies, 528C)

Containers for urine collection

- 25 ml urine containers

Bar-coded labels:

The unique numbers from the data management core will be used to generate 2D labels for each site. The sticky labels are cryo-resistant but do not have a transparent plastic strip. We have to stick the label and then put one layer of cello tape around it (not more as it will make it too bulky for the slot in the box). This is to prevent scratches while putting it into the box. Otherwise, the scanner may not read correctly later.

1.3. Obtaining samples

1.3.1. Requirements prior to collection

- Sample tubes should be labelled with the bar-code prior to collection
- Blood should be taken at the clinic / hospital
- For those subjects undergoing neuroimaging assessments, blood should be collected at the end of the last imaging session.
- Urine sample should be collected after blood collection

1.3.2. Details to be recorded:

- Samples collected (i.e. blood [which tubes and how many and in which order]).
- Date and time the samples were taken (blood).
- Last time the subject consumed food/drink.

Equipment

- The equipment used throughout the study for sample collection and storage should be the same as outlined in this protocol.
- Any significant variations in equipment must be recorded (e.g. blood tube number or type).
- Details of any new purchase of tubes should be recorded (e.g. lot/batch no. and expiry date).

1.4. Protocol for taking blood

1.4.1. General precautions

- Gloves must be worn at all times when handling specimens. This includes during removal of the rubber stopper from the blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and clean up of any spills. Tubes, needles, and pipets must be properly disposed of in biohazard containers, in accordance with institutional requirements.
- Universal precautions and institutional requirements should be followed, including gloves, eye protection or working in a biosafety cabinet for blood processing. All equipment (storage, shipping, and centrifuge) must be labeled as biohazard.
- It is important to take steps to prevent hemolysis in blood samples. A vacutainer is recommended. If a needle is used, a 21 gauge needle is recommended.
- Contents of tubes that contain chemical additives may be irritating to eyes, respiratory system and skin.
- For safety information regarding sample tubes and risks associated with venepuncture please refer to the manufacturer's product guidelines and/or your institution's risk assessment.
- The sample tubes contain additives. It is important to follow the correct order of draw to prevent contamination of samples.

1.4.2 Guidelines for venepuncture

- Select a vein by observation and palpitation (lightly tapping the vein). Listen to the subject as they will often advise on the best or only site for venepuncture.
- Place the tourniquet around the subject's upper arm or lower arm 10 cm above the venepuncture site. Note: To avoid pinching of the skin, place the tourniquet over the subject's sleeve. Alternatively, place a cotton wool pad under the buckle of the tourniquet.
- Ask the subject to clench their fist. If the vein is not visible or palpable, ask the subject to "pump hand" or dangle arms over hand rest of chair.
- If still not visible, lightly tap antecubital fossa or dorsal surface of hand.
- Disinfect the puncture site well with alcohol skin wipe and allow the skin to dry. Note: It is important that the skin is completely dry to avoid discomfort when inserting the needle.
- Ask subject to relax hand/arm in a downward position.
- Remove the cap from the needle, position the needle parallel with the vein with the bevel uppermost and insert it into the vein.
- Push the first tube into the needle holder and onto the needle valve, puncturing the rubber stopper. Hold the tube vertically, below the donor's arm during blood collection.
- Release the tourniquet as soon as blood appears in the tube. Note: The tourniquet should not be applied for longer than one minute as this can affect blood samples.
- Allow at least 10 seconds for a complete blood draw to take place. When the first tube is full and blood flow ceases, remove it from the holder and introduce the next tube into the holder. The order of draw should be:
    1) 1 x 6 ml or 10ml EDTA (lavender lid) for plasma and buffy coat
    2) 1 x 3 ml Tempus tube for RNA
    3) 1 x 4 ml blood tube for blood count

1.4.3 Guidelines for avoiding hemolysis

- It is important to take steps to prevent hemolysis in blood samples (see Figure 1). *In vitro* hemolysis can be caused by improper technique during collection of blood specimens (e.g., difficult collections, incorrect needle size, improper tube mixing and incorrectly filled tubes) or by the effects of mechanical processing of blood (e.g., too hot or too cold). This can cause inaccurate laboratory test results by contaminating the surrounding plasma with the contents of hemolyzed red blood cells. For example, the concentration of potassium inside red blood cells is much higher than in the plasma and so an elevated potassium level is usually found in biochemistry tests of hemolyzed blood.

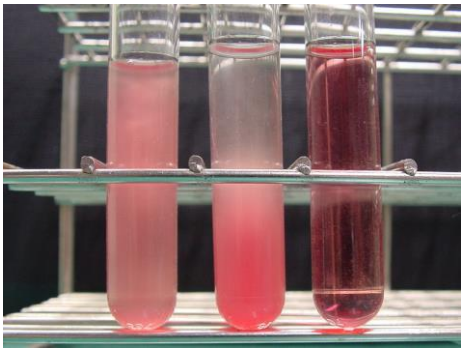


Figure 1: Hemolysis of blood samples: Red blood cells without (left and middle) and with (right) hemolysis. If as little as 0.5% of the red blood cells are hemolyzed, the released hemoglobin will cause the serum or plasma to appear pale red or cherry red in color. Note that the hemolyzed sample is transparent, because there are no cells to scatter light. [By Y tambe - Y tambe's file, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=989846].

Techniques to Prevent Hemolysis (which can interfere with many tests):

- Mix all tubes with anticoagulant additives gently (vigorous shaking can cause hemolysis) 5-10 times.
- Avoid drawing blood from a hematoma; select another draw site.
- If using a needle and syringe, avoid drawing the plunger back too forcefully.
- Make sure the venipuncture site is dry before proceeding with draw.
- Avoid a probing, traumatic venipuncture.
- Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute is optimal).
- Avoid massaging, squeezing, or probing a site.
- Avoid excessive fist clenching.
- If blood flow into tube slows, adjust needle position to remain in the center of the lumen.

Preparation

- Label the tubes with the provided labelled. The labels should be applied along the tube and NOT around the tube.
- Ensure subject is sat comfortably in a chair/lying down on a bed.
- Explain procedure to the subject (to obtain consent and co-operation).
- Select the appropriate blood tubes and place within reach. Ensure sharps container and all necessary equipment are close to hand.
- Wash and thoroughly dry hands. Put on disposable gloves.
- Remove the cover from the valve section of the multi-sample needle.
- Thread the needle into the needle holder and ensure that the needle is firmly seated in the holder.

1.4.4. Notes on EDTA tube(s)

- Aim to completely fill all tubes. EDTA tubes contain a certain amount of anticoagulant that needs to be mixed in an exact proportion to the blood.
- Since the EDTA tubes contain chemical additives, precautions should be taken to prevent possible backflow from the tubes during blood drawing.

1.4.5. Notes on Tempus tube(s)

With Tempus™ Blood RNA Tube, the blood is drawn directly into a reagent that stabilizes RNA at room temperature for up to five days.

- Do not use the Tempus tubes if they are discolored or contain precipitates
- Do not use the Tempus tubes after their expiration date.
- Filling up the tube to the black mark on the tube label indicates the collection of approximately 3 ml of blood.
- Draw blood directly into TempusTM Blood RNA Tubes

  Shake vigorously or vortex for 10 sec (Process immediately or store at room temperature for up to 5 days, or at 4 °C for up to 7 days, or –20 °C indefinitely).

1.5. Immediately following blood sample collection.

- **EDTA tubes:** it is imperative to **gently invert** the EDTA (lavender lid) blood tubes at least 10 times. **Do NOT shake**. Afterwards, store the vacutainer tubes upright at 4ºC until centrifugation. These blood samples should be centrifuged within two hours of blood collection.
- **Tempus tubes: shake vigorously or vortex** the Tempus tube for 10s and store the whole tube immediately at -80C. [These samples can be kept upright and stored at room temperature for up to 5 days, or at 4 °C for up to 7 days].
- Safely discard the used needle holder and syringe into the sharps bin.
- Ensure all sample tubes are labelled with the date, subject code and the study visit number.
- The time of phlebotomy, processing and final storage should be logged as well as the date and any unusual conditions in the lab (failure of temperature control etc.)
- Urine must be transferred into a monovette (see below) then transported and stored at 4°C until processing.
- Send the small EDTA 4 ml tube to the appropriate laboratory for routine hematology – can be transported at room temperature.
- Samples should be moved between laboratories in a sealed container.

1.6. Protocol for collecting urine

- Ensure sample pots are labelled correctly
- Provide patient with two sample pots and ask them to fill them (2 x 25 ml mid-stream urine in clean, disposable, capped container)

1.7. Protocol for buccal swab saliva collection

- Ensure subject has not eaten for 1hr.
- Keep tube with bar code label ready. Ask person to rinse mouth with water. Use the swab to collect cells by moving the swab firmly against the inner cheek and inner lip for about a minute.
- Air dry the swab for 60 minutes carefully and replace into the tube. Transfer to 4 degree refrigerator until shipment.
- Note : Keep swab and labelled tubes next to each other in a stand or rack to prevent sample mix up at all costs.

NOTE: This is not the ideal option to obtain DNA from subjects. The buccal sample should be collected only if blood sample cannot be obtained from a person to be recruited.

2. Sample processing

The time elapsed between the taking of the blood, urine or saliva samples and sample processing and freezing must be recorded for all samples

2.1 Plasma and Buffy Coat preparation from EDTA tubes

2.2.1 Equipment list

- 2 x EDTA tube for plasma and buffy coat (lavender lid)
- Centrifuge with swinging bucket rotor
- 15 ml polypropylene conical tubes (for example, Corning 430052, Fisher cat #05-538-53D)
- sterile screw-cap cryotubes (1,5 or 2 ml; e.g., VWR, Ref. 720-0516)
- Filter pipette tips
- Small ice bucket
- General lab equipment

2.1.2 Requirements

- The samples should be processed as soon as possible after blood taking and within 2 hours of blood collection.
- The time elapsed between the taking of the blood and sample processing must be recorded for this sample
- Label the cryotubes with barcode label (the label should go around the tube such that the 2D label is clearly seen and covered with transparent cello tape).

Instructions for removal of BD Hemogard closure:

- Grasp the blood tube with one hand, placing the thumb under the closure. With the other hand, twist the closure while simultaneously pushing up with the thumb of the other hand, only until the tube stopper is loosened.
- Move thumb away before lifting closure. Caution: Do not use thumb to push closure off tube. If the tube contains blood, an exposure hazard exists.
- Lift closure off tube. In the unlikely event of the plastic shield separating from the rubber stopper, do not reassemble closure. Carefully remove rubber stopper from tube.

2.1.3 Procedure

- After collection, gently mix the blood by inverting the tube 8 to 10 times. Store the vacutainer tubes upright at 4ºC until centrifugation. Blood samples should be centrifuged within two hours of blood collection.
- Maintain sample at 4°C throughout processing.
- Upon arrival at the lab, centrifuge the sample in a horizontal rotor (swing-out head) at 2000*g* for 10 min**, at 4°C**. This causes separation of the sample into 3 distinct phases: the upper layer is the plasma (contains clotting factors), the narrow middle layer is the 'buffy coat' (white blood cells), and the bottom layer is the red blood cells.
  Warning: Excessive centrifuge speed (over 2000 g) may cause tube breakage and exposure to blood and possible injury. If needed, RCF for a centrifuge can be calculated. For an on-line calculator tool, please refer to:http://www.sciencegateway.org/tools/rotor.htm

- After centrifugation, plasma layer will be at the top of the tube. Mononuclear cells and platelets will be in a whitish layer, called the “buffy coat”, just under the plasma and above the red blood cells (Figure 1).

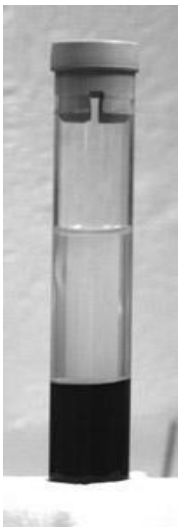


Figure1. This is what the tube should look like after centrifuging

- Carefully collect the plasma layer with an appropriate transfer pipette without disturbing the buffy coat layer. Do not take all the plasma; collection of all plasma may cause contamination with the underlying buffy coat and red blood cell layers. If more than one tube is collected, pool the plasma samples from both tubes into a 15 ml conical tube and mix. Pipette the plasma into 250 µl aliquots in labeled cryovials (maximum of 5 aliquots/per subject). Aliquot volume is recommended to be 500 µl or less; however, some sites may determine that larger aliquot sizes (1 ml or more) are needed (e.g., if amount of blood collected is 20 ml). Close the caps tightly and place on ice.

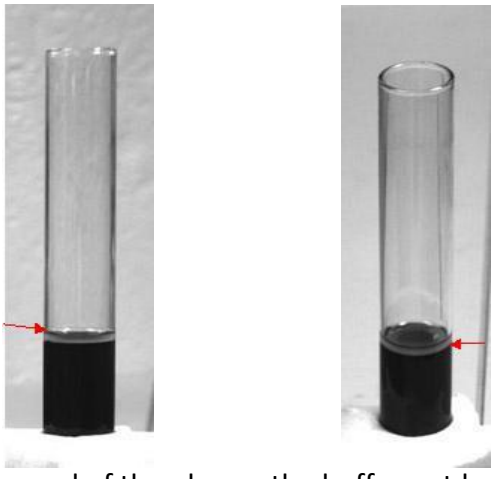


Figure 2. After removal of the plasma the buffy coat layer is left (red arrow)

- Using a cut-off 1ml pipette tip, collect the buffy coat layer (Figure 2) into a separate cryotube and mix by pipetting up and down a number of times. The resulting sample will be enriched for white blood cells, but will also contain some of the overlying plasma and underlying red blood cells. Aliquot this into 2 appropriately labeled

cryovials, approximately 200 µl in each/each 10 ml blood tube (i.e., 4 aliquots/subject). Close the caps tightly and place the cryovials on ice.

- Take 1 ml of red cells and pipette into a labeled cryotube, close the cap tightly and place the cryovial on ice.
- All processing should be completed within 1 hour of centrifugation.
- Check that all aliquot vial caps are secure and that all vials are labeled. Place all aliquots upright in a specimen boxes (i.e., 10x10 boxes (VWR 211-9001), using different boxes for the plasma and the buffy coats and freeze immediately at -80°C. All specimens should remain at -80ºC prior to shipping. The samples should not be thawed prior to or during shipping.

  Samples will be shipped on dry ice. Refer to "Shipping" instructions, below.

Notes

- Plasma and buffy coat should not undergo freeze-thaw cycles, so choose aliquot volume carefully.
- For sites not likely to have a refrigerated centrifuge (e.g., Mysore and West Bengal): Centrifugation will be performed at room temperature at these sites after pre-chilling blood tubes on ice.

2.2. Processing of Tempus RNA tubes

No particular processing: Freeze immediately at -80°C after collection.

- All times of incubations and freezer transfers should be documented

2.3 Collection of urine (for Hydrocarbons, Cotinine, Arsenic, Fluoride)

2.3.1 Equipment list

- 2 x 25 ml mid-stream urine in clean, disposable, capped containers (monovette: yellow cap or clean, disposable, capped container)

2.3.2 Requirements

- Label cryotubes with 2D barcode label (label should go around the tube, followed by cellotape)

2.3.3 Procedure

- Follow procedure described in section 1.6. The sample can be maintained at 4°C for up to 7 days before long-term storage at -20°C.

3. Sample storage

3.1. General guidelines

- For immediate storage, prior to transportation, the 10 mL EDTA, Tempus and urine tubes should be stored upright at 4°C. The 4 mL EDTA tubes should be stored upright at room temperature (ideally 18-22°C).
- For immediate storage, aliquoted samples and EDTA tubes for DNA need to be labelled with cVEDA barcode and sample type (e.g. serum, plasma), and assigned box numbers to aid location in the freezers.
- Samples should be stored at -80 °C (cryovials with buffy coat for DNA, plasma, red

cells and tempus blood samples for RNA) or -20 °C (urine samples) and sent regularly to NIMHANS in dry ice for long-term storage.

3.2. Storage prior to shipment

3.2.1 Plasma, Buffy coat, Red cells, Tempus blood for RNA: Frozen at -80 °C.

3.2.2 Urine: Frozen at -20 °C.

4. Transport and Shipping instructions

Samples should be kept frozen at all times during transport and shipped in dry ice. They should be packaged appropriately to prevent breakage or leakage and shipped with sufficient dry ice to ensure samples do not thaw during transport.

Important: avoid direct contact of samples with dry ice

4.1 Transport and shipping for long-term storage at NIMHANS (DrMeera Purushottam (cVEDA), c/o Prof Sanjeev Jain, Molecular Genetics Laboratory, Neurobiology Research Centre, National Institute of Mental Health and Neuro Sciences(NIMHANS), Hosur road, Bangalore- 560029.):

Samples (i.e., frozen aliquots of Plasma, Buffy coat, Red cells and Tempus blood tubes) should be sent every three months or when you have a sufficient number of samples.

4.2 Collection and transport of urine samples and plasma aliquot for lead estimation to the West Bengal (WB) laboratory (only those samples from subjects undergoing deep phenotyping). These samples will be collected by WB technicians when during their visit to the sites. Contact Amit Chakrabarti amitc@icmr.org.in for details.

5. Data to be recorded

For each type of biological sample to be collected, the following needs to be recorded (in an excel file):

1. Subject ID
2. Type of samples collected (blood, urine, saliva)
3. Date and time of sample collection
4. Last time the subject consumed food/drink.
5. Number and volume of aliquots prepared
6. Date and time into -80ºC
7. Date and time of shipping
8. Any freeze-thaw that occurs with a sample for any reason
9. Any variations or deviations from the SOP, problems, or issues

Notes

• Freezers need to have a backup generator or other emergency system Options: Create emergency management plan, such as moving to a new freezer or adding dry ice in the event of a freezer failure.

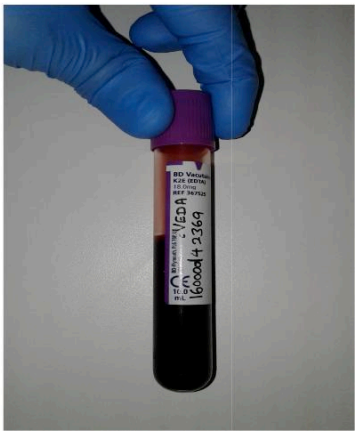
BIOMARKER SOP – PART 2

PROCESSING OF BLOOD SAMPLES, SAMPLE STORAGE & SHIPMENT

2A: Sample collection and processing

At the time of sampling

Fix **paper label** with PSC1 code on **1 EDTA tube**.

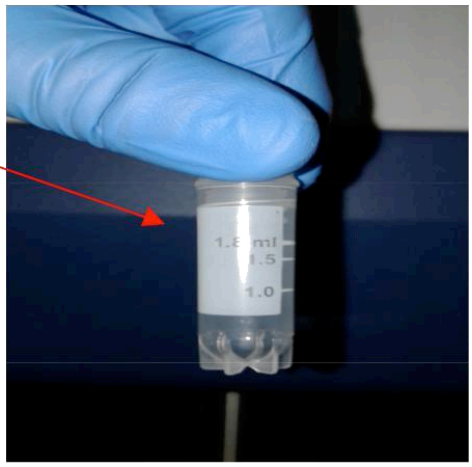


Fix corresponding **cryo labels** containing the same PSC1 code on **1 tempus tube** and **1 urine container**

- Blood sample collected in one or two **10ml/6ml EDTA** tubes to be placed **on ice**.
- **Tempus tubes,** placed in the emptied tempus tube racks, to be stored at **-80 deg C** directly
- **Urine sample** in a 50ml container to be directly stored at **-20 deg C**

Preparation in the lab for processing blood in EDTA tubes

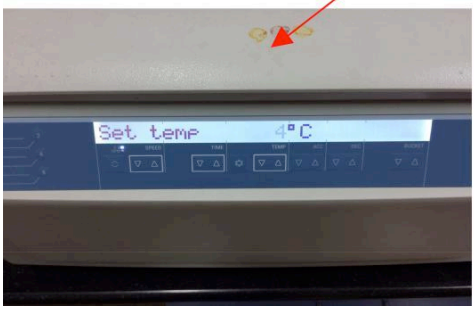
Select the PSC1 **cryo labels** for the recruited subject. Trim the labels to fit the **cryo tubes lengthwise**. Apply the labels onto 8 cryo vials such that the **graduation on the tubes is visible.**



Use marker pen to write P1, P2, P3, P4 and P5 for the 5 plasma vials. Write BC1 and BC2 for the buffy coat vials and R for the RBC vial. Next apply cello tape

EDTA Blood Processing

Centrifuge blood tubes at **4°C, 2000g for 10minutes**.



Use 1ml pipette to transfer the Plasma to the 15ml falcon tube placed on ice. Mix to make homogenous. **Divide the plasma equally** (approx. 4-5 ml/10ml blood) in to 5 pre labeled cryo tubes named as P1, P2, P3, P4 and P5. About 0.5 ml of the plasma layer maybe left behind above the buffy coat.

Cut the tip of the 200ul tips carefully using a sterile blade

To collect the buffy coat, first gently re-suspend creamish coloured buffy coat layer into the remaining plasma, using the cut 200ul tips by repeated pipetting. Next, transfer this layer to ensure all the WBCs are collected. If some RBCs get transferred it's OK. Transfer the entire buffy coat layer into BC1 tube at first. After mixing to make homogenous, transfer half the contents to BC2 – approximately 0.5-1ml in each tube.

Take out 1ml RBC from bottom of the EDTA tube in 1 pre labelled Cryotube (R), using 1ml pipette.

Store the Cryo tubes at -80°C.

2B: Sample storage

2B.1.a – Tempus tubes

- These have to be directly stored at -80 deg C soon after blood collection.
- The tubes can be placed in the emptied tempus tubes rack. The tubes placed in these racks can be placed inside the tempus tube cardboard boxes. So, tempus tubes with blood samples have to be packed as they were received.
- The tempus tube boxes can be labeled with a marker pen and a cello tape affixed on the label.
- A log of the 50 PSC1 codes in each box has to be maintained.

2B.1.b – Urine samples

- Urine samples (1 sample from each individual in a 50ml container) have to be stored in the -20 deg C freezers, soon after sample collection.
- Please arrange the samples in a manner that you can identify which PSC1 codes are stored where.
    - *Samples could either be stored in cardboard boxes and the boxes labeled or samples can be placed in thermo-labile plastic covers and these packages labeled, etc*.
- For the MRI participants, a second urine sample, in a 50ml container, can be collected when they come for the MRI scanning.
    - These second samples are to be stored separately. They can be labeled as 'b' samples, next to the PSC1 code label.

2B.1.c – Buccal swabs

- Buccal swabs are only to be collected if the participant does not consent for a blood sample despite our best efforts.
- Buccal swabs have to be air dried and placed in their covers.
- The PSC1 code labeled buccal swabs are to be placed inside thermolabile plastic covers.
- The individual packages have to be labeled (with centre name, sample and package no.) and stored at -80 deg C.
- Paper records of which package contains which PSC1 codes have to be kept.

2B.1.d – Cryotubes with Plasma/Buffy coat/RBC samples

- Use one cryobox for the plasma tubes and another for the BC/RBC tubes
    - 5 plasma tubes, from 20 individuals (i.e. 100 tubes in all) can fit into one 10*10 cryobox
    - 2 BC tubes + 1 RBC tubes, from 33 individuals (i.e. 99 tubes in all) can fit into one 10*10 cryobox
- Label the lid and at the side of the 10*10 cryoboxes with name of centre, and serial number to prevent mixup.

- Eg. For Plasma boxes: RIMS-P1, RIMS-P2 …. or ROHCK-P1, ROHCK-P2 ….
  For BC/RBC boxes: RIMS-BR1, RIMS-BR2… or ROHCK-BR1, ROHCK-BR2…

- Print two copies of the template shown on the next page (one for each box). Use the extra cryo labels to paste on the grid as shown. This will help us to identify the positions of the tubes in the boxes for our record at NIMHANS. Please keep a copy in your files. A copy of this may be sent along with the consignment.

Box No :

Date of dispatch:

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

PLASMA BOX

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

Each row of the plasma box will have 2 subjects,
i.e. 20 individuals' samples/box

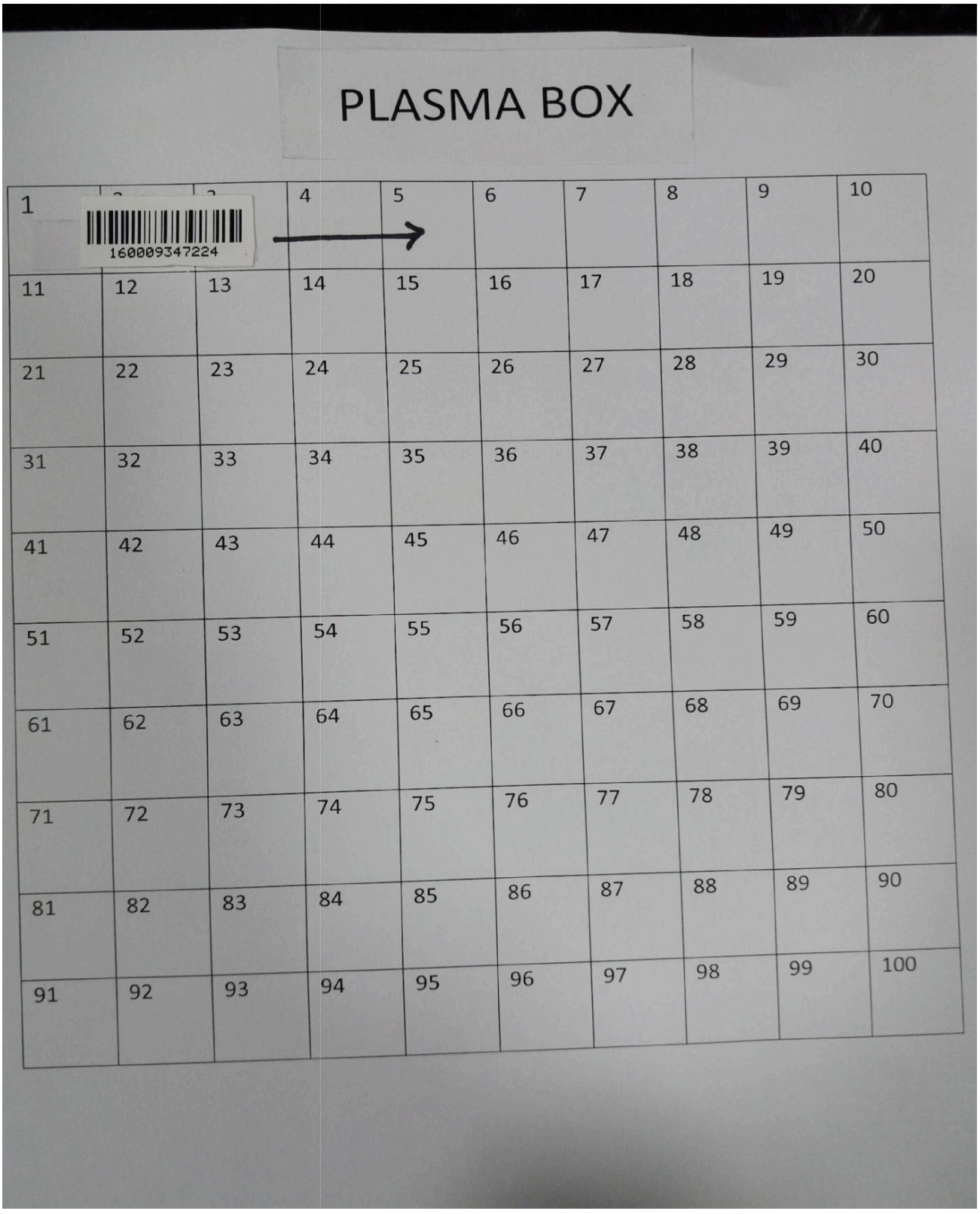
BUFFY COAT AND RBC BOX

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

The Cryo tubes filled with Buffy coat and RBC named BC1, BC2, R will be placed in this box.

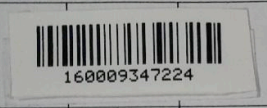
Each row will have 3 subjects, after reaching the last row, we can then use the last column as shown above.

So each box can take (3x10) +3 subjects = 33 subjects



Arrow indicates same number in slots 4 and 5 also. The next subject's tubes will be placed from slot 6-10 and the corresponding PSC code should be stuck on squares 6,7,8.

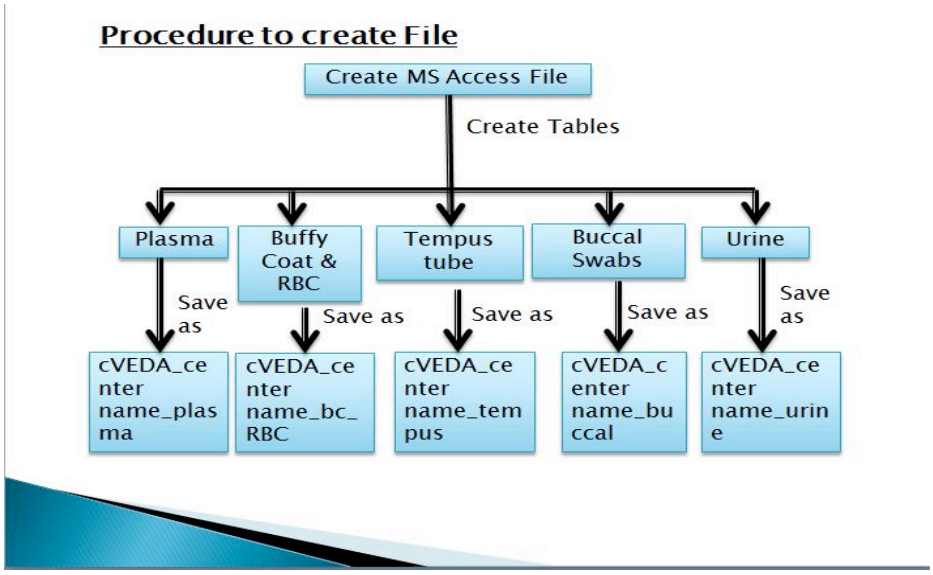
BUFFY COAT AND RBC BOX

1 			4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

2B.2 – Maintaining a log of sample acquisition

Create an MS Access file

1. Open an MS Access file (**Start-> All programs -> Microsoft office -> Microsoft Access**),
2. Name it as "cVEDA_center name", e.g. 'cveda_nimhans'
3. Create tables in MS Access, as follows:
    - "cVEDA_center name_plasma", e.g. 'cveda_nimhans_plasma'
    - "cVEDA_center name_bc_RBC" e.g. 'cveda_nimhans_bc_RBC'
    - "cVEDA_center name_tempus" e.g. 'cveda_nimhans_tempus'
    - "cVEDA_center name_urine" e.g. 'cveda_nimhans_urine'
    - "cVEDA_center name_buccal" e.g. 'cveda_nimhans_buccal'



4. Label columns in the **plasma/bc_RBC/tempus** tables as follows (Snapshot below):
    a) Date - Mention sample collection date.
    b) Box No. - mention sample box number.
    c) Location - mention the sample location (as per the grid shown below – cryoboxes for the plasma and bc_RBC tubes, and the tempus tube racks for the tempus tubes).
    d) PSC1 code - mention the PSC1 code (the barcode scanner can be used to enter this)
    e) Volume - mention volume of sample in each tube, in microlitres for e.g., 900ul p1, 900ul p2, 900ul p3, 900ul p4, 900ul p5 etc.
    f) Remarks - mention if any remarks while collecting/storing samples.

CVEDA NIMHANS PLASMA BOX 1

ID	DATE	BOX NO	LOCATION	PSC CODE	VOLUME	REMARKS	DONE BY
1	01-12-2016	1	1	160001091813	900UL P1		
2		1	2	160001091813	900UL P2		
3		1	3	160001091813	900UL P3		
4		1	4	160001091813	900UL P4		
5		1	5	160001091813	900UL P5		
6	02-12-2016	1	6	160007698397	600UL P1		
7		1	7	160007698397	600UL P2		
8		1	8	160007698397	600UL P3		
9		1	9	160007698397	600UL P4		
10		1	10	160007698397	600UL P5		
11	03-12-2016	1	11	160007535191	500UL P1		
12		1	12	160007535191	500UL P2		
13		1	13	160007535191	500UL P3		
14		1	14	160007535191	500UL P4		
15		1	15	160007535191	500UL P5		
16	03-12-2016	1	16	160007544148	500UL P1		
17		1	17	160007544148	500UL P2		
18		1	18	160007544148	500UL P3		
19		1	19	160007544148	500UL P4		
20		1	20	160007544148	500UL P5		
21	03-12-2016	1	21	160007524979	500UL P1		
22		1	22	160007524979	500UL P2		
23		1	23	160007524979	500UL P3		
24		1	24	160007524979	500UL P4		
25		1	25	160007524979	500UL P5		

Grid for Plasma boxes
(5 plasma cryotubes per subject)

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

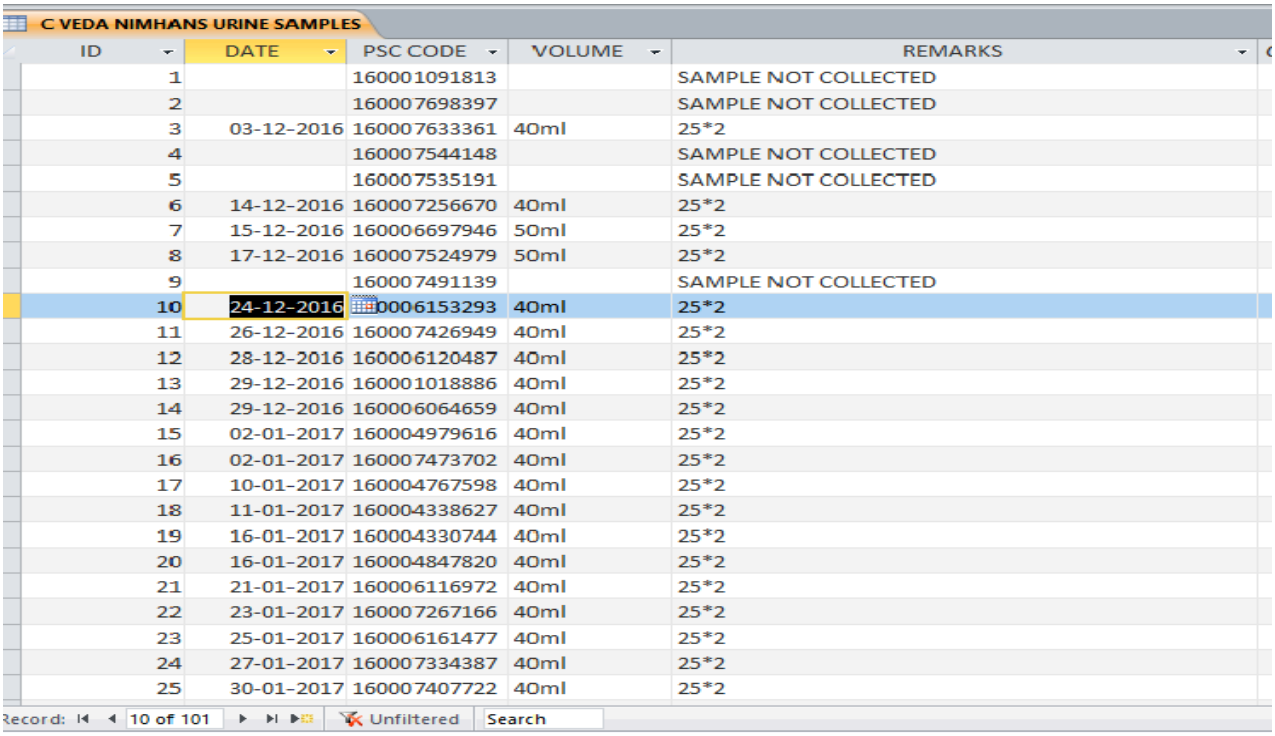
Grid for bc_RBC boxes
(2 buffy coat cryotubes + 1 RBC cryotube per subject)

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

Grid for Tempus Tube
(1 Tempus tube per subject)

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50

4. Label columns in the **urine** table as follows (Snapshot below):
    a) Date column - Mention sample collection date.
    b) PSC1 code - mention the PSC1 code (barcode scanner can be used to enter this)
    c) Volume - mention volume of each sample in milliliter for e.g., 40 ml.
    d) Remarks - mention how many samples have been collected for per subject for e.g., 25ml A & 25 ml B, and if there are any other remarks during the sample collection/storage



C VEDA NIMHANS URINE SAMPLES

ID	DATE	PSC CODE	VOLUME	REMARKS
1		160001091813		SAMPLE NOT COLLECTED
2		160007698397		SAMPLE NOT COLLECTED
3	03-12-2016	160007633361	40ml	25*2
4		160007544148		SAMPLE NOT COLLECTED
5		160007535191		SAMPLE NOT COLLECTED
6	14-12-2016	160007256670	40ml	25*2
7	15-12-2016	160006697946	50ml	25*2
8	17-12-2016	160007524979	50ml	25*2
9		160007491139		SAMPLE NOT COLLECTED
10	24-12-2016	0006153293	40ml	25*2
11	26-12-2016	160007426949	40ml	25*2
12	28-12-2016	160006120487	40ml	25*2
13	29-12-2016	160001018886	40ml	25*2
14	29-12-2016	160006064659	40ml	25*2
15	02-01-2017	160004979616	40ml	25*2
16	02-01-2017	160007473702	40ml	25*2
17	10-01-2017	160004767598	40ml	25*2
18	11-01-2017	160004338627	40ml	25*2
19	16-01-2017	160004330744	40ml	25*2
20	16-01-2017	160004847820	40ml	25*2
21	21-01-2017	160006116972	40ml	25*2
22	23-01-2017	160007267166	40ml	25*2
23	25-01-2017	160006161477	40ml	25*2
24	27-01-2017	160007334387	40ml	25*2
25	30-01-2017	160007407722	40ml	25*2

Record: 10 of 101 Unfiltered Search

5. Label columns in the **buccal swabs** table as follows:
    a) Date column - Mention sample collection date.
    b) PSC1 code - mention the PSC1 code number.
    c) Remarks - mention if any remarks while collecting samples.
    d) Done by - Mention the name of the person done the sample process.

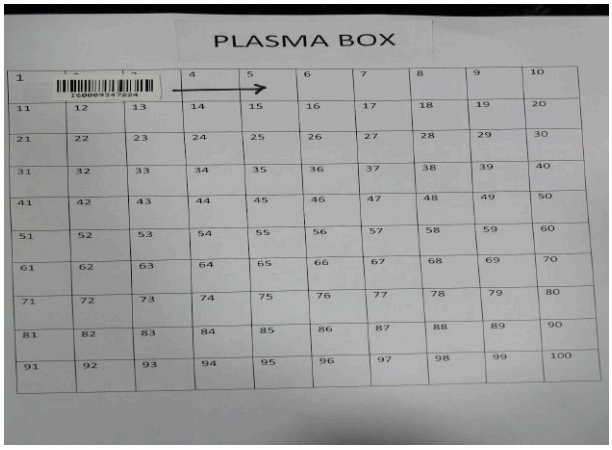
2C: Sample shipment

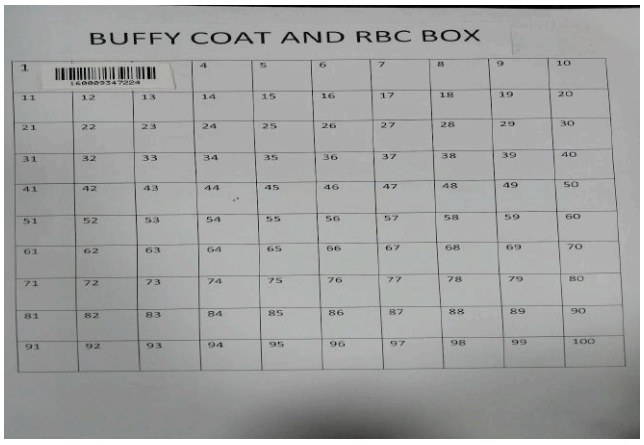
a. Shipment of **plasma, bc_RBC, tempus and buccal swabs.**

These samples will be periodically shipped to NIMHANS (once in every 2-3 months, or as required by the sample holding capacity of the site). One person from the NIMHANS/site team will physically carry the samples to the sample repository at NIMHANS. The sites are to ensure the steps below so that samples are systematically transported.

Before putting them in thermacol dry ice boxes:

a) Make sure that all **cryoboxes** are properly labeled with the centre name, sample and box no. Be ready with hard copy catalog for plasma, bc_RBC boxes (image below).





b) Place Buccal swabs in a thick thermo liable cover (e.g. micro tip covers). Enclose a separate sheet of paper mentioning the PSC1 codes in each package.

c) The tempus tubes in the tempus tube racks and cardboard boxes. Label each package serially, with the centre name, sample and box no. Enclose a separate sheet of paper mentioning the PSC1 codes in each package.

Placing the boxes/packages in the thermacol dry ice boxes:

a) Add one layer of dry ice at the bottom of the thermocol box.

b) Place the boxes/packages of plasma, bc_RBC and tempus one after one.

c) Again add dry ice and place the remaining plasma, bc_RBC and tempus boxes/packages.

d) Place the Buccal swabs packages in the sides of the box.

e) At last, fill up the space with dry ice and seal the thermacol box tightly with brown cello tape.

b. Shipment of **Urine** samples.

- All the urine samples collected at a site have to be stored in the -20 deg C deep freezers that will be shortly supplied. These samples will be shipped to NIMHANS (one sample from all participants) or Kolkata (deep phenotyping set, i.e. 'b' samples) at the end of the baseline data collection phase.
- The sites are requested to ensure that the samples are stored in a systematic manner such that PSC1 code locations can be easily identified.
- Details on shipment strategy will be shared later.

Documents to be sent along with the samples:

A. Paper logs of the plasma/bc_RBC boxes (10*10 grid with cryolabels indicating location of PSC1 codes)
B. Paper record of tempus tubes package nos and the PSC1 codes they contain
C. Paper record of buccal swabs package nos and the PSC1 codes they contain
D. Paper record of urine cardboard box nos. and the PSC1 codes they contain