HELIX Subcohort Common SOP: Sample Collection, processing and shipping

V7. 20 January 2014

Cohorts must ensure that they are following the most up-to -date version of this SOP during fieldwork

Contenido

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BTHFT : Bradford Teaching Hospitals NHS Foundation Trust ; INSERM: Institut National de la Sante et de la Recherche Medicale; CREAL: Centre for Research in Environmental Epidemiology; VDU: Vytauto Didziojo Universitetas; UoC: University of Crete; CRG: Centre for Genomic Regulation; NIPH: Norwegian Institute of Public Health, Division of Environmental Medicine; ICL: Imperial College London; APA: Apa Laboratoris Clínics

Summary of samples to be collected during the HELIX Subcohort follow-up:

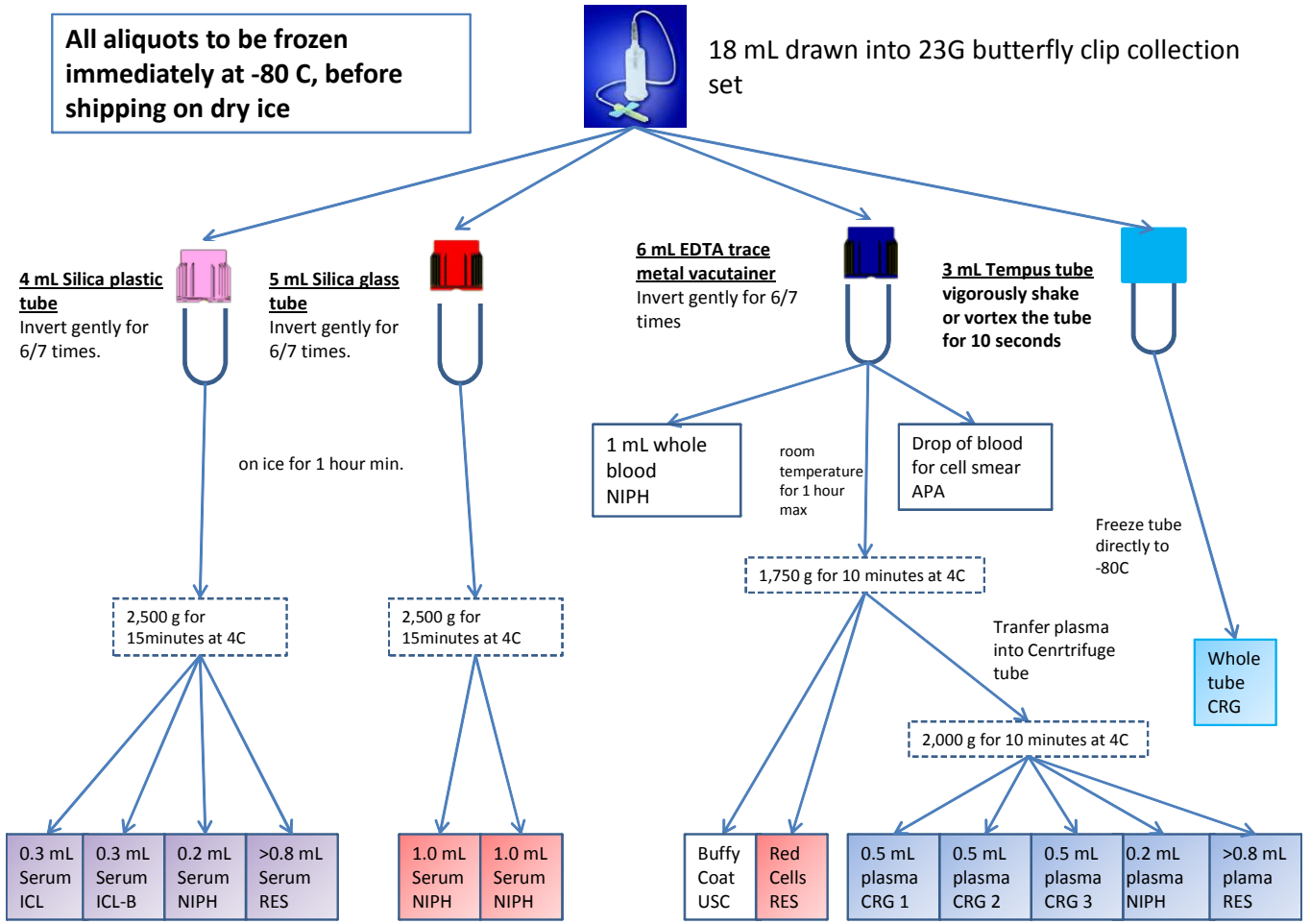
Type of sample	N*	Collection Tube	Sample processing	Sample quantity Required, mL	Purpose
Urine	1200	High quality polypropylene 70 mL collection containers (collected at two time points)	Urine	1.75	Metabonomics
				0.35	Phthalates
				0.5	Phenols
				0.5	OP Pesticides
				1.0	Metals
				0.2	Cotinine
				0.5	Creatinine, specific gravity
Blood (18 mL)	1200	4 mL silica vacutainer **368813**	Serum	0.6	Metabonomics
				0.2	HDL, Cholesterol, trigycerides, glucose
				>0.8	spare
		5mL silica glass vacutainer (no additive) **367614**	Serum	2.0	PCBs, DDE, HCB, PBDE
		6 mL EDTA (trace metal tube) **368381**	Whole blood	0.9	Heavy metals
			Blood smear	0.1	Cell differentiation
			DNA	2.5	Methylomics
			Plasma	0.5	Proteomics
				1.0	miRNA
				0.2	PFASs
				>0.8	spare
		3 mL Tempus	RNA	3	Transcriptomics

* From across all HELIX cohorts

Materials:

- BD Vacutainer blood collection set (1/subject, BD: 368655, pre-attached holder, butterfly clip, 23G needle).
- tube rack
- cuff
- Gloves
- disinfectant
- local anaesthetic patch (ELMA)
- gauze bandages
- medical tape
- Disposal box for needles.
- Tubes for blood collection (1/subject, NOTE: BD tube references are the Spanish reference numbers, check number against description)
  - 6 ml EDTA vacutainers for plasma and DNA isolation (BD: 368381, dark blue top, trace element plastic, K_2EDTA coated)
  - Tempus tubes for RNA isolation (Life Technologies Cat No: 4342792, light blue top)
  - 4 ml plastic silica vacutainers for serum (BD: 368813, pink top, silica coated, clot activator)
  - 5mL glass silica vacutainer for serum (BD: 367614, red top, silica coated, no activator)
- Centrifuge
- Centrifuge tube : 15 ml falcon tube or 5 mL tube (SARSTED: 55.475.001)
- Micropipette and pipette tips (HDPE, LDPE, PP, or PS plastic (recycle mark 2,4,5,and 6, check material with Cathrine Thompsen))
- For urine collection to be sent out to each family prior to subcohort visit:
  - 70 mL urine collection tubes (Sarstedt: 75.9922.744)
  - Small fridgebox (a Tupperware box that urine tubes are placed inside)
  - Cool bag
  - Ice pack
  - Printed labels for families to fill in themsleves
- 1.2 ml vials (nominal volume 1ml) external threads: (SARSTED: 72.377).
- 2.0 ml vials (nominal volume 1.8 ml) external threads (SARSTED: 72.379)
- SARSTED Coloured cap inserts: (red (5/subject), 65.386.002), yellow (7/subject, 65.386.003), green (5/subject, 65.386.004), blue (5/subject, 65.386.005), violet (5/subject).
- Plastic Boxes cryovial boxes, 9x9 samples: (SARSTED 93.877)
- Boxes for Tempus tubes (Nirco, Cat No. B77)
- Printed labels with aliquot codes as specified in this SOP
- Dry ice for eventual shipping
- For Blood smear:
  - Glass slides (26mm x76 mm 1 mm thick polished edges frosted 20 mm vacuum packed, Deltalab, box of 50 units: D100003)
  - Capillary glass tube (Sarstedt, Ref num: 51.931.100)
  - Slide box for 50 slides Blue Company (Sharlab, Ref num: 027-19277A).
  - Quick panoptic number 1 (Química Analítica Aplicada, Ref num: 991681)

Blood collection and processing



Procedure for blood collection:

- Blood should be collected only by trained personnel using aseptic methods.
- An EMLA plaster will be applied to child before their clinical examination at least one hour before blood collection and before blood collection following manufacturer instructions.
- Sampling location should be an isolated, peaceful area (e.g., a separate room) with all the necessary equipment prepared beforehand.
- Date and time of blood sampling should be noted on the sample data worksheet (Annex to this document). Food and medicines consumed that day should be specified on sample data worksheet.
- To avoid hemolysis (lysis of red blood cells) when collecting blood samples, we recommend the following procedures:
    - Follow manufacturer's instructions
    - Avoid drawing blood from a hematoma
    - Avoid frothing of the sample
    - Make sure the venipuncture site is dry
    - Avoid a probing, traumatic venipuncture
    - Avoid prolonged tourniquet application or fist clenching
    - Vacuum tubes should be filled completely

- To prevent backflow of tube additives from the tube into the individual's arm, observe the following precautions:
    - Place the individual's arm in a downward position.
    - Hold the tube with the cap up.
    - Release the tourniquet as soon as the blood starts to flow into the tube.
    - Make sure the tube contents do not touch the cap or the end of the needle during venipuncture.

- Blood will be collected into the four tubes in the following order:

    1. Plastic serum vacutainer,
    2. glass serum vacutainer
    3. EDTA,
    4. Tempus,

- The serum vacutainers and EDTA tubes should be filled completed. Blood should be drawn into the Tempus tube up to the black line on the tube.
- Immediately after collection into all tubes, The serum and EDTA tubes should be gently inverted 6-7 times. The Tempus tube should be **vigorously shaken or vortexed for 10 seconds**

Blood processing protocol

It is very important to document the time of collection of the sample, the time of the start of centrifugation, the time of freezing to -80°C. Moreover, the patient ID, the date sample taken and the type of sample should be noted in the **sample data worksheet**.

The blood will be processed in a variety of ways: Serum (silica vacutainers), Plasma and DNA (EDTA), RNA (tempus) and blood cells (future lipid metabolomics or adductomics) will be extracted. A blood smear will be made from a drop of whole blood in order to count white blood cell types.

Serum vacutainers should be put on ice or in the fridge while you proceed with processing of other samples. Tempus tubes should be put directly into the freezer.

Aliquot racks should be placed on ice while they are prepared for placing in the freezer

EDTA vacutainer: plasma and DNA

- Collect blood in 1 EDTA vacutainer (dark blue top)
- Invert gently for 6/7 times.
- Aliquot 1 mL whole blood for lead measurement into 1.8 mL cryovial
- Label this aliquot HELIXID-WB-1. Do use colour insert. Put in first column of NIPH storage box.
- Take out a drop of blood using a sterile capillary glass tube and proceed with cell count smear below.
- The remaining blood in EDTA vacutainer may be left at room temperature for a maximum of 1 hour before centrifugation
- Centrifuge the rest of the EDTA blood samples in a horizontal rotor (swing-out head) at 1,750 g for 10 minutes at 4 ^{o}C.
- Transfer the supernatant (plasma, approximately 50% of the volume) to a new pre-labelled 15 ml falcon tube. Leave a very small amount of plasma on top of the buffy coat layer, so that the buffy coat is not being disturbed and no cells are contaminating the plasma.

  Warning: Do not discard the vacutainer tube!

- Centrifuge the falcon tubes with plasma at 2,000 g for 10 minutes at 4 ^{o}C.
  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.changbioscience.com/cell/rcf.html

- Aliquot the plasma as follows. Be sure of not transferring any pellet residuals.
  - 3 x 0.5 mL. BLUE colour insert. Labels: HELIXID-P1, HELIXID-P2 and HELIXID-P3. Put in first columns of separate CRG storage boxes A, B and C
  - 0.2mL. BLUE colour insert. Label : HELIXID-P4. Put in second column of NIPH storage box.
  - Remaining plasma. (Use 1.8 mL cryovial if necessary). BLUE colour insert. Label: HELIXID- P5. Put in first column of Reserve box.
- Store the storage boxes at -80°C. Prior to shipment

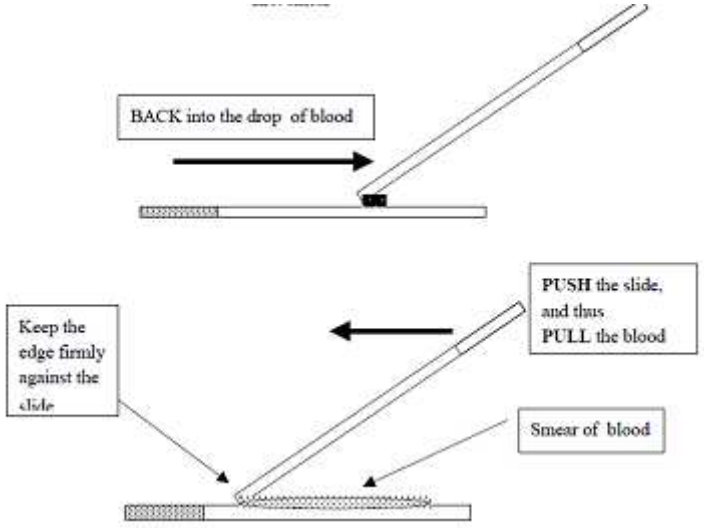
- Transfer the buffy coat (grayish layer and a small amount of the red blood cells) from the first centrifugation round, into a 1mL cryovial. **Be sure you get the entire buffy coat.** The small amount of plasma left in the vacutainer may be taken as well as the buffy coat
- Label this aliquot HELIXID-BC-1. Do not use colour insert. Put in first column of USC storage box.

- Transfer the remaining contents on the vacutainer (the red blood cells) into a 1.8 mL cryovial. Label this aliquot HELIXID-RC-1. Red colour insert. Put in second column of Reserve box
- Store the storage boxes directly to -80C, prior to shipment
- Fill the sample collection document.
- **Always record exact time of collection (venipucture), centrifugation and storage to -80°C (on attached worksheet), and always record any deviation from the procedure.**

Blood smear for cell count protocol

1. Write in pencil the id of the child on the slide matte label.
2. Perform the blood draw as shown above.
3. Invert the EDTA tube several times to homogenize the proportion of cells. Take one drop of blood from the tube using a sterile capillary glass tube.
4. Place a drop of blood approximately 4 mm in diameter on the slide (near the end).
5. Smear the blood droplet (requires training): Spread the drop by using another slide (called here the "spreader"), placing the spreader at a 45° angle and BACKING into the drop of blood. The spreader catches the drop and it spreads by capillary action along its edge. To make a short smear, hold the spreader at a steeper angle, and to make a longer smear, hold it closer to the drop. Now, push the spreader across the slide; this PULLS the blood across to make the smear. **Do not push the blood by having it ahead of the smearing slide!** It should take about one second to smear the drop. A smooth action is required, with the edge of the spreader held against the slide. This will yield a nice, even smear. Take a look at the figures and for extra information visit

http://www.youtube.com/watch?v=O3d_4dkVVSE,
http://www.youtube.com/watch?v=XBCxusLUe68&feature=endscreen&NR=1



6. Allow to dry at least 30 minutes. Be sure that the slide is completely dry!

7. Submerge the slide in the panoptic number 1 (blue, fixation solution) **for 30 seconds** and eliminate the excess of liquid by contacting the extreme of the slide with a paper.

8. Air dry until completely dried. To accelerate drying, keep the sample leaning.

9. Store it inside the slide box and leave at room temperature. They are stable for at least one month.

10. Ship slides to Apa Laboratoris Clínics every three weeks. Follow instructions for shipment in next sections.

Tempus tube: RNA

- Draw 3 mL of blood directly into each Tempus Blood RNA.
  Note: The black mark on each tube label indicates approximately 3 mL.
- Immediately after filling each Tempus tube, vigorously shake or vortex the tube for 10 seconds to ensure that the Applied Biosystems Stabilizing Reagent makes uniform contact with the sample.
  IMPORTANT! Failure to mix the stabilizing reagent with the blood leads to inadequate stabilization of the gene expression profile and the formation of microclots that can potentially compromise the RNA purification procedure.
- Label the tube HELIXID-R1. Store in Tempus tube storage box
- Store the tubes directly at -80 ºC. Prior to shipment to CRG.

IMPORTANT: Do not let the samples come into direct contact with the dry ice.

- Fill the sample collection document.
- Follow instructions for shipment in next sections.

Serum samples

- Collect blood in 1 plastic silica vacutainer (pink top) and 1 glass silica vacutainer (red top).
- Invert both gently for 6/7 times.
- Allow both to clot for **1 hour on ice.**
- Centrifuge the vacutainers at 2500g for 15 minutes, at 4^{o}C.

- Aliquot the serum from the plastic vacutainer as follows into 1 mL cryovials:
  - 0.2mL. VIOLET colour insert. Label : HELIXID-SP1. Put in third column of NIPH storage box.
  - 0.3mL. VIOLET colour insert. Label : HELIXID-SP2. Put in first column of ICL storage box.
  - 0.3mL. VIOLET colour insert. Label : HELIXID-SP3. Put in first column of ICL_BACKUP storage box.
- Transfer remaining serum into 1mL cryovials . VIOLET colour insert. Label: HELIXID-SP4. Put in third column of Reserve box

- Aliquot the serum from the glass vacutainer as follows into a 1.8 mL cryovials:
  - 1 mL. RED colour insert. Label: HELIXID-SG1. Put in fourth column of NIPH storage box
  - Remaining serum. RED colour insert. Label: HELIXID-SG2. Put in fifth column of NIPH storage box

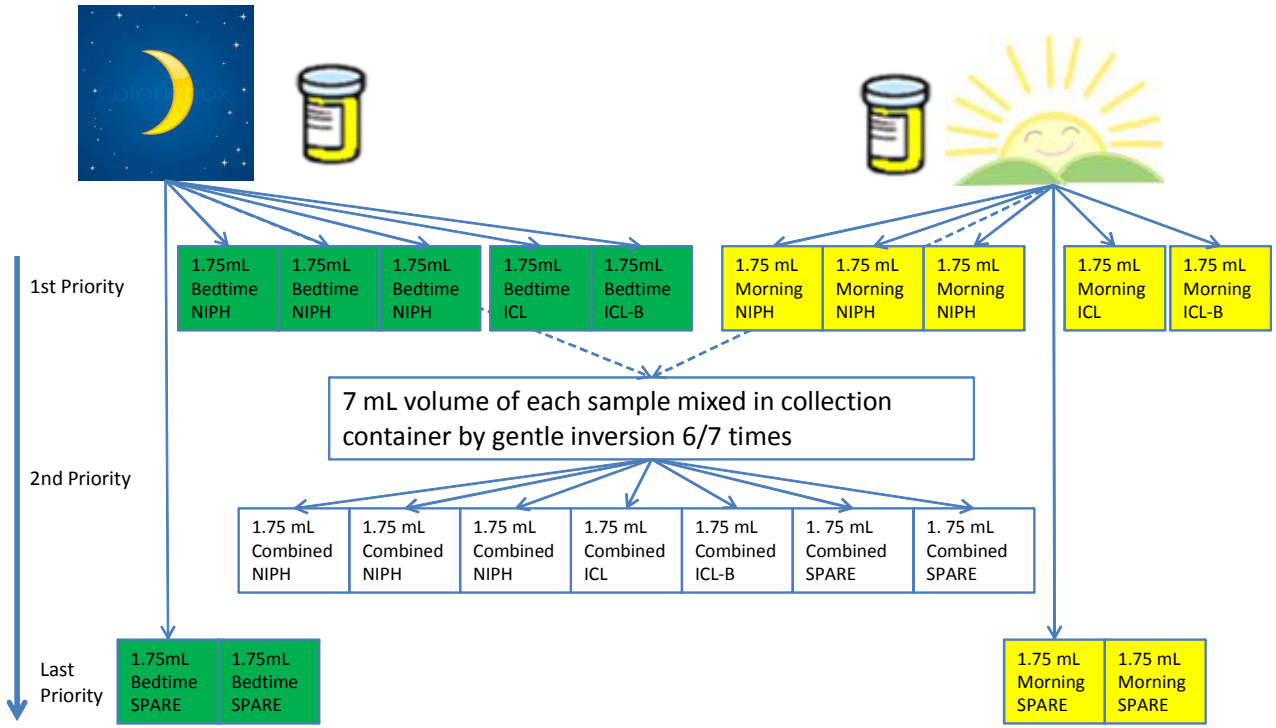
- Store the cryovials at -80°C prior to shipment
- **Always record exact time of collection (venipucture), centrifugation and storage to -80°C (see annex 10), and always record any deviation from the procedure.**
- Keep 3 full sets of disposable equipment used for sampling and storage for blank control. Ship to NIPH together with last samples.

Technical Quality Control samples

Technical quality control samples will be distributed to each of the cohorts. These should be shipped 'blind' to the analysing laboratories. Further information on this process will sent on receiving the technical control samples.

Urine Collection and processing

Samples collected in 70 mL containers
Samples stored at 4C after collection (>24h)
Aliquots frozen at -80C before shipping on dry ice



Urine collection

Prepare the 70 mL urine collection tubes tubes to be sent out to families:

- Stick two printed labels (See example) on two collection tubes. Place tubes into fridgebox. Place fridgebox into cool bag alongside ice pack. This will be sent to families as part of information pack prior to visit (See subcohort 'information to families').
- Parents will be instructed how to fill and store the samples (See subcohort 'information to families').

Subject ID: 123

Date: _ _ / _ _ / 20 _ _

Time of urination: _ _ : _ _ hours

Infancia y Medio Ambiente

On arrival at study centre:

- Parents will bring two samples with them to centre (inside their cool bag), which will be placed in 4°C fridge on arrival. Check labels are completed correctly (ask mother for to help fill in if it is not completed).
- If the family has not brought samples, ask child to provide new urine sample in collection tube when given opportunity to go to toilet (this sample should be labelled as CUx samples and a note made on comment section of sample data sheet).

Urine sample processing

- The samples that children have bought with them that day will be collected from fridge.
- Aliquot in the order specified below.
- Aliquot the urine from the 'bedtime sample' tube as follows into 2 mL cryovials:
    - 3 x 1.75 mL. GREEN colour insert. Label HELIXID- NUx-01, HELIXID-NUx-02 and HELIXID-NUx-03. Put in first, second and third columns of NIPH storage box (below blood aliquots for that individual)
    - 1 x 1.75 mL. Green colour insert. Label HELIXID- NUx-04. Put in second column of ICL storage box
    - 1 x 1.75 mL. Green colour insert. Label HELIXID- NUx-05. Put in second column of ICL_BACKUP storage box
- Aliquot the urine from the 'morning sample' tube as follows into 2 mL cryovials:
    - 3 x 1.75 mL. YELLOW colour insert. Label HELIXID- MUx-01, HELIXID- MUx-02 and HELIXID-MUx-03. Put in fourth, fifth and sixth columns of NIPH storage box (below blood aliquots for that individual)
    - 1 x 1.75 mL. YELLOW colour insert. Label HELIXID- MUx-04. Put in third column of ICL storage box
    - 1 x 1.75 mL. YELLOW colour insert. Label HELIXID- MUx-05. Put in third column of ICL_BACKUP storage box
- Add 7 mL 'First morning sample' and 7mL 'Bed time sample' to a pp collection container. Invert the container 6 or 7 times to mix.
- From this mixture aliquot out the following into 2 mL cryovials:
    - 3 x 1.75 mL. Do not use colour insert. Label HELIXID- CUx-01, HELIXID-CUx-02, HELIXID-CU3. Put in seventh, eighth and ninth columns of NIPH storage box (below blood aliquots for that individual)

  - 1 x 1.75 mL. Do not use colour insert. Label HELIXID- CUx-04. Put in fourth column of ICL storage box
  - 1 x 1.75 mL. Do not use colour insert. Label HELIXID- CUx-05. Put in fourth column of ICL_BACKUP storage box
  - 2 x 1.75 mL. Do not use colour insert. Label HELIXID- CUx-06 and HELIXID-CUx-07. Put in eighth and ninth columns of Reserve storage box.
- Then, if urine left in the 'first morning sample' and 'bed time sample', do following aliquots:
  - 2 x 1.75 mL 'bed time sample'. GREEN colour insert. Label HELIXID- NUx-06 and HELIXID-NUx-07. Put in fourth and fifth columns of RESERVE storage box.
  - 2 x 1.75 mL. 'First morning sample' YELLOW colour insert. Label HELIXID- MUx-06 and HELIXID-MUx-07. Put in sixth and seventh columns of RESERVE storage box.
- Freeze the cryovials at -80°C. Prior to shipment.
- **Always record exact time of urine collection and storage to -80 ºC (on attached worksheet), and always record any deviation from the procedure.**

Blank Control (for urine samples)

Keep 3 full sets of disposable equipment (pipette tips, 1mL and 1.8mL cryvials, 100 mL collection tubes) used for sampling and storage for blank control whenever the batch is changed. Note the HELIX-IDs of sample using that batch of equipment. Ship to NIPH together with the last samples.

Technical Quality Control samples

Technical quality control samples of urine, plasma and serum will be distributed to each of the cohorts. These should be shipped 'blind' to the analysing laboratories. Further information on this process will sent on receiving the technical control samples.

Labelling of samples

All aliquots should be labelled with pre-printed labels containing just the aliquot code.

The aliquot code will be made up as follows

Cohortcode _ Existing Cohort specific ID Number _Study code_ (these three parts will make up the HELIX subject ID) _aliquot type_aliquot number

Note all children taking part in the HELIX subcohort (who are not also taking part in the panel studies) will have study code 1x.

Aliquot Coding Key:

HELIX ID Number			**Aliquot specific code**	
Cohort Code	Existing Cohort specific ID Number	Study code	Sample type	Aliquot number
SAB= INMA -Sabadel	xxxx	0 = Mother (archived)	NUx= Bedtime urine	01
EDP= EDEN - Poitiers	xxxx	1x = Child participating in subcohort only	CUx= Combined urine	02
KAN = KANC	xxxx	1a= Child (finishing first period of panel study)	MUx= Morning urine	03
RHE= RHEA	xxxx	1b= Child (finishing second period of panel study)	BC=Buffy coat	...
BIB= BiB		2a= Pregnant women first period panel study	WB=Whole Blood	
MOB = MoBa		2b= Pregnant women second period panel study	P=Plasma	
OSL = Oslo			R=RNA (tempus tube)	
BCN = Barcelona			SP=Serum (from plastic tube)	

GRE = Grenoble			SG=Serum (from glass tube)	
			RC=Red blood cells (from EDTA tube)	

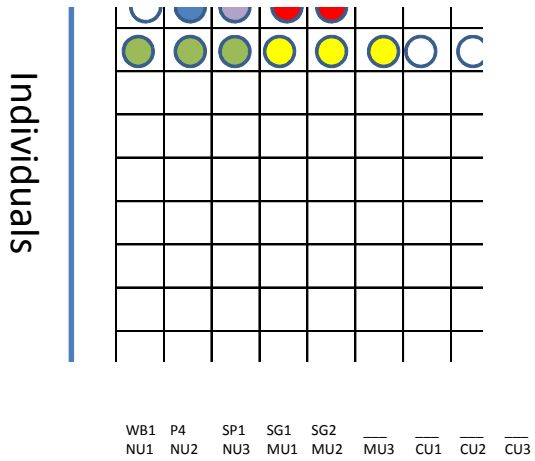
Example:

SAB-223-1x-NUx-01 indicates the **first aliquot** made of the **bedtime urine** sample from **child 223** collected at the **subcohort follow-up** in the **INMA – Sabadell** cohort.

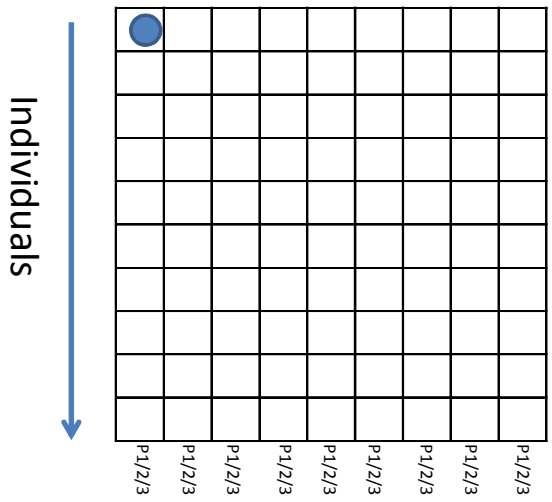
Storage of samples:

Each centre should have the following box ready in their freezers to be filled as follows. They should be number consecutively as they are filled to keep track of samples within each box (eg NIPH Storage Box 1, NIPH storage box 2 etc..)

NIPH STORAGE BOX

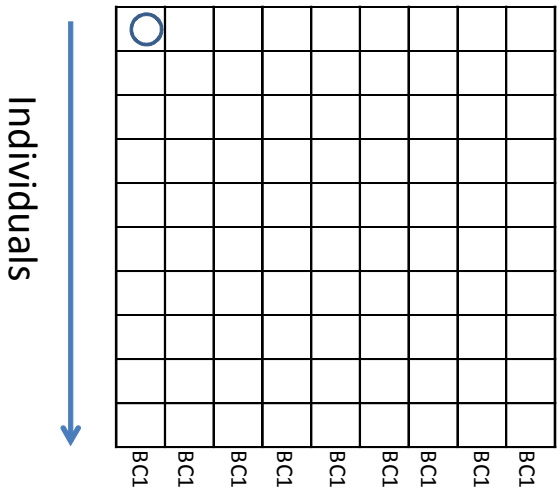


CRG STORAGE BOX A/B/C



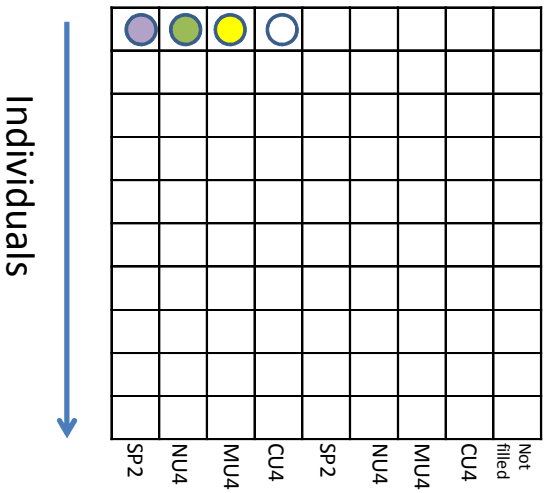
NB: Filled down rows first with consecutive individuals , before new column started. Same scheme followed for CRG storage boxes A,B and C

USC STORAGE BOX



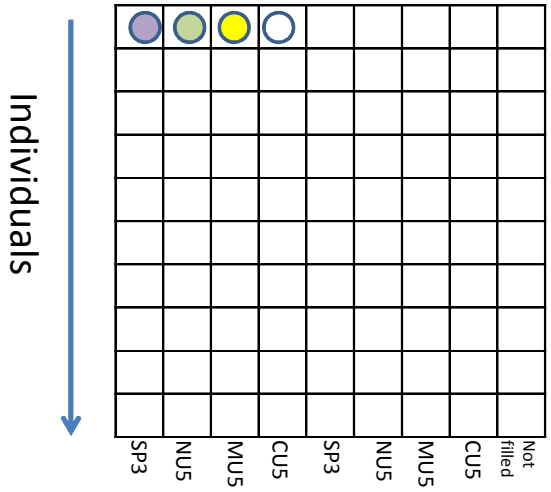
NB: Filled down rows first with consecutive individuals , before new collumn started

ICL STORAGE BOX



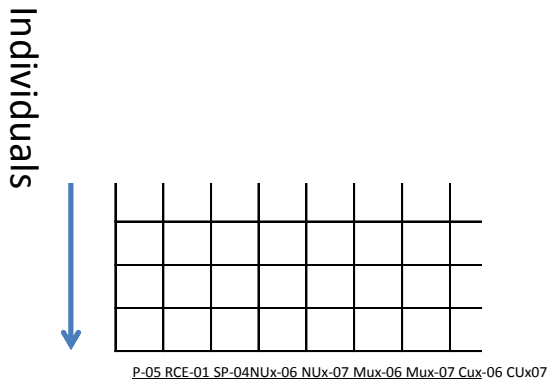
NB: Filled down rows first with consecutive individuals , before new collumn started

ICL_BACKUP STORAGE BOX



NB: Filled down rows first with consecutive individuals , before new collumn started

RESERVE STORAGE BOX



In addition there should be a:

- TEMPUS tube box (stored at -80C until shipment to CRG Epigenetics)
- Cell Smear Slide Box (Stored at Room temperature before shipment to **Apa Laboratoris Clínics** every three weeks)

Tracking and shipping of samples

Central database

As samples are collected, aliquot information should be entered onto the sample data form. This data can be entered immediately or at a later date into the HELIX questionnaire application.

At the same the 'Sample tracking Excel' should be completed. This should be completed and uploaded periodically onto the HELIX website (WP1). The Excel should be saved as 'Sample tracking_Child panel 1 (*or 2)_Cohort code_date uploaded* '

Shipping

ALWAYS give notice of shipment date before sending samples and let laboratories know the tracking number. Send the receiving laboratories the 'Sample tracking Excel' listing all the boxes contained in that shipment.

Samples should be shipped on a Monday, or Tuesday at the latest

Blood and urine samples should be shipped according to the instructions below, frozen with **sufficient dry ice** to prevent thawing

- NIPH (NIPH Storage box) when enough samples are collected for suitable shipment
- ICL (ICL STORAGE Box) on completion of the subcohort sample collection (eg 200 samples).
- CRG epigenetic laboratory (CRG Storage Box A, CRG Storage Box B and Tempus Tubes, sent in separate package)s on completion of the panel study
- CRG proteomics laboratory (CRG storage box C sent separately) on completion of the subcohort sample collection (eg 200 samples).
- USC (USC storage box) on completion of the subcohort sample collection (eg 200 samples).

Blood smear slides should be **sent every three weeks** to **Apa Laboratoris Clínics**

Cohorts should keep the Reserve and ICL-backup boxes in their respective cohorts until required by the HELIX project.

Once child samples are shipped, their corresponding archived mother samples are to be retrieved and shipped (*According to SOP outlined in separate protocol*)

Packaging of samples

According to the **WHO/HSE/GCR/2012.12** "*Guidance on regulations for the Transport of Infectious Substances 2011-2012"* the sample materials are to be considered as infectious substances of category B. This implies the packaging and shipment of samples to follow the "Basic triple packaging system" as described below:

Primary receptacle:
All samples should be placed in specified cryovials sealed by water-proof screw caps. All cryovials should further be placed in a storage box with a capacity of 81 tubes.
The storage boxes containing the cryovials are to be considered as primary receptacles.

Secondary packaging:
The storage boxes must be enclosed by a secondary durable and watertight packaging. Between the first and second layer of packaging there should be enough **absorbent material** to ensure that all liquid sample material will be absorbed in case of brakeage or leakage.

Outer packaging:
The samples should further be packed in a sturdy outer packing of Styrofoam boxes of sufficient size to give room for enough dry ice to keep samples cooled/frozen during transport, as well as to give room to enough cushioned material to insulate samples from physical damages. They should preferably have think walls (eg 6 cm) to prevent thawing during shipping (that may take up to a week for South to North Europe)

Dry ice:
Plenty of dry ice should be used to prevent thawing.

Documentation:
Outside outer packaging should be a Proforma invoice (low value) and a print of the Waybill that has been filled in online (full description). Both Proforma invoice and the Waybill should have "UN3373, BIOLOGICAL SUBSTANCE, CATEGORY B, and UN1845 dry ice"!) written on it, (a letter from the university/inst explaining that the samples will be used for research only, to speed up Customs.

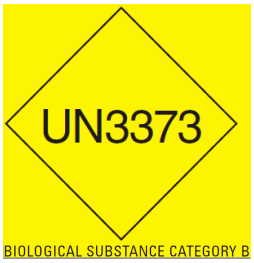
A separate document inside the box should list all the codes of aliquots contained in package to aid receiving laboratories

Marking of the outer packaging

1. Name and address of the sender of the samples. This *must* also include phone numbers, e-mail addresses and name of contact person with knowledge of the shipment.

2. Name and address of the receiver of the samples. This should also include phone numbers, e-mail addresses and name of contact person.

3. A sticker containing the UN 3373-symbol (see below) as well as the proper shipping name (PSN) "BIOLOGICAL SUBSTANCE, CATEGORY B" next to the sticker.



BIOLOGICAL SUBSTANCE, CATEGORY B

4: Since the package also includes dry ice and will be transported by aircraft a sticker containing the “UN-1845 Carbon Dioxide, solid (dry ice)”-symbol, as well as the UN1845-sticker containing information on the amount of dry ice in the package.

5: All irrelevant labels and marks should be removed

Shipping Address and Contacts:

ALWAYS give notice of shipment date before sending samples and let laboratories know the tracking/ Airway Bill numbers numbers. WorldCourier is the recommended courier service.

NIPH:

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Sample Data Sheet (HELIX Subcohort)

Fieldworker (processing samples): _____

Day of week: _____

Date: | | | | | 2 | 0 | | |

(day) (month) (year)

Helix ID (as written on aliquot labels): _____

Blood sampling

Vacutainers collected (Tick all that apply):

- ☐ EDTA,
- ☐ Tempus,
- ☐ Plastic serum vacutainer,
- ☐ glass serum vacutainer

Action	Time performed (24 hours)	
Blood sampling (venopunture)		
Start of Centrifugation of EDTA tube		
Freezing of aliquots from EDTA tube to -80°C		
Freezing Tempus tube to -80°C		
Start of centrifugation of plastic serum tube		
Start of centrifugation of glass serum tube		
Time of freezing of serum aliquots to -80°C		

Blood Aliquots made (Tick all that apply):

- ☐ WB-01
- ☐ Blood Smear
- ☐ P-01
- ☐ P-02
- ☐ P-03
- ☐ P-04
- ☐ P-05
- ☐ BC-01
- ☐ RC-01
- ☐ SP-01
- ☐ SP-02
- ☐ SP-03
- ☐ SP-04
- ☐ SG-01
- ☐ SG-02

Comments on blood processing (Eg. Any deviation from protocol)

_____
_____
_____

<u>Urine sampling</u>

Did the family bring the following urine samples to clinic (Tick all that apply):

- ☐ Night before sample
- ☐ Morning sample

How had the parents stored 'Night before sample' before coming to clinic:

- ☐ Stored at room temperature
- ☐ Stored in fridge
- ☐ Stored in freezer

How had the parents stored 'morning sample' before coming to clinic:

- ☐ Stored at room temperature
- ☐ Stored in fridge
- ☐ Stored in freezer

Action	Time performed (24 hours)	
Time of night before collection		
Time of morning urine collection		
Time of transfer into fridge at clinic		
Time of new urine collection (if needed):		
Time of aliquotting urine		
Time of freezing of urine aliquots to -80°C		

Urine Aliquots made (Tick all that apply):

☐ NUx-01	☐ MUx-01	☐ CUx-01
☐ NUx-02	☐ MUx-02	☐ CUx-02
☐ NUx-03	☐ MUx-03	☐ CUx-03
☐ NUx-04	☐ MUx-04	☐ CUx-04
☐ NUx-05	☐ MUx-05	☐ CUx-05
☐ NUx-06	☐ MUx-06	☐ CUx-06
☐ NUx-07	☐ MUx-07	☐ CUx-07

Comments on urine processing (Eg. Any deviation from protocol)

_____
_____
_____