Preparation of blood and tissue samples

I. BLOOD SAMPLES

Material used (provided by University of Utah and CIHCAMS):
- 6ml Vacutainer tubes (with EDTA)
- Nunc 1.8ml cryotubes (coded with red, yellow, and purple tops)
- 1 Gilson pipette and tips for Gilson pipettes or polyethylene liquiptettes
- Vacutainer needles G20 ½
- Vacutainer Brand Needle Holders

Blood extraction:
Two 6-ml tubes (5mls of blood in each) of blood should be obtained. The first 6ml tube should be labelled with the barcode (ID+B1) and the second 6ml tube should be labelled with the barcode (ID+B2). Please make sure to place the barcode as shown in the figure below. Once collected blood can stay at room temperature for a maximum of 12 hours before being processed in a laboratory.

During the processing, please change your gloves if it becomes contaminated with blood (to avoid cross contamination).

Blood processing (shown in figures 3a and 3b)

1. First 6ml vacutainer tube:
   a. If the blood in the 6ml vacutainer tube has separated into layers, invert a few times so that it is mixed.

   b. Transfer 1.8ml of the whole blood sample into a 1.8ml cryotube with a red top and label with a barcode (ID+W1) and put in the freezer

   c. Centrifuge the remaining blood in the 6ml vacutainer tube for 10 minutes at 2000rpm to get 3 layers (plasma, white blood cell and red blood cells), as shown in the figure.

   d. Separate the plasma (about 50% of sample and 1.8ml max) out with a pipette and transfer to a 1.8ml cryotube with a yellow top and label with the barcode (ID+P1). Do not disturb the white blood cell layer. It is fine if some

   e. The remaining red blood cells + white blood cells should be transferred to 1-2 cryotube(s) with a purple top and labelled with the barcode (ID+R1).
2. For the second 6ml vacutainer tube, process in the same way. But use the corresponding barcode labels (figure 3b):
   a. 6ml EDTA tube: ID+B2
   b. Whole blood 1.8ml cryotube: ID+W2
   c. Plasma in 1.8ml cryotube: ID+P2
   d. RBC+WBC mix: ID+R2

**Sample storage:**
All samples should be stored at -80°C or at least -70°C depending on centre freezer. If possible they should be equally distributed between different freezers in order to avoid the loss of material in case of any problem with one of the freezers.
II : SNAP FROZEN FRESH TISSUE SAMPLES (OESOPHAGUS, ORAL, LARYNX and PHARYNX)

Whenever possible fresh tumour tissue will be collected. This will require a high level of coordination with surgeons.

Material used
Store in a cryotube (blue tops) or in plastic bags.

Sample collection
Obtain at least 0.5g of fresh tumour tissue from biopsy or surgery and place it in a cryotube or small sterile plastic bag. Close the tube or bag and label with identification labels provided. Cover the identification label with transparent tape to protect it. In order to snap freeze the tissue it should be placed in a container with liquid nitrogen within 20 minutes. If it is not possible to snap freeze the tissue, ensure that it is placed in a freezer within 24 hours of collection. It should be noted on the logsheet whether the tissue is snap frozen or not.

Sample storage
If sample collection takes place in an area far from the freezer use small “cold bags” to keep the specimen cool.
If the samples have not been snap frozen, place them in an ice-bath prior to freezing. Move the samples to the freezer the same day they are obtained.

Record keeping
Record the availability of fresh tissue on the case log sheet.
iii. PARAFFIN EMBEDDED TISSUE

Sample collection and preparation
Identify all paraffin-embedded blocks -histology and cytology- related to the disease of interest.
Priorities are:
1. The whole block of paraffin-embedded tissue (PET)
2. Part of the block of PET
3. Shavings of PET from the block,
   note: shavings must be at least 10μm thick. This is 2-3 times the usual thickness.

Sample storage
1. Whole blocks of PET should be stored in plastic bags, part blocks (where small enough) and
   shavings (10 shavings per tube) should be stored in cryotubes (green top).
2. Samples from different blocks should be stored separately.
3. Every tube/ plastic bag should be labelled with the labels provided.
4. The PET should be kept at 4°C.

Sample shipment
1. Include the pathological diagnosis report of all PET samples shipped.

IV. HISTOLOGICAL / CYTOLOGICAL SLIDES

If at all possible the histological slide on which the diagnosis is based should be obtained from the
hospital, or if not then another histological slide. If diagnosis is by cytology only and no tissue samples
are available then the pathologist should be asked to provide an extra cytological slide for the purpose
of this study.

Sample storage
1. Store slides from each paraffin-embedded block in a separate box.
2. Label every box with the hospital, center, subject, and block code numbers.
3. Keep the boxes at 4°C, otherwise, store in a safe cupboard placed in a cool area.

Sample shipment
1. Send packages of 10-20 boxes and include the pathological diagnosis report of all blocks shipped.
**Identification numbers**

Each subject will receive a unique identification number. The ID numbers will be assigned in the order of interviews from 1 to 1300. This ID number should be consistent for each study subject across the lifestyle questionnaire database and the logsheet database.

The ID number for nonparticipant cases should start at 8000.
The ID number for nonparticipant controls should start at 9000.

**Labels**

Labels that have the ID numbers with serial coding will be provided by University of Utah. The labels will be used on the paper questionnaire and biological samples.

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|__|__|     |__|__|__|__|     -  |__|__|__|
Center code   Subjects number    specification of sample
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Specifications, with number of labels provided:

- **Q** = questionnaire (n=1)
- B1 and B2 = 6ml blood sample (n=2)
- W1 and W2 = whole blood in 1.8ml cryotube (n=2)
- R1 and R2 = red blood cells + white blood cell in 1.8ml cryotube (n=2)
- P1 and P2 = plasma in 1.8ml cryotube (n=2)
- TF = tissue, fresh-frozen
- TP = paraffin-embedded tissue
- TB = biopsy tissue

**Example:** Subject number 233 at center 1 will have the following ID numbers for the questionnaire and biological samples:

010233Q
010233B1
010233B2
010233W1
010233W2
010233P1
010233P2
010233R1
010233R2
010233TF
010233TP
010233TB
INFORMATION FOR SHIPMENT OF ALL SAMPLES

Record keeping
Make a record of the number of samples available on the case/control logsheet. This should be updated when samples are sent to CICAMS.

Shipment of samples to CICAMS
One half of the samples from each subject should be transported to CICAMS, assuming local ethical committee approval. Before packing for shipment, sample tubes should be checked to ensure that they are tightly closed. If, before packing, the outside of tubes have been contaminated with blood, this must be removed with a dilute chlorine bleach solution.

Samples should be securely stored in cardboard boxes with a polystyrene lining containing a sufficient quantity of dry ice (solid CO2). For example, for each kg of material travelling for 2 days, 7 kg of dry ice is needed. Ensure that all samples are frozen solid before they are placed in a plastic bag in the dry ice, as a sudden change in temperature may crack the tubes. Seal the box with heavy packing tape. Indicate on the box: “To be kept frozen at -20°”. For customs, specify also: “No commercial value: Material for scientific research”. Make sure that accompanying customs documents are complete. A list of sample ID numbers and a copy of the records should be enclosed in each batch shipped. This material needs to be placed in a plastic bag. Another copy of the list and records should be kept at the local centre. It is also necessary to provide a copy of the records to the contact person at CICAMS prior to shipment.

Shipment must be made without delay. If there is a transit time, it should be ensured that the parcel is stored in a cold room. Please send the parcel at the beginning of the week, preferably on a Monday.

At least one week before the expected day of shipment, the contact person at CICAMS should be informed by e-mail or fax of all details of the impending shipment. Please do not ship samples without having received confirmation from him that your message was received and that the proposed shipment details are acceptable.
### SHIPMENT OF BLOOD AND FROZEN TUMOUR TISSUE

<table>
<thead>
<tr>
<th>ID-No.</th>
<th>Cryotubes with whole blood</th>
<th>Cryotubes with plasma</th>
<th>Cryotubes with RBC and WBC mix</th>
<th>Samples of fresh tumour tissue</th>
<th>Remarks</th>
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## Shipment of paraffin-embedded tissue samples

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<tr>
<th>ID-No.</th>
<th>Path.No.</th>
<th>Paraffin embedded tissue Blocks No.</th>
<th>Tumor cytology No.</th>
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