SECTION 4.

Selected protocols

4.1 Processing of blood specimens

If serum and plasma samples are being collected, the blood should be centrifuged as soon as possible after blood collection and separated immediately after centrifuging so that the samples can be frozen as soon as possible. This is critical for time-sensitive samples for protein studies, for example. For the processing of blood specimens, the following protocols are recommended.

4.1.1 Filter paper dried blood spot collection, processing, storage, and shipment

Dried blood spot (DBS) is an easy and inexpensive means of collection and storage of peripheral blood specimens in settings where collection and storage of plasma is not optimal. DBS can be used for molecular biology techniques and other diagnostic assays. The cost and difficulty of cold chain shipping of plasma samples are greatly reduced by the use of DBS, which can be shipped as non-dangerous goods.

Always wear gloves when handling filter papers, and hold them only by the upper corner, marked out for labelling. Do not allow the card to come into contact with any unclean surface (e.g. bench, base of hood).

The procedure should be performed in accordance with the relevant health and safety practices specific to specimen handling and waste disposal. A minimum level of training is required to perform the procedure.

Reagents and materials required:

- finger prick device (e.g. Unistik 2 device; Fisher Scientific, 22-0227);
- alcohol swab;
- Whatman Protein Saver Card (e.g. Whatman, 903; 10534612);
- gas-impermeable storage bag (e.g. Fisher Scientific, NC9307519);
- desiccant pack (e.g. Whatman, 10548234);
- humidity indicator cards (e.g. Multisorb Des Manufacture, MS200032);
- card drying rack (e.g. VWR, 89015-592) (optional; cards can be placed on a dry worktop if a drying rack is not available);
- gloves, preferably powder-free; and
- sample label.

4.1.1.1 Collection of DBS from finger prick

i. Disinfect the selected site and prick it using a lancet/needle.
ii. Uniformly saturate the entire circle by quickly and gently touching, not pressing, the puncture site to the filter paper.

iii. Note: Do not touch the Whatman card at any stage of collection.
iv. After the collection of five blood spots, clean the site and leave it unbandaged.
v. Allow the blood spots to air-dry, without the flap covering the spots, in a clean dry place that is protected from rodents or insects and direct sunlight, for at least 4 hours (overnight drying may be needed in areas with higher humidity).
vi. Do not heat or stack DBS cards or allow them to touch other surfaces during the drying process.
vii. Tuck in the flap of the card as indicated on the card.
viii. Clearly label the card with the patient identifier and date, or label it with a prepared barcode label.
ix. Be sure the DBS card is completely dry before packing.
x. Insert the DBS card into a gas-impermeable plastic bag containing a desiccant pack and a humidity indicator. Do not store more than one card per bag.
xi. Ensure that the patient identifier and date (or the prepared barcode label) are on the outside of the bag as well as on the DBS card.
xii. Seal the plastic bag.
xiii. Place the sealed bag containing the DBS card in a clean, dry area of the laboratory with no exposure to direct sunlight, free of insects or rodents, and where ambient temperatures will not exceed 30 °C.
xiv. The room should be temperature- and humidity-controlled (temperature of 20–22 °C and humidity of not more than 22%).
xv. Check the desiccant pack before shipment of the DBS card and replace it if the colour of the humidity indicator has changed from blue to pink or colourless.
xvi. Ship the DBS cards at ambient temperature.

4.1.1.2 Preparation of DBS from EDTA, ACD, or heparin tubes

DBS can also be prepared from EDTA, ACD, and heparin blood tubes.

i. Before starting, mix vacutainers containing anticoagulated blood by inversion.
ii. Wipe the top of the vacutainer with 70% ethanol before opening the lid.
iii. Using a micropipette, apply 40 µL of blood onto the circle on the DBS card.
iv. Air-dry the filter paper thoroughly by following step v above (Section 4.1.1.1), and continue with the rest of the protocol as described above.

4.1.1.3 Shipping of DBS cards

DBS are classified as "Exempt Biological Specimens" according to the ICAO and IATA shipping regulations. DBS bags should be shipped in courier envelopes or boxes under ambient conditions according to the triple packaging system (see Section 3.9.2).

i. Provide a shipping manifest for all boxes. The shipping manifests must exactly match the label information and the order in the associated shipment, including the global specimen IDs.
ii. Provide a box map for all boxes. The box maps must exactly match the label information and the order in the associated shipment, including the global specimen IDs.
iii. Record the courier service and the courier air bill number on the specimen shipment notice.
iv. Advance notification of shipment must be made to the recipient.

4.1.2 Whole blood

Whole blood is to be prepared from EDTA tubes. The anticoagulated blood can be snap-frozen as it is. If the blood cells are needed intact, DMSO is needed to keep them alive while freezing.

i. Dispense 50 µL of DMSO into two 1 mL sterile cryovials.
ii. Invert the EDTA tube twice, and then add 450 µL of blood to each cryovial.
iii. Invert the cryovial to mix the whole blood with the DMSO. Note: DMSO is cytotoxic at room temperature; therefore, as soon as it is mixed with blood, it should be placed in a controlled-rate freezer.
iv. Transfer to −80 °C after at least 4 hours.

4.1.3 Plasma

Plasma collected in EDTA or ACD tubes can be used for bioassays, plasma DNA isolation, proteomic analysis, and biomarker discovery.

i. Spin the vacutainer (about 9 mL) at 815*g* for 10 minutes at 4 °C to separate plasma from blood cells.
ii. After wiping each tube with 70% alcohol, remove about 3 mL of plasma. (The tube can be retained for white blood cell extraction.)
iii. Transfer to a labelled 15 mL tube, and centrifuge at 2500*g* for 10 minutes at 4 °C.
iv. Aliquot plasma into 1 mL labelled cryovials (three or four aliquots).
v. Place in LN_2 dewar to snap-freeze.
vi. Store at −80 °C or in LN_2.

The purpose of double-spinning the plasma is to remove all cellular contaminants so that the plasma is suitable for plasma DNA analysis. Therefore, it is extremely important not to disturb the buffy coat after the first spin and any pellet after the second spin.

4.1.4 Platelet-poor plasma

Platelet-poor plasma can be used for the isolation of plasma DNA (from EDTA tubes).

i. Spin blood at 3200*g* for 12 minutes at room temperature.
ii. Pipette off plasma using a plastic Pasteur pipette. Transfer into a tube.
iii. Spin plasma at 2000*g* for 10 minutes at 4 °C.
iv. Aliquot into 1 mL aliquots in labelled cryovials.
v. Store at −80 °C.

4.1.5 Buffy coat cells

The buffy coat is a thin, greyish-white layer of white blood cells (leukocytes and lymphocytes) and platelets covering the top of the packed red blood cells after centrifugation at 450*g* (from EDTA- or ACD-containing blood tubes).

i. After having spun the blood, take the buffy coat off with about 100 µL of plasma using a disposable sterile Pasteur pipette; be careful not to lift red blood cells.
ii. Lyse the remaining red blood cells by addition of red blood cell lysis buffer at room temperature.
iii. Spin the tube at 450*g* for 10 minutes at room temperature.
iv. Resuspend the pellet.
v. Aliquot as appropriate into labelled cryovials.
vi. Place in LN_2 to snap-freeze.
vii. Store in LN_2.

4.1.6 Blood pellets (white blood cells)

Blood pellets can be used for the isolation of DNA (from EDTA or ACD tubes).

i. Transfer blood from the original tube to a labelled 50 mL tube.
ii. Fill the tube with Tris-EDTA buffer (formula) and mix vigorously. Place on ice for 5–10 minutes.
iii. Spin at 1200*g* for 10 minutes.
iv. Carefully pour off the supernatant into a waste container containing chlorine bleach. Briefly vortex the pellet and add 50 mL of Tris-EDTA buffer. Shake vigorously.
v. If division of the sample is necessary, at this point pour 25 mL of the sample into another centrifuge tube.
vi. Spin both tubes at 1200*g* for 10 minutes.
vii. Repeat the washing if red blood cells persist.
viii. Carefully pour off the supernatant.
ix. Using a swirling motion, remove the pellet with a pipette and transfer it to a labelled cryovial.
x. Store at −80 °C or in LN_2 until further use.

As an alternative, red blood cells can be lysed by using an ammonium-containing lysis buffer.

4.1.7 White blood cells

White blood cells collected in EDTA or ACD tubes can be used for DNA extraction and the creation of cell lines.

i. Transfer the remaining blood from the plasma spin to a labelled 50 mL tube containing 10 mL of RPMI 1640.
ii. After swabbing the lid of this tube with alcohol, aliquot 3 mL of Ficoll into each of two clearly labelled 15 mL tubes.
iii. Carefully layer 9 mL of diluted blood onto each tube of Ficoll. Treat gently, and do not mix, but spin as soon as possible.
iv. Spin at 450*g* for 30 minutes. Note: When centrifuging, do not use the brake.
v. Remove most of the top layer (RPMI 1640) using a 1 mL Eppendorf tip, and discard about 3–4 mL into a waste container containing chlorine bleach.
vi. Collect white blood cells with the same Eppendorf tip using a swirling motion to "vacuum up" white blood cells. Do not take too much Ficoll (third layer), because it is toxic to the cells. Place the white blood cells into a labelled 15 mL tube containing 10 mL of RPMI 1640.
vii. Spin at 450*g* for 10 minutes.
viii. Pour off the supernatant into a waste container containing chlorine bleach. Add 3 mL of cold freezing mix (10% DMSO, 20% fetal calf serum [FCS], RPMI 1640) and resuspend.
ix. Dispense the white blood cells into three 1 mL labelled cryovials that have been sitting on ice.
x. Place on ice. Place vials in a controlled-rate freezer so as to cryopreserve cells under conditions that maintain cell viability. This should be done as soon as possible, because DMSO is toxic at room temperature.
xi. Transfer on a weekly basis to LN_2 tanks.

Instead of a separation based on Ficoll, a Percoll separation can be used.

4.1.8 Serum

The blood is collected into tubes without addition of anticoagulants. Then, two phases are distinguishable: a solid phase containing fibrin and cells, and a fluid phase containing the serum.

This process should be completed after 30 minutes at room temperature, after which the process described below should start.

i. Spin blood at 1500*g* for 10 minutes at room temperature.
ii. Aliquot 1 mL portions of the supernatant into labelled cryovials.
iii. Place into LN_2 dewar or dry ice to snap-freeze.
iv. Transfer to −80 °C freezer or LN_2.

4.2 Processing of solid tissue

Careful and well-documented processing of tissue specimens is crucial to the overall usefulness of the biobank as a resource for scientific research. Detailed records of the first steps in the process of sample handling include the times of anaesthesia administration, ligation of

vessels, and specimen removal from the patient.

These factors are important to assess tissue quality, because they affect the quality of the resulting biomolecules. The most commonly described factor that has an impact on tissue quality is the warm ischaemia time, which is the period from when the blood supply is ligated until the specimen is placed in fixative in the pathology laboratory. Studies have demonstrated that changes in both RNA and protein occur during this time (Dash et al., 2002).

These protocols for collecting and freezing tissue samples were developed within the European Human Frozen Tumour Tissue Bank (TuBaFrost) project (Riegman et al., 2006b) and the Standardisation and Improvement of Generic Pre-analytical Tools and Procedures for In Vitro Diagnostics (SPIDIA) project (Malentacchi et al., 2015).

These recommended protocols contain choices and recommendations for preserving solid tissue, and describe the roles of key people involved in the process. Consult the CEN norms for more detailed information on the processing of snap-frozen tissue and FFPE samples for protein DNA and RNA isolation (CEN/TS 16826-1–2 and CEN/TS 16827-1–3).

4.2.1 Snap-freezing

Snap-freezing is the process by which samples are lowered to temperatures below −70 °C very rapidly using dry ice or LN_2. This method can provide excellent sample integrity and a wide array of options for tissue analysis, including extraction of proteins, RNA, and DNA for use in research diagnosis. Before tissues are stabilized by freezing, the protein, RNA, and DNA profiles can change, and these changes depend on the duration of warm and cold ischaemia and the ambient temperature before freezing. All the different pre-analytical conditions and durations should be documented.

4.2.1.1 Safety

All procedures should be carried out in accordance with the local codes of practice. Working with LN_2 and isopentane is hazardous; all procedures must comply with local safety rules specific to these chemicals. All tissue must be handled as if it is potentially infectious.

4.2.1.2 Hospital ward

Consent must be obtained from the patient before surgery (if applicable, according to the law and procedures in the country where the samples are being collected).

4.2.1.3 Operating theatre

i. Deliver the notification of tissue collection (and the consent form, if needed) to the surgeon, or highlight on the operating list.
ii. The surgeon should:
    a. complete the pathology form (if possible, in advance);
    b. perform the operative procedure and record the time of arterial clamping and of excision of the specimen; and
    c. place the specimen in a labelled sterile pot or bag and put it on ice.
iii. The operating theatre staff should send the fresh tissue specimen to the pathology department immediately.

4.2.1.4 Histopathology department

i. Notify the pathologist and the tissue bank research technician (if not already present).
ii. Check the paperwork and allocate a pathology number to the specimen as routine.

4.2.1.5 Role of the pathologist

i. Macroscopically describe the specimen as usual.
ii. Using clean instruments and on a clean surface (sterile foil or clean dissection board), dissect the tissue specimen. Clean or change instruments between dissecting normal tissue and tumour tissue.
iii. Take representative parts of tissue for routine diagnosis (for fixation and embedding) as a priority, and decide whether there is sufficient material available for the tissue bank.
    a. Supply the research technician with a tissue sample or samples for biobanking representative parts of the lesion, normal tissue, and pre-malignant conditions.
    b. Perform QC of frozen tissue and annotation.

4.2.1.6 Role of the technician

i. Prepare the tissue sample for snap-freezing on a clean surface and using clean instruments; change instruments between preparing normal tissue and tumour tissue. The minimum volume of tissue for snap-freezing is approximately 0.5 cm^3, although the amount of tissue available will differ depending on the sample site. Smaller fragments should still be snap-frozen and stored in the tissue bank; if there is sufficient material, freeze duplicate samples.
ii. Pre-cool the freezing medium isopentane (2-methylbutane) until opaque drops begin to appear in the isopentane and the solution becomes misty; this will bring the isopentane towards its freezing point (−160 °C), the optimal freezing point for the tissue. Options:
    a. LN_2: suspend a vessel of isopentane in LN_2.
    b. Dry ice: add dry ice (cardice) to the isopentane until a slush is

formed, or suspend a vessel of isopentane in dry ice.

iii. Label cryovials, cryomoulds, or cryostraws with a barcode and/or sequential code (depending on local laboratory practice). Use a waterproof pen with ink that is able to withstand long-term storage at low temperatures. The sequential code is the local inventory code and must not relate to the pathology number or other identifiers. If a barcode is used, readable recognition must also be included to make the sample identifier readable at institutions where there are no barcode readers.

iv. Record the local sequential code, the pathology number, the date, the lag time from arterial clamping and excision to freezing, and the type of tissue (the site, and also whether the sample is tumour, normal, and/or pre-malignant) in the inventory book. If a barcode system is in use, the barcode can be scanned into the LIMS and the above-mentioned data recorded.

v. Freeze directly in isopentane. Do not remove the tissue from the isopentane until freezing is complete (5 seconds or less, depending on size), but ensure that the sample does not crack. Remove the sample from the isopentane and enclose it in the labelled cryovial. It is good practice to strive to snap-freeze all tissue within 30 minutes of excision from the patient. Tissue subject to a delay of up to 2 hours should still be collected and the delay noted within the local inventory database. Options for freezing:
   a. Embed the tissue samples in OCT compound and freeze in isopentane, or freeze directly in isopentane. The isopentane used is cooled either by suspension in LN_2 or through addition of dry ice.
   b. Orientate the tissue on a piece of cork and an equally sized piece of Whatman paper soaked in physiological salt solution.
   c. If the cryostraw system is used to introduce a carrot of tissue into the straw, thermally seal each extremity and place in LN_2.

4.2.2 Storage of tissue

Storage of tissue can be done according to different protocols according to the equipment available in the facility. Options for storage:

i. Transfer the snap-frozen sample from the isopentane to a pre-chilled storage container for transfer to either a locked −80 °C freezer or a LN_2 storage facility in the liquid or vapour phase. For storage for longer than 5 years, LN_2 in the liquid or vapour phase is recommended.

ii. Place cryostraws in a designated visotube within a goblet (removable LN_2 storage elements) and place in the locked LN_2 repository.
   a. Store duplicate samples in a different storage facility if this is available.
   b. Check the backup system for the storage repository – either a backup freezer running constantly or adequate supplies of LN_2.
   c. Record the storage details in the inventory system, and check earlier data that were entered. At a minimum, the information recorded will include the inventory number (local sequential code), the location, the pathology number, the type of tissue (the site, and also whether the sample is tumour, unaffected/normal, and/or pre-malignant), the lag time between excision and freezing, and the date.

4.2.3 Storage of FFPE blocks and slides

i. FFPE blocks and sections mounted on slides can be stored at room temperature. Prevent exposure of blocks to sun or extreme temperature variation or humidity.

ii. Store blocks in moisture-resistant cardboard boxes or plastic storage boxes.

iii. Transfer details to the computerized database system.

iv. Update the database when samples are moved or depleted.

4.2.4 Formalin fixation

Formalin fixation is standard practice in most routine histopathology laboratories. The following guidelines address specific issues related to preservation of formalin-fixed specimens in biobanks. Table 6 provides information on the composition of neutral buffered formalin.

i. Tissue specimens should not be bigger than 3 cm × 2 cm × 0.5 cm.

ii. Specimens should be fixed in fresh 10% neutral buffered formalin for a minimum of 4 hours and a maximum of 48 hours, after which they should be embedded in paraffin in accordance with conventional techniques.

iii. All reagents should be DNase- and RNase-free (e.g. prepared using diethylpyrocarbonate [DEPC] water).

iv. Fixation media, such as Bouin's solution, that contain picric acid should be avoided, because this compound interferes with subsequent PCR analysis of extracted nucleic acids.

v. Alcohol fixation may be used as an alternative to formalin fixation. For this, tissue is placed into 70% alcohol (diluted with DEPC water) for a minimum of 4 hours.

Because of the chemical hazards of formalin, it can be desirable to use alternatives to formalin as a routine

Table 6. Composition of neutral buffered formalin and 70% ethanol

Composition		Total volume
10% neutral buffered formalin (in 40% formaldehyde)		100 mL
100% formaldehyde	37–40 mL	
Na_2HPO_4 (anhydrous)	6.5 g	
NaH_2PO_4	4.0 g	
Distilled water	900 mL	
70% ethanol		
100% absolute alcohol	70 mL of absolute alcohol + 30 mL of water	100 mL
96% ethanol	73 mL of 96% ethanol + 27 mL of water	100 mL

fixative. However, the effect of long-term storage with these alternative fixatives on the desired macromolecules is not always known and should be established empirically.

*4.2.5 RNA*later

This substance protects RNA in fresh specimens. It eliminates the need to immediately process or freeze samples.

4.2.5.1 Tissue

Cut the tissue to be smaller than 0.5 cm in at least one dimension, and then submerge the tissue in 5 volumes of RNA*later* (e.g. a 0.5 g sample requires about 2.5 mL of RNA*later*).

4.2.5.2 Cells

Resuspend the pelleted cells in a small volume of phosphate-buffered saline (PBS) before adding 5–10 volumes of RNA*later*.

4.2.5.3 Storage

RNA*later*-treated tissue and cell samples can be stored at 4 °C for 1 month, at 25 °C for 1 week, or at −20 °C for an indefinite period. For RNA isolation, simply remove the tissue from RNA*later* and process.

4.2.6 Shipment of tissues and slides

FFPE tissues and slides are shipped at ambient temperature in accordance with the established shipment guidelines and protocols of the sending and recipient institutions. Please refer to Section 3.9 for more details.

4.2.7 Quality control for tissue samples

i. Ensure that the reagents have not expired and are of the correct composition and volumes.
ii. Keep high-quality records on all variables related to specimens, FFPE tissues, and slides, including the time of tissue collection, the processing time, and the period of storage before shipment and/or use.

4.2.8 Data to be recorded

i. Date and time of tissue collection.
ii. Number of unprocessed samples, FFPE blocks, and slides prepared.
iii. Date and time of shipping.
iv. Any variations or deviations from the protocol, problems, or issues related to the collection and storage.

4.3 Processing of urine and buccal cells

The following protocols for processing of urine and buccal cells contain recommended procedures.

4.3.1 Urine

i. Plastic or glass containers for collection of urine should be clean and dry, should have a 50–3000 mL capacity, a wide mouth, and a leakproof cap, and should be clearly labelled.
ii. When in transit, urine collections should be maintained on ice or refrigerated.
iii. Urine should be aliquoted according to the volume needed for analysis or storage.
iv. Depending on the analyte to be measured, a preservative may be added during collection or before aliquoting.
v. Store urine at −80 °C or below in LN_2.

4.3.2 Buccal cells

i. A collection kit (containing mouthwash, a 50 mL plastic tube, a

plastic biohazard bottle, and courier packaging) is mailed or given to the participant, along with an instruction sheet. The participant is to brush their teeth as usual, rinse their mouth out well with water twice, and then wait 2 hours. The participant should not eat or drink anything other than water during this time.

ii. After 2 hours, 10 mL of commercial mouthwash should be poured into the tube, and then 10 mL of tap water should be added. This diluted mouthwash should be placed into the mouth (without swallowing) and swished around vigorously for 30 seconds.
iii. The mouthwash should then be spat back into the plastic tube, and the tube should be sealed tightly.
iv. The sample should be sent back to the biobank immediately for processing, or stored at 4 °C until it is sent, but it should be sent within 24 hours.
v. When the sample arrives at the laboratory, transfer the mouthwash to 15 mL conical test tubes.
vi. Add 35 mL of Tris-EDTA to the mouthwash sample and spin at 450*g* for 5 minutes.
vii. Decant the supernatant and discard.
viii. Wash the cells twice, each time with 45 mL of Tris-EDTA.
ix. Resuspend the cell pellet in 50 µL of Tris-EDTA and transfer to 2 mL labelled cryovials.
x. Store the sample at −80 °C or in LN_2.

Note: Buccal cells can also be collected with other means, such as brushes.

4.4 Collection and processing of saliva

A research consortium at the University of California, Los Angeles was funded by the United States National Institute of Dental and Craniofacial Research to investigate the human saliva proteome. The protocol for collection and processing of saliva is derived from the consortium's *Salivary Proteome Handbook Procedures and Protocols* (Hu et al., 2007).

i. Saliva collection should be done in the morning (aim for 10:00–11:00 am if possible). Ask the subject to refrain from eating, drinking, or oral hygiene procedures for at least 1 hour before the collection.
ii. The subject should be given drinking water (bottled) and asked to rinse their mouth out well (without drinking the water).
iii. Five minutes after this oral rinse, the subject should be asked to spit whole saliva into a 50 mL sterile centrifuge tube. The subject should refrain from talking. It is better for the subject to drop their head down and let the saliva run naturally to the front of the mouth, hold this position for a while, and spit into the tube provided. The subject will spit into the collection tube about once a minute for up to 10 minutes. The goal for each whole saliva donation should be about 5 mL. Require that the tube be placed on ice while collecting whole saliva. Remind the subject not to cough up mucus, so that saliva is collected, not phlegm.
iv. For the collection of submandibular saliva, use 2 × 2-inch cotton gauze to block the opening of each parotid duct. Dry up the floor of the mouth, and block the openings of the sublingual gland (both sides), and have the subject raise their tongue slightly to elevate the opening to the submandibular gland. Begin to collect submandibular saliva by using a sterilized Wolf device. A sterilized and disposable yellow tip (for pipette P200) should be connected into the device and changed after every collection. During the collection, at 2-minute intervals, a few grains of citric acid powder should be swabbed with a moistened cotton applicator onto the lateral dorsum of the tongue to stimulate the secretion. Aim to collect at least 200 µL of submandibular saliva.
v. For the collection of sublingual saliva, the protocol is similar to that described above for collection of submandibular saliva. The only difference is that the ductal orifices of the submandibular gland are blocked off. Aim to collect > 100 µL of sublingual saliva every time.
vi. For the collection of parotid saliva, use a parotid cup to collect the saliva. Parotid cups may be placed bilaterally if the clinical investigator so chooses. This will enable the simultaneous collection from each parotid gland. The citric acid stimulation should be performed as described above. Aim to collect > 1 mL of parotid saliva. The first 0.1 mL of parotid saliva collected should be discarded, to ensure that fresh parotid saliva is obtained.

   Note: The collected samples should be kept on ice at all times before processing.
vii. For sample processing using proteinase inhibitors, to each 100 µL of saliva:
   a. Add 0.2 µL of proteinase inhibitor cocktail from standard stock solution (Sigma, P8340), and invert gently.
   b. Add 0.3 µL of sodium orthovanadate (Na_3VO_4) (Sigma, S6508) from standard stock of 400 mM, and invert gently.
viii. Centrifuge the specimens at 2600*g* for 15 minutes at 4 °C (if you note that incomplete separation has occurred, increase the spin time to 20 minutes). Then:
   a. Remove the supernatants from the samples and label them with the term "super", which stands for the supernatant phase of the saliva.

b. Taking care not to disturb the pellet and keeping the pellet as is in the original tubes, label the original tubes as “pellet”.

ix. Freeze the samples at −80 °C.

4.5 Processing of cervical cells

In a Pap smear test, a sample of cells is taken from the uterine cervix using a wooden spatula or a brush, smeared onto a slide, and examined under a microscope for abnormal cells (precancer or cancer). This protocol is a selected protocol from diverse collection procedures.

Note the following:

i. It is best not to take a smear from a woman who is actively menstruating or has symptoms of an acute infection. Slight bleeding is acceptable.
ii. Pregnancy is not an ideal time for a Pap smear, because it can give misleading results.

4.5.1 Taking the sample of cells

Insert the long tip of the spatula into the cervical os, and rotate the spatula through a full circle (360°). If the cervical broom brush is used, place the tip of the brush into the cervical os, and rotate the brush gently through three 360° circles.

4.5.2 Taking the Pap smear

i. Smear both sides of the spatula (or the contents of the brush) onto the glass slide with one or two careful swipes. If any abnormalities are seen outside the area sampled, take a separate specimen and smear it onto another slide.
ii. Immediately fix each slide. Either use spray fixative, at a right angle to, and at a distance of 20 cm from, the slide, or immerse the slide in a container of 95% ethanol for at least 5 minutes. If the slide is not fixed immediately, the cells will dry and become misshapen; it will then not be possible to read the slide accurately in the laboratory.
iii. Gently close and remove the speculum.
iv. Place all used instruments in decontamination solution.

4.5.3 After taking the smear

i. Label the frosted edge of each slide carefully.
ii. On the patient record, note and illustrate any features you have noted: visibility of the transformation zone, inflammation, ulcers or other lesions, or abnormal discharge. Note whether other samples were taken, for example Pap smears of other areas, and if the woman has been referred elsewhere, note to whom and when.

4.6 Processing of hair and nails

These protocols are recommended for collecting hair or nail specimens.

4.6.1 Hair

Head hair may be collected as follows.

i. Along an imaginary line drawn across the middle of the back of the head from the centre of one ear to the centre of the other, gather a lock of hair at least the thickness of a pencil, and tie it together near the root end (near the scalp) using a small string or a rubber band.
ii. Cut the hair as close to the scalp as possible without cutting the scalp.
iii. Maintain the horizontal position of the hairs in the bundle by wrapping the cut section in aluminium foil or plastic wrap.
iv. Indicate the root end and the tip end by marking the foil or plastic wrap with a permanent marker or with a paper label. **Do not use tape on the hair itself.**
v. Place the specimen in a clean, dry, labelled paper envelope for shipment to the laboratory. Note whether bleaches, hair dye, or medications (e.g. selenium or minoxidil) were used.

Please note that hair from other sources (pubic, axillary, beard, moustache, chest, etc.) may also be analysed if head hair is not available.

4.6.2 Nails

A clean pair of nail clippers should be used. To clean nail clippers thoroughly, they should be rubbed with alcohol swabs. Nails should be clean of all polish, dirt, and debris. Nail clippings from each finger or toe should be collected and packaged separately in plastic bottles. Each bottle should be labelled with the mass of the nail collected and its source (e.g. right index finger) (NMS Labs and ExperTox).