SOP Name	Sample Handling
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1. **SCOPE:** This SOP applies to all staff, visitors, researchers and research staff working inVACCELERATE affiliated labs and handling samples.

2. **PURPOSE:** To outline appropriate policies and standards for handling, including labelling,transit, storage, logging and inventory of clinical samples within VACCELERATE affiliated labs.

3. **POLICY:** VACCELERATE affiliated labs work within the guidelines and regulations of theEU CT Directive 2001/20/EC, GCP Commission Directive 2005/28/EC, ICH/GCP and with all other local and international applicable regulatory requirements.

4. **ROLES AND RESPONSIBILITIES**

    1. The investigators will stipulate the appropriate labelling transit, storage, logging and tracking requirements for all prospective studies with VACCELERATE affiliated lab staff prior to thestudy's initiation.
    2. Once adequate training and support has been provided, the ultimate responsibility for both health and safety and sample integrity rests with the investigator.
    3. It is the responsibility of the research personnel carrying out this procedure toensure that all steps are completed both competently and safely.
    4. VACCELERATE affiliated lab staff should undertake to facilitate the provision of all appropriate training to investigators regarding sample labelling, transit, storage, logging and tracking

5. **DEFINITIONS**

6. **RELATED DOCUMENTS**

7.0 PROCEDURES

7.1 LABELLING

7.1.1 Use of Patient Identifier codes (PID)

1. In order to preserve patient confidentiality, samples for processing and storage must not be labelled with patient name or medical record number (MRN).

2. Each patient in the study should be assigned a unique patient identifier upon enrolment. All samples collected should be labelled with both the PID and the sample type, which will be used to identify the stored samples in the storage inventories.

3. The copy of the consent form should be retained by the clinical team with the PID.As this document has to be retained by the clinical team. This provides a hard copy record of PID assignment.

4. PID should also be included with the study database.

7.1.2 Label Design and Type

1. Labels should be printed centrally and distributed to sites to include labels for blood bottles, consent forms, and lab aliquot cryovials.

2. Only freezer-proof, resin labels should be used. Paper labels are not suitable.

3. The information on the label should be double checked to tally with the information included in the processing and sample logs.

7.1.3 Barcoding

1. The possibility of applying a bar-code label to samples should be discussed with VACCELERATE affiliated lab staff before the commencement of the study.
2. Ideally barcodes should encode enough information to identify study type, PID and sample type.

7.2 Sample Transfer

7.2.1 Transfer of samples from the clinic to the VACCELERATE affiliated lab for processing and storage

The details of how samples are to be handled after initial processing is outlined in appendix 1.

While transferring and storing samples the integrity of the sample itself, the health and safety of those working /handling the samples, and the ability of staff to identify and track the sample should be taken into consideration.

Investigators should consult the relevant VACCELERATE lab staff in advance of the sample transit to ensure that all study needs are met in the most efficient manner.

Insert the biological samples alongside with the lab requisition form inside the specimen transport bag (A), then place the bag inside a specimen transport box, cardboard (B), or Plastic (C). Note: Please contact the central laboratory to obtain all the necessary materials to ship biological samples.


A


B


C

1. Samples should be transported in the appropriate containers labelled as "containing biohazardous material".

2. Samples should be appropriately contained and bagged in biohazard bags.

3. Tissue specimens and cell suspensions for primary cell culture should be transported in appropriate media.

4. Tissue specimens and cell suspensions for genomics and proteomics analysis should be transported refrigerated.

5. Blood samples should be transported at room temperature for short times, or refrigerated for longer transport times.

6. All other human samples (body fluids, faeces, etc) should be transported in correct media in accordance with study requirements.

7.2.2.1 Fresh samples

1. If samples are being shipped *off* site they should be placed in insulated Styrofoam containers, kept at approx. 4°C by use of pre-cooled freezer blocks. Boxes should be clearly labelled and sealed.
2. Particular care to seal, label and package samples should be taken if samples are to be transported by third parties (ie taxis, couriers etc).
3. Before sending samples the Scientific Services Manager or other lab staff

member should contact the person due to receive the shipment. If the tracking number is available this should also be sent by email.

7.2.2.2 Frozen Samples

1. Samples stored at -80°C should only be transported on dry ice (solid CO_2).
2. Dry ice should only be transported in Styrofoam boxes.
3. Particular care to seal, label and package samples must be taken if samples are to be transported by third parties (ie taxis, couriers etc.). Courier companies must be notified in advance that the package contains dry ice, and the package should also be labelled accordingly.
4. Air transport of dry ice should be in accordance with the IATA guidelines (http://www.iata.org/Pages/default.asp). If in doubt the courier company should be contacted for advice.
5. Before sending samples the Scientific Services Manager or other lab staff member should contact the person due to receive the shipment. If the Tracking number is available this should also be sent by email.

7.3 SAMPLE STORAGE

7.3.1 1 Cryo-storage definition

The common temperature ranges for cryostorage are:

- **Liquid nitrogen (<-150°C):** for samples where biological activity must be retained (ie cell stocks, some tissue samples).
- **Dry Ice (<-70°C}:** for temporary storage, (ie shipping, of cryopreservedsamples).
- **Ultra low freezer (<-70°C}:** for general long term storage of clinical samples,(ie serum/plasma, urine, RNA, DNA and protein).
- **Standard freezers (-20 to -40°C}:** for short to medium term storage of clinical samples (ie DNA).
- **Cold rooms/fridges (2 to 8°C):** for DNA in solution, short term storage of clinical samples, samples in RNAlater.
- **Room temperature (region 15°C):** for FFPE samples, lyophilised DNA

samples, buffers.

732 Inventory and organisation

Investigators using VACCELERATE cryostorage space are instructed to closely follow the above recommendations:

1. Individual samples should be clearly and adequately labelled.
2. Sample should be stored in adequate cryovials and placed in boxes. Every box should be identified by a unique number. Excel based inventories should be used record position of samples in the box.
3. Multi-box racking should be used wherever possible. Each space on the rack should be assigned an identifier (ie box 2, rack 3).

All this information should be included on the shipping log. When samples are removed for further testing or processing, the inventories should be updated.

733 Appropriate storage conditions for various sample types

The choice of storage condition depends on

- sample type/stage of preparation
- eventual use of sample (ie which assay/procedure)
- length of time sample will be stored

734 Cryopreservation of cells:

1. In order to ensure the long-term viability of cryopreserved cells, suspend the cells in a storage medium that includes DMSO (containing low concentrations of DMSO (i.e. 10%)) to reduce ice-crystal formation when freezing. Cells stored in DMSO should be thawed as quickly as possible and removed from the storage medium.
2. Cells should be stored in LN_2 for later culturing of for a stable and long-term storage. Before placing the cells in Liquid N_2, they should first be stored at -80°C overnight in order to reduce thermal shock.
3. For cell isolation from blood (i.e. PBMCs), the whole blood should not be refrigerated and instead be kept at room temperature (18 -25°C) until the blood is processed. Cellular viability and expression can be affected by change in temperature. The blood should be processed as soon as possible, within 1 hour should be preferable.

7.3.5 Cryopreservation of tissue:

1. For storage of tissue samples in RNAlater, surface area of the tissue samples and tissue-to-RNAlater ratio are important in ensuring that tissue RNA is stabilized. Tissue stored in RNAlater is stable for up to 4 weeks at 2 - 8°C and can be stored long-term at -80°C.
2. Tissue should be placed in RNAlater and transferred to -80°C storage as soon as possible to ensure sufficient RNA stabilization.
3. For snap-freezing tissue samples, these should be frozen as quickly as possible as they are not protected against protein degradation unlike RNAlater stabilised tissues. Snap-frozen samples can be stored at either -80°C or in Liquid N_2.

7.3.6 Cryopreservation of blood for downstream DNA/ RNA isolation:

1. Blood without stabilisation in PAXgene tubes should be transported at 4°C and stored at -80°C as soon as possible to reduce the risk of DNA/ RNA degradation. It is also important to ensure the blood tube has been inverted and the blood is homogenous before storage.

7.3.7 Cryopreservation of plasma/ serum and urine:

1. Plasma, serum and urine can be stored at -20°C for short time, storage at -80°C is preferred to ensure little or no degradation of biomarkers during storage. Blood and urine used for biomarker analysis should be transported and stored temporarily at 4°C and processed as soon as possible due to the instability of heat-labile proteins found in blood and urine.
2. Samples for downstream miRNA analysis should be handled using pipettes and cryovials that have been DNase and RNase treated to reduce degradation.

7.3.8 Storage of Nucleic Acids

1. Genomic DNA should not be overdried after ethanol precipitation prior to storage, to do so let it air dry instead of using a vacuum system.
   Genomic DNA should be stored at 2 to 5℃. Storing genomic DNA at -15 to -25°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Freeze small aliquot for single analysis. Avoid freeze-thaw cycles.
2. Plasmid DNA and other small circular DNAs should be stored at 2 to 5℃ or at -15 to -25°C. Store small aliquot for single analysis. Avoid freeze-thaw cycles. Plasmid DNAand other small circular DNAs can be vacuum dried.

3. To dissolve the DNA, carefully invert the tubes several times after buffer and/or tapthe tube gently on the side. Alternatively let the DNA stand in buffer overnight at 2 - S°C. Avoid vortexing.

4. Extract RNA as quickly as possible after obtaining samples. Use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -70°C.

5. Store RNA at -70° to -80°C, as aliquots in water. Most RNA is relatively stable at this temperature. RNase inhibitors can be used to protect RNA from degradation both during isolation and purification and also in downstream applications (i.e. reverse transcription into cDNA by RT-PCR, in vitro RNA transcription/translation reactions, RNA-dependent in vitro functional assays).

739 Storage of Protein:

1. Protein solutions should be prepared in high concentration, preferably 1 mg/ml or greater. The high concentration tends to stabilize the protein's native structure as well as inhibiting protein "sticking" to surfaces such as glass and plastic.
2. While working with proteins in the laboratory, they should be kept on ice. Since proteins are generally more stable at colder temperatures, maintenance at low temperatures even for short duration is recommended. Proteins are stored lyophilized, frozen in an appropriate buffer, or refrigerated at 4°C. For short term storage (hours to days), a standard laboratory refrigerator at 4°C is satisfactory providing the buffer used to solvate the protein provides all the necessary components required to stabilize the protein of interest (i.e. reducing agents, hydrophobic additives, and protease inhibitors).
3. Proteins can be stored long term (days to weeks) by quick freezing the sample followed by storage at -20°C. Addition of stabilizers such as glycerol helps prevent damage to the protein during freezing and thawing. The rapid freezing process is typically performed by immersing the protein solution in a dry ice bath containing either acetone or ethanol followed by frozen storage at -20°C. For long term storage of proteins samples should be stored at -80°C.

7.4 SAMPLE LOGGING AND INVENTORY

The sample log will include the shipping log and the inventory and will be applicable to each institution receiving and storing samples. This will take the form of an eCRF which will be uploaded onto the central VACCELERATE database the same day.

7.4.1 Processing Logs

The processing log should include:

- Sample custody trail
- Record of processing
- Time between sample being taken and processed
- Sample storage location

7.4.2 Inventory

The inventory may be initially kept as an excel spreadsheet, which allows the users to enter relevant information about sample processing storage etc. They can also track the further processing, use or disposal of samples, and thus provide a complete inventory.

These databases should contain enough information to allow samples to be located, indicate how many samples remain, and contain some information regarding sample processing A VACCELERATE template processing/storage log is available should be modified to accommodate field requirement specific to each site.

8. REVIEW AND REVISION

Review date – May 2022

9. DOCUMENT HISTORY

Version Number	Effective Date:	Summary of changes from previous version:	Edited by: (name and role)

10. Appendix

Appendix 1.