HRE Exploration Biobank

Deliverables [D-01] and [D-02]

[D-01]: Mandatory standardised protocols and SOPs for ESA-directed research samples.

[D-02]: Recommended protocols for enabled research samples.

SOP

- SOP.BIO/ESA.001 – Collection and Transportation of Biological Samples
- SOP.BIO/ESA.002 – Processing and Storage of Biological Samples
- SOP.BIO/ESA.004 – Shipment of Biological Samples to GIMM Biobank in European Space
- SOP.BIO/ESA.005 – DNA Extraction from Biological Samples
- SOP.BIO/ESA.006 – RNA Extraction from Biological Samples
- SOP.BIO/ESA.007 – Peripheral Blood Mononuclear Cells Isolation
- SOP.BIO/ESA.008 – Establishment of Primary Fibroblast Cell Culture from Skin Biopsies

SOP.BIO/ESA.001.V2 – COLLECTION AND TRANSPORTATION OF BIOLOGICAL SAMPLES

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VERSION CONTROL

Number of Version	Date of Version	Summary of Changes
V1	19-09-2025	Original version
V2	12-12-2025	Added “Indirect-Human Samples” category; ESA Harmonized Unique Identifier (UID) Scheme in annexes; minor text updates.

This document will be reviewed and updated if any quality control issues are identified during operations. Additionally, since this SOP primarily addresses human samples, future revisions are planned to incorporate additional sample categories, namely indirect human samples and non-human samples.

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1. SCOPE

This Standard Operating Procedure (SOP) establishes the protocol for the collection of biological samples in ESA research facilities, and their transportation to sample processing sites. The inclusion of samples in the HRE Exploration Biobank will only be carried out upon the donor's signing of the informed consent form, ensuring compliance with the ethical and legal regulations governing the collection and use of biological samples.

2. SAMPLE COLLECTIONS

Sample collections conducted at ESA research facilities must comply with the GIMM Biobank standards. For direct-, indirect- and non-human samples, it must be ensured that all relevant ethical approvals are in place prior to collection, and approval from the ESA Medical Board is also required. The following formal documentation (will be requested as evidence: 1) Statement confirming ESA Medical Board approval, provided by ESA; 2) Statement confirming that informed consent was obtained in accordance with legal and ethical regulations, provided by ESA Research/Study Sites (STATEMENT_ESA Research or Study Site, annexed to this SOP); 3) Blank copy of the Consent Form template that is given to participants, provided by ESA Research/Study Sites.

2.1 Procedure for Sample Collection Performed by ESA Research Sites

2.1.1 Appropriate Material

Biological samples may pose an infectious risk and must always be handled with maximum safety precautions. The use of gloves and a laboratory coat is mandatory throughout the entire procedure. Blood must be collected exclusively by personnel with specialized training.

To ensure the highest possible quality of the collected samples, it is essential that all study sites strictly follow the recommended biosafety and handling practices. Consistent application of these measures safeguards both the integrity of the samples and the safety of the personnel involved. The appropriate materials to collect the samples should be selected according to the type of samples to be collected, and the biological material to be preserved. **Table 1** summarizes the required collection containers and safety information to collect each sample type. For non-human subject samples (e.g., habitat samples), precautions appropriate to the specific environment must also be applied.

2.1.2 Sample Identification

Samples must be unequivocally identified at the time of collection:

- Samples must be labelled in a manner that safeguards donor privacy.
- Label all tubes with the donor's unique identification number.
- Documentation of Relevant Information:

  Record the number and type of tubes collected, specific conditions if applicable (as anticoagulants used, preservative medium, etc.), and the exact time of collection in the Sample Information Form (SIF) – annexed to this SOP. Instructions for generating a structured pseudonymous Donor ID are provided in the ESA Harmonized Unique Identifier (UID) Scheme, also annexed to this SOP.

2.1.3. Sample Storage and Processing

To preserve sample integrity and minimize degradation risk, sample processing should occur as promptly as possible, ideally within 30 to 60 minutes post-collection. If immediate processing is unfeasible, samples must be stored according to the recommendations listed in **Table 2**, with processing completed no later than 4 hours after collection.

Table 1. Summary of collection containers and safety precautions recommended for the collection of each sample type.

Sample Type	Collection Container	Recommended Safety & Handling Information
Direct-Human Samples		
Whole Blood / Plasma / Serum	EDTA tube (buffy coat), Serum tube, Hep-Li tube, Citrate tube, Plasma card, Dry blood drops	Must be collected by trained personnel; Standard PPE (gloves, lab coat); dispose of sharps appropriately
DNA Samples (genomic DNA)	EDTA tube (buffy coat)	Must be collected by trained personnel; Standard PPE (gloves, lab coat); dispose of sharps appropriately
cfDNA (cell-free DNA from plasma)	Cell-free DNA blood collection tubes	Must be collected by trained personnel; Standard PPE (gloves, lab coat); dispose of sharps appropriately
RNA (PAXgene, tissue, cells)	PAXgene Tubes	Must be collected by trained personnel; Standard PPE (gloves, lab coat); dispose of sharps appropriately
Cell Pellets (from blood, urine, etc.)	RNase/DNase-free tubes	Must be collected by trained personnel; Standard PPE (gloves, lab coat); dispose of sharps appropriately
Cultured Cells / Skin Fibroblasts	Falcon tube with fibroblast culture medium	Standard PPE; risk of contamination requires careful sealing and storage
Saliva	Salivette, Drooling into tube, Dry saliva filter	Standard PPE; ensure secure sealing of collection device
Hair	Bulk hair	Standard PPE; handle with clean scissors/collection tools
Stool	Stool tube	Standard PPE; risk of contamination requires careful sealing and storage
Urine	12–12h urine tube, 24h urine tube	Standard PPE; avoid direct contact; transport in sealed secondary container
Tissue (Skin, Biopsies)	Skin patches	Standard PPE; avoid skin-to-skin contact during application/removal
Stem Cells (urine, skin, cord blood, etc.)	RNase/DNase-free tubes with culture medium	Standard PPE; risk of contamination requires careful sealing and storage
CSF / Pleural / Cystic Fluids	RNase/DNase-free tubes	Standard PPE; risk of contamination requires careful sealing and storage
Cell Pellet – Urine	Large volume collection (100–400 mL)	Standard PPE; risk of contamination requires careful sealing and storage
Stem cells – Keratinocytes (hair follicles)	Primary container: Sterile screw-cap tube (1.5–2 mL microtube for few follicles; 15 mL conical for larger volume), prefilled with transport medium. Transport medium: For tissue or cell samples, a defined keratinocyte medium such as Keratinocyte Serum-Free Medium (KSFM) is recommended. KSFM typically contains a basal medium supplemented with epidermal growth factor (EGF), bovine pituitary extract (BPE), calcium, and optionally antibiotics/antimycotics. Serum or other substitutes may be added if required. This medium supports cell viability during transport prior to processing	Standard PPE; risk of contamination requires careful sealing and storage

This table continues in the next page.

Sample Type	Collection Container	Recommended Safety & Handling Information
Indirect-Human Samples		
DNA	Purified DNA in sterile microtube or plate wells	Use standard PPE. Avoid aerosol formation and cross-contamination. Applications include genomic sequencing, bisulfite sequencing, and methylation arrays.
RNA (mRNA, miRNA)	Purified RNA in sterile RNase-free microtube	Use standard PPE. Prevent RNase contamination; store at −80 °C. Used for transcriptomic sequencing (bulk or single-nuclei RNA-seq).
Secretome (Extracellular Vesicles, Proteins, Metabolites, Lipids)	Isolated fraction from cell culture media or biofluids	Use standard PPE. Handle under sterile conditions. Analyses include immunochemistry (Western blot, ELISA), mass spectrometry, metabolomics, lipid panels, or sequencing.
Lipids (from cell lysates)	Extracted lipid fractions in glass vials or microtubes	Use standard PPE. Handle with solvent safety precautions; applicable for lipidomics.
Microorganisms (e.g. Bacteria, Viruses, Fungal Spores)	Sterile tube, swab, or culture plate	Use biosafety level–appropriate PPE (BSL-2 minimum). Handle in biological safety cabinet. Dispose of waste as biohazardous material.
Non-Human Samples		
Water	Water sample in tube/bottle; if grey water system exists → N/A	Standard PPE; avoid aerosol formation
Air	Filter-based or settle plates	Standard PPE; handle filters carefully
Surface/vents	Swabs	Standard PPE; avoid cross-contamination
Plant Leaves	Sterile tube or envelope	Standard PPE; avoid cross-contamination; handle with sterile forceps; minimize exposure to heat/sunlight
Plant Seed	Sterile tube or Falcon tube	Standard PPE; ensure seeds are dry; avoid contamination; label clearly
Plan Roots	Sterile tube or Falcon tube	Standard PPE; remove excess soil gently; rinse with sterile water if needed; avoid aerosol formation
Plant Substrate (soil or growth medium)	Sterile Whirl-Pak® bag or screw-cap tube	Standard PPE; avoid inhalation of dust; measure and record moisture; handle in clean area; if microbiological analysis, maintain humidity (20–40%)

Table 2. Summary of the material requirements and storage conditions for each sample type.

Sample Type	Primary Collection Container/ Material	Storage Condition (until processing)	Critical Notes
Direct-Human Samples			
Blood	EDTA tube (DNA)	2–8 °C	Invert tubes as per manufacturer
	PAXgene Blood RNA tube (RNA);	2–8 °C	10× PAXgene). Collect ≥10 mL for adults
	EDTA or Citrate (plasma)	2–8 °C	Invert tubes as per manufacturer
	SST with clot activator/gel (serum);	2–8 °C	-
	Dry blood spot card	Room Temperature	-
	CPT or Heparin (PBMC)	Room Temperature	-
Plasma (cards)	Plasma card	Room temperature	Protect from moisture.
CSF / Pleural / Cystic Fluids	Sterile Falcon Tube	2–8 °C	Handle gently to preserve integrity.
Stool	Stool collection kit with stabilization buffer (e.g., OMNIgene-GUT) or stool tube	Room temperature or 2–8 °C (per kit instructions)	Donor must avoid urine contamination; mix thoroughly with buffer.
Urine	Sterile pre-labelled container (with or without preservative, e.g., boric acid)	2–8 °C (fresh); preservatives allow delayed analysis	Collect midstream (20–50 mL).
Saliva	Saliva DNA kit (e.g., Oragene), salivette, or sterile tube with stabilizer	Room temperature	Donor fasting: no food, drink, smoking, or gum 30 min before. Shake tube after stabilization.
Hair	Clean scissors; paper envelope (no plastic)	Room temperature, dry, protected from light/moisture	Collect at posterior vertex, ~3–5 cm, align roots, label growth direction.
Skin (patches)	Adhesive skin patches	Per protocol (ambient or refrigerated)	Label site of application.
Skin biopsies	Sterile surgical container with 10% formalin (histology)	Room temperature	Typically 2–4 mm punch biopsy. Aseptic field required.
	RNA-later / DNA/RNA Shield (molecular);	2–8 °C	
	Culture medium (cell isolation)	2–8 °C	
Stem cells – Urine	Large sterile container (100–400 mL, TBD)	2–8 °C	Handle gently to preserve integrity.
Stem cells – Keratinocytes (hair follicles)	Sterile screw-cap tube (1.5–2 mL microtube or 15 mL conical) with transport medium	2–8 °C	Transport medium defined by protocol.
Indirect-Human Samples			
Soluble Proteins	Sterile microtube or cryovial containing aliquot of plasma, serum, or cell culture supernatant	-20 °C (short-term) or -80 °C (long-term)	Avoid repeated freeze–thaw cycles. Handle gently to preserve protein integrity.
DNA	Sterile microtube or plate wells containing purified DNA	-20 °C (short-term) or -80 °C (long-term)	Avoid aerosol formation and cross-contamination. Label with extraction date and concentration

This table continues in the next page.

Sample Type	Primary Collection Container/ Material	Storage Condition (until processing)	Critical Notes
RNA (mRNA and miRNA)	RNase-free microtube containing purified RNA	-80 °C	Protect from RNases; store in aliquots to prevent degradation. Maintain cold chain during handling.
Secretome (Extracellular Vesicles, Proteins, Metabolites, Lipids)	Sterile microtube or cryovial containing isolated extracellular fraction	-80 °C	Handle under sterile conditions. Avoid freeze–thaw cycles. Ensure protocol-specific isolation method.
Lipids (from Cell Lysates)	Glass vial or microtube with extracted lipid fraction	-20 °C or -80 °C (depending on solvent)	Handle with solvent safety precautions; avoid evaporation and oxidation.
Cell Cultures	Sterile microtube or cryovial containing isolated cell cultures.	-80 °C or LN_2	Handle under sterile conditions. Avoid freeze–thaw cycles. Ensure protocol-specific cell culture.
Microorganisms (e.g Bacteria, Viruses, Fungal Spores) - stool	Sterile tube, swab, or culture plate	2–8 °C (short-term) or per biosafety protocol	Use BSL-2 minimum precautions. Handle in biological safety cabinet. Dispose of waste as biohazard.
Non-Human Samples			
Water	Sterile tube or bottle	2–8 °C	If no grey water system: N/A.
Air	Filter device or settle plates	Room Temperature	Handle aseptically; protect from contamination.
Surface/vents	Sterile swabs	2–8 °C	Avoid desiccation; use transport medium if delay expected.
Plant Leaves	Sterile tube or envelope	2–8 °C	Avoid desiccation;
Plant Seed	Sterile tube or falcon tube	2–8 °C	Avoid desiccation;
Plan Roots	Sterile tube or falcon tube	2–8 °C	Avoid desiccation;
Plant Substrate (soil or growth medium)	Sterile Whirl-Pak® bag or screw-cap tube	2–8 °C	Maintain Humidity levels between 20–40% (for long term storage maintain humidity lower than 10%)
Microorganisms (e.g Bacteria, Viruses, Fungal Spores) - surfaces	Sterile tube, swab, or culture plate	2–8 °C (short-term) or per biosafety protocol	Use BSL-2 minimum precautions. Handle in biological safety cabinet. Dispose of waste as biohazard.

3. TRANSPORT FROM COLLECTION FACILITIES TO PROCESSING LABORATORY

3.1. Transport of Tubes

Upright Position: tubes must be transported in an upright position to preserve sample integrity.

Appropriate Container: An appropriate container must be used to ensure safety during transport.

Secondary Containment: Whenever possible, the container should include secondary containment to provide additional protection.

3.2. Protection Against Shocks and Vibration

Cushioning: Tubes must be cushioned during transport to minimize vibrations and prevent damage, such as hemolysis of red blood cells.

3.3. Absorption in Case of Leakage

Absorbent Material: Adequate absorbent material must be placed around the blood tubes to immediately absorb any potential leakage during transport.

3.4. Labelling of the Outer Container

Required Information: The outer transport container must be clearly labeled with the following information:

- Name of the collection center
- Contact person in case of loss or incidents
- Recipient of the sample
- Safety markings, including:
  - *"Biological Material"*
  - *"Handle with Care"*
  - Biohazard symbol

Other labelling may be required by legislation in your country of origin.

4. QUALITY CONTROL

Any deviation to this SOP must be documented by the ESA Research Site in the SIF to ensure traceability and protocol compliance.

5. RECORDS GENERATED BY THIS PROCEDURE

Records' Identification	Indexation	Archive Responsible
Sample Information Form (SIF)	Medical Life Science Data Management System (MLS-DMS)	José Maximino, GIMM Biobank Data Manager
ESA statement confirming Medical Board Approval	GIMM - ESA SharePoint	Ana Rita Cruz, GIMM Project Manager
Blank Copy of the Consent Form Template	GIMM - ESA SharePoint	Ana Rita Cruz, GIMM Project Manager
STATEMENT_Informed Consents	GIMM - ESA SharePoint	Ana Rita Cruz, GIMM Project Manager

6. ANNEXES

- Sample Information Form (SIF).xlsx
- ESA Harmonized Unique Identifier (UID) Scheme
- STATEMENT_Informed Consents

ESA Harmonized Unique Identifier (UID) Scheme

This scheme provides a simple, consistent, and privacy-preserving approach for creating a unique subject identifier that can be used across multiple centers and studies.

1. Identifier Structure (Subject ID; SID)

A structured pseudonymous identifier is proposed:

[Biobank Code]-[ESA Center Code]-[Study Code]-[Sequence Number]

- Biobank Code: 3-character code representing the consortium/biobank.
- ESA Center Code: 3-character site identifier.
- Study Code: 4-character code for the specific study.
- Sequence Number: A 5-digit sequential number used to uniquely identify each study subject within the same center.

Example: BIO-MED-VIV3-00001

The SID is used only in secure internal systems and never printed or shared externally.

2. Internal Master ID (MID)

Each subject is assigned a MID, generated by GIMM Biobank LIMS software (typically in the format of AXXXXX). The MID is used only in secure internal systems and shared with approved users.

A central mapping table links MID ↔ SID ↔ local patient identifiers. By this scheme:

MID ↔ SID: GIMM Biobank

SID ↔ local patient identifiers: Study Center

The MID ↔ SID mapping table is stored securely within the LIMS database as part of the GIMM Biobank-controlled data environment. It is maintained in a relational database format (SQL), ensuring structured relationships and traceability. Access is restricted to authorized users under role-based permissions, and the table is never exported or shared externally except through approved, encrypted reports.

The SID ↔ local patient identifiers mapping table is stored securely in the ESA Center.

This approach guarantees data integrity, privacy, and compliance with ESA and biobank governance policies.

Key Principles

• Standardization: All centers use the same format and code registries.

• Privacy Protection: No personal identifiers or sensitive data appear in the SID.

• Uniqueness: Sequence numbers ensure uniqueness per study within the same center.

• Traceability: MID enables secure re-identification when legally authorized.

Governance Rules

• Maintain registries for biobank, center, and study codes.

• Allocate sequence numbers centrally.

• Store all mappings in a secure, access-controlled environment with audit logging.

• Codes and identifiers must remain immutable once assigned.

Operational Guidelines

• Barcodes/labels use the SID only; MID remains at GIMM Biobank.

• Existing IDs can be harmonized by generating new SIDs and storing mappings.

• Provide SOPs to all centers for consistent enrolment and identifier usage.

Benefits

• Uniform identifier format across all studies and centers.

• Improved data interoperability and sample tracking.

• Stronger privacy protection and reduced risk of misidentification.

• Scalable structure suitable for long-term, multi-site biobanking projects.

STATEMENT

Regarding the Human Biological Material and associated personal data collected by us, _____ [official designation of the ESA Research/Study Site], as ESA Research/Study Site, we hereby declare to have obtained all necessary Informed Consents from donors according to the ethical and legal rules applicable to donor's jurisdiction, regarding the participation on the research project and regarding the processing of participant's personal data.

All the subsequential forwarders and GIMM rely on the warranty provided by the ESA Research/Study Site, in the above paragraph, which is an essential component of their operations. Therefore, the undersigned entity assumes all liabilities for the lack of compliance with the stated above.

Place_____, Date ____/___20____

Signor name _____, Position: _____

Signature _____

HRE Exploration Biobank

Deliverables [D-01] and [D-02]

[D-01]: Mandatory standardised protocols and SOPs for ESA-directed research samples.

[D-02]: Recommended protocols for enabled research samples.

SOP

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1. SCOPE

This Standard Operating Procedure (SOP) establishes technical protocols and guidelines for the processing, handling, storage, and freezing of biological samples. These samples shall be preserved and stored in accordance with the specific requirements of the GIMM Biobank, ensuring the integrity and traceability of the biological materials throughout all stages of the process.

2. SAFETY INFORMATION

Given that the samples are of human biological origin, it is not possible to guarantee the complete absence of biological or infectious risk. Therefore, it is essential that all samples are handled and processed in accordance with applicable biosafety guidelines, adhering to the minimum requirements of Biosafety. Specifically, it is recommended that processing take place within a Biosafety Level 2 (BSL-2) environment.

Sample handling shall be performed exclusively by technically trained and certified personnel, to ensure safety of the personnel and the environment. During all handling procedures, strict use of Personal Protective Equipment (PPE) is mandatory, including laboratory coats, disposable gloves, protective goggles, or face masks, as specified in biosafety guidelines. These measures minimize contamination risk and ensure compliance with Good Laboratory Practices (GLP).

3. QUALITY CONTROL

All equipment used, including pipettes, centrifuges, and ultra-low temperature freezers must undergo regular inspections, cleaning, and disinfection, in accordance with the manufacturer's guidelines and recommendations. Routine maintenance is essential to ensure proper equipment performance and to safeguard the integrity of the processed samples, thereby ensuring compliance with established quality and safety standards.

3.1 Sample Category Quality Requirements

This section outlines the quality requirements applicable to the designated sample category. The purpose is to ensure that all samples meet defined standards of consistency, compliance, and reliability, thereby supporting accurate and reproducible outcomes. To facilitate clarity and practical application, a summary table is provided, consolidating the key quality requirements for this category. **Table 1** serves as a reference point for both operational execution and quality assurance review.

4. BACKUP STORAGE

Samples shall be stored in two sets of aliquots, with each set placed in a separate freezer. This strategy prevents sample loss in the event of technical failure or malfunction of one of the freezers.

5. ADVISED INFRASTRUCTURE

Biosafety Level 2 (BSL-2)

6. REQUIRED EQUIPMENT

- Centrifuge with a swing-bucket rotor and aerosol-tight buckets (to prevent aerosol dispersion);
- Refrigerator at 4 °C;
- Freezer at -20 °C;
- Ultra-low temperature freezers at -80 °C (confidence limits -65 °C to -85 °C);

Table 1. Sample category quality requirements.

Sample Type	Acceptance Criteria	Rejection Criteria	Notes / QC Parameters
Direct-Human Samples			
Whole Blood / Plasma / Serum	Standard collection and processing within SOP timelines	Clotted, hemolyzed, wrong tube, gross contamination	Hemolysis should be documented (visual scale)
DNA Samples (genomic DNA)	Yield ≥ 50 ng/µL, purity A260/280 ~ 1.8, no visible degradation (agarose gel or TapeStation), OD 260/230 > 1.5	Degraded DNA smear, A260/280 < 1.6 or > 2.0, low concentration < 10 ng/µL	Long-term stability best at -80 °C
cfDNA (cell-free DNA from plasma)	Yield ≥ 1 ng/mL plasma, distinct cfDNA peak (~170 bp) on Bioanalyzer/TapeStation	High molecular weight contamination (cellular DNA), hemolyzed plasma	Requires rapid processing and double centrifugation
RNA (PAXgene, tissue, cells)	RIN (RNA Integrity Number) ≥ 7, yield ≥ 50 ng/µL, A260/280 ~ 2.0	RIN < 5, low concentration (< 10 ng/µL), degradation smear	Store at -80 °C; avoid freeze–thaw
Cell Pellets (from blood, urine, etc.)	Viability ≥ 70% (Trypan Blue or flow cytometry), pellet intact, sterility maintained	Viability < 50%, contaminated, pellet lost during processing	Use cryomedium with 10% DMSO; record viability before freezing
Cultured Cells / Skin Fibroblasts	Mycoplasma-negative, viability ≥ 80% pre-freezing, correct morphology	Mycoplasma-positive, viability < 60%, abnormal morphology	Controlled-rate freezing, LN_2 storage
Stem Cells (urine, skin, cord blood, etc.)	CD marker profile consistent (flow cytometry), viability ≥ 80%, sterility confirmed	Loss of stemness markers, viability < 60%, contamination	Record passage number and freezing medium
CSF / Pleural / Cystic Fluids	Clear or slightly turbid, cell count within physiological range, volume ≥ 0.5 mL	Grossly blood-stained, insufficient volume, bacterial overgrowth	Can be processed for DNA/RNA or cytology
Saliva (tube, salivette, filter)	No visible blood, ≥ 0.5 mL, DNA yield ≥ 20 ng/µL	Blood contamination, insufficient volume, degraded nucleic acids	Stabilization buffer recommended
Tissue (Skin, Biopsies)	Snap-frozen within 30 min, fragment intact, RIN ≥ 6 (if RNA extraction planned)	Delayed freezing > 2 h, degraded RNA/DNA, fragmented tissue	Document ischemia time
Hair (DNA)	Follicles intact, DNA yield ≥ 10 ng/strand	No follicles, insufficient DNA, contamination	Useful for forensic or genetic analysis
Indirect-Human Samples			
Soluble Proteins (plasma, cell supernatants)	Detectable signal within assay range; no hemolysis or protein precipitation	Degraded/denatured proteins, microbial contamination	Suitable for ELISA, Western blot, Mass Spectrometry, Metabolomics arrays
DNA (indirect, from cells or media)	Yield ≥ 20 ng/µL, A260/280 ~ 1.8, no degradation	A260/280 < 1.6, degraded DNA, low yield < 5 ng/µL	For genomic sequencing, bisulfite sequencing, methylation array
RNA (mRNA, miRNA)	RIN ≥ 7, yield ≥ 20 ng/µL	RIN < 5, low concentration, degradation smear	For transcriptomics, bulk/single-nuclei RNA-seq; avoid freeze–thaw
Secretome (extracellular vesicles, metabolites, lipids)	EV integrity confirmed (NTA or EM), protein yield ≥ 50 µg, no hemolysis	Hemolyzed supernatant, low yield, contamination	For EV proteomics, miRNA sequencing, lipidomics, metabolomics arrays
Lipids (from cell lysates or media)	Adequate lipid yield (OD/fluorometric quantification), absence of debris	Contaminated or oxidized sample, low recovery	Lipidomics; use antioxidant stabilizers, store -80 °C
Microorganisms (e.g. Bacteria, Viruses, Fungal Spores)	Target organism detected, purity confirmed (culture or qPCR), sterility for non-infected samples	Cross-contamination, misidentified species, insufficient biomass	For microbiome, metagenomics, pathogen detection; observe biosafety level (BSL) requirements

Sample Type	Acceptance Criteria	Rejection Criteria	Notes / QC Parameters
Non-Human Samples			
Habitat Samples (water, air, swab)	Adequate volume/surface, DNA yield above assay limit, no contamination with inhibitors	Insufficient biomass, inhibitory contaminants, wrong container	Requires QC depending on target (DNA, RNA, microbial culture)
Plant material samples (leaves, roots, seeds)	Adequate volume, DNA yield above assay limit, no contamination with inhibitors	Insufficient biomass, inhibitory contaminants, wrong container	Requires QC depending on target (DNA, RNA, microbial culture)
Microorganisms (e.g. Bacteria, Viruses, Fungal Spores)	Target organism detected, purity confirmed (culture or qPCR), sterility for non-infected samples	Cross-contamination, misidentified species, insufficient biomass	For microbiome, metagenomics, pathogen detection; observe biosafety level (BSL) requirements

7. REQUIRED MATERIAL

- Personal Protective Equipment;
- Pipettes;
- Sterile pipette tips;
- Sterile 2 mL cryovials;
- Cryovial storage boxes;
- Sterile 15 mL and 50 mL Falcon tubes;
- Specific reagents according to each protocol.

8. PROCEDURE

8.1 Verification of Donor and Sample Documentation

Prior to sample processing, the documentation accompanying the samples must be verified by the sample collection site.
Mandatory documents to be checked:

- Informed Consent Form, signed by the donor and the responsible physician. *(non applicable for non-human biological samples)*
- Donor information pertaining to the donor up to the time of collection. *(non applicable for non-human biological samples)*
- Sample Information Form (SIF) - Annexed to this SOP

Note: These documents must be generated by the sample collection site, and delivered to ESA/data handler/processing site.

8.2. Verification of Sample Identification

To ensure traceability and compliance with data protection requirements:

- The collection site must verify that all samples received are properly identified and correspond to the accompanying documentation.
- Each donor and related samples are assigned unique internal codes, managed centrally in the database.
- Upon processing, aliquots generated receive sequential sample codes assigned by the database.

Note: Detailed procedures for donor and sample identification, coding, and traceability are described in SOP.BIO/ESA.001.

8.3 Guidelines to Process Biological Samples

Processing must be performed according to harmonized standards to guarantee sample quality, aliquot consistency, and compliance with downstream applications.

- **Table 2** outlines the mandatory procedures for sample processing and storage, in accordance with the protocol requirements described in this section.
- **Table 3** outlines the recommended reagents, consumables, and materials, in accordance with the protocol requirements described in this section.
- Unless otherwise specified, sterile 2 mL screw-cap cryovials must be used, and samples stored following gradual freezing (-20 °C for 48 h, followed by -80 °C).
- Whenever more than one applicable protocol exists, the choice must be documented in the SIF.
- All sample processing must be carried out under sterile conditions.

8.3.1 Processing of Blood

8.3.1.1 Processing of Whole Blood:

Materials

- K2EDTA anticoagulant tubes (recommended for collection)
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 2 aliquots, 1 mL each.

Methods

i) Gently invert the anticoagulant tube (K2EDTA anticoagulant tube is recommended for collection) approximately 5 times; vigorous mixing may compromise sample integrity.

ii) Transfer the whole blood into a pre-labeled 2 mL cryovial with an external screw cap, aliquoting the appropriate volume.

iii) Securely close the cryovial and proceed with gradual freezing (-20 °C for 48 hours, followed by storage at -80 °C).

8.3.1.2 Processing of Blood for Serum Separation

Materials

- Sample tubes containing blood (with clotting completed)
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 6 aliquots, 250 µL each.

Methods

i) Verify that the centrifuge is in good working condition and that the tubes are properly closed and balanced to prevent breakage and spillage.

ii) Centrifuge at 2000 × g, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), for 10 minutes at room temperature.

iii) Carefully remove the tubes from the centrifuge.

iv) Recover the serum using a micropipette with sterile disposable tips. Transfer the serum to a pre-labeled 2 mL screw-cap cryovial (recommended: 6 aliquots of 250 µL each).

v) Securely close the cryovial and proceed with gradual freezing (-20 °C for 48 hours, followed by storage at -80 °C).

8.3.1.2.1 Processing of Blood for Serum Separation for Biomarker Research:

Materials

- Sample tubes containing blood (with clotting completed)
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Protease inhibitor cocktail for use with mammalian cell and tissue extracts, diluted 1:10 from 1× concentrated solution
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 5 aliquots, 1 mL each.

Methods

i) Keep samples at 4 °C after the 30 minutes room temperature recommend in SOP.BIO/ESA.001 and process them within a maximum of 4 hours after blood collection.

ii) Ensure that the centrifuge is in good working condition and that the tubes are properly closed and balanced to prevent breakage and spillage.

iii) Centrifuge the tubes at 1800 × g for 30 minutes at 4 °C, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum)

iv) After centrifugation, transfer 1000 µL of serum into a sterile cryovial preloaded with 100 µL of protease inhibitor cocktail (Protease Inhibitor Cocktail for use with mammalian cell and tissue extracts, SIGMA; diluted 1:10 from 1x concentrated solution).

v) Securely close the cryovial and store at -80 °C in the ultra-low temperature freezer.

8.3.1.3 Processing of Blood for Plasma Separation:

Materials

- K2EDTA anticoagulant tubes (recommended for collection)
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox

- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 2 aliquots, 500 µL each.

Methods

i) Gently invert the anticoagulant tube approximately 5 times; vigorous mixing may compromise sample integrity (K2EDTA anticoagulant tubes are recommended for collection; however, the anticoagulant used may be selected to best suit the expected downstream applications).

ii) Ensure that the centrifuge is in good working condition and that the tubes are properly closed and balanced to prevent breakage and spillage.

iii) Centrifuge at 2000 × g, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), for 20 minutes at room temperature.

iv) Carefully remove the tubes from the centrifuge.

v) Recover the plasma using a micropipette with sterile disposable tips. Collect plasma from the surface, leaving a 1 cm margin above the buffy coat to avoid platelet contamination. Transfer the plasma to a pre-labeled 2 mL screw-cap cryovial and aliquot in the appropriate volume.

vi) Securely close the cryovial and proceed with gradual freezing (-20 °C for 48 hours, followed by storage at -80 °C).

8.3.1.4 Processing of Blood for RNA extraction:

Materials

- PAXgene RNA tubes
- Upright tube racks compatible with PAXgene tubes (for room temperature incubation and freezing)
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: primary tube.

Methods

i) Incubate the PAXgene RNA tube upright at room temperature for 2–4 hours (do not exceed 8 hours). This allows the PAXgene reagent to fully penetrate the blood cells.

ii) Transfer the tube to a -20 °C freezer for 24 hours to allow gradual freezing and prevent tube breakage.

iii) Subsequently, transfer the tube to a -80 °C freezer for long-term storage.

Note: Freezing must be performed with the tubes in a vertical position. Do not use polystyrene (Styrofoam) racks, as they may damage the tubes.

8.3.1.5 Processing of Blood for DNA Extraction:

Materials

- PAXgene DNA tubes
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 4 aliquots, 1 mL each.

Methods

i) Centrifuge the PAXgene DNA tube at 1900 × g with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), for 15 minutes at room temperature.

ii) Carefully transfer the plasma volume to a pre-labelled 2 mL screw-cap cryotube, ensuring that the visible cellular layer is not disturbed. Record the exact volume of plasma aliquoted into the cryotube.

iii) Close the cryotube tightly and proceed with gradual freezing (-20 °C for 48 hours, followed by storage at -80 °C).

8.3.1.6 Processing of Blood for Separation of Plasma for Circulating DNA Extraction:

Materials

- K2EDTA anticoagulant tubes (recommended for collection)
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 15 mL Falcon tubes
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 4 aliquots, 1 mL each.

Methods

i) Gently invert the tube approximately five times; vigorous mixing may compromise sample integrity.

ii) Ensure that the centrifuge is in proper working condition and that the tubes are securely closed and properly balanced to prevent breakage and leakage.

iii) Centrifuge at 1600 × g, with acceleration set to 9 maximum and deceleration set to 0 minimum, for 10 minutes at room temperature (it is important to ensure that the brake is turned off to avoid disruption of the cellular layer).

iv) Carefully remove the tubes from the centrifuge.

v) Transfer the supernatant to a 15 mL Falcon tube, taking care not to disturb the buffy coat.

vi) Centrifuge again at 3000 × g, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum) for 10 minutes at room temperature.

vii) Transfer the plasma volume to a pre-labelled 2 mL screw-cap cryotube, taking care not to aspirate the visible cellular layer. Record the exact plasma volume aliquoted into the cryotube.

viii) Close the cryotube securely and proceed with gradual freezing (-20 °C for 48 hours, followed by storage at -80 °C).

8.3.1.7 Processing of Blood for Erythrocyte Separation:

Materials

- K2EDTA anticoagulant tubes (recommended)
- Physiological saline solution (0.9% NaCl, sterile)
- Micropipette
- Sterile disposable pipette tips

- 2 mL screw-cap cryovials (pre-labeled)
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 2 aliquots, 500 µL each.

Methods

i) After a 2-hour rest period at room temperature, centrifuge the anticoagulated blood sample (collection in K2EDTA tubes is recommended) at 800 × g, with acceleration set to 9 maximum and deceleration set to 2, for 10 minutes at room temperature;

ii) Discard the plasma and wash the erythrocyte pellet with an equal volume of physiological saline (0.9% aqueous NaCl solution);

iii) Centrifuge at 700 × g, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), for 5 minutes at room temperature;

iv) Wash the pellet again with 3 mL of physiological saline and centrifuge at 800 × g, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), for 5 minutes at room temperature;

v) Resuspend the pellet in 1 mL of physiological saline;

vi) Transfer the suspension to a pre-labelled 2 mL cryotube and proceed with gradual freezing (-20 °C for 48 hours, followed by storage at -80 °C).

8.3.1.8 Processing of Blood for Separation of Platelet-Rich Plasma (PRP) and Platelet Pellet:

Note: For each product (PRP and platelet pellet), collect at least one 9 mL K2EDTA blood tube to ensure a final yield of 1–3 × 10^8 platelets/mL. Donors must not have taken antihistamines, aspirin, or anti-inflammatory medication for at least two weeks prior to sample collection. This protocol is optimized to isolate non-activated platelets.

Materials

- K2EDTA anticoagulant tubes
- Phosphate-buffered saline (PBS)
- Prostaglandin E1 (PGE1) stock solution (10 mg/mL)
- Pasteur pipettes
- Micropipette
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials (pre-labeled)
- Cryobox
- 15 mL Falcon tubes
- CoolCell container (for controlled freezing)
- MilliQ water (for 50% saline solution preparation)
- Sodium chloride (NaCl) for saline solution
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 2 aliquots, 1 mL each.

Methods

Prepare in advance: 1 µM PGE1 in PBS (2 µL of PGE1 at 10 mg/mL in 20 mL of PBS)

i) Gently homogenize the K2EDTA tubes by fully inverting them 10 times;

ii) Centrifuge the K2EDTA tubes at 100 × g for 10 minutes at room temperature (RT), with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum)

iii) In a laminar flow hood, transfer the plasma into a 15 mL Falcon tube, without disturbing the buffy coat, using a Pasteur pipette;

iv) Add an equal volume of 1 µM PGE1 in PBS to the plasma in the Falcon tube to achieve a 1:1 (v/v) ratio. Gently mix the plasma and 1 µM PGE1 solution by inversion;

v) Centrifuge the 15 mL Falcon tubes at 200 × g for 15 minutes at RT, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum)

vi) Discard the upper two-thirds of the supernatant using a Pasteur pipette.

vii) To prepare Platelet-Rich Plasma (PRP):

a) Resuspend the pellet in the remaining lower third of the supernatant using a Pasteur pipette, and transfer the entire suspension into a cryotube;

b) Place the cryotube in a CoolCell container and freeze overnight at -80 °C;

viii) To prepare Platelet Pellet:

Prepare in advance: 50% saline solution (dissolve 5 g of NaCl in 10 mL of MilliQ water)

a) Resuspend the pellet in the remaining lower third of the supernatant using a Pasteur pipette;

b) Add 0.9% saline solution to the platelet suspension (add 37 µL of 50% saline solution to 2 mL of suspension), and transfer the full suspension to a cryotube;

c) Centrifuge the cryotube at 800 × g for 15 minutes at RT, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum)

d) Discard the supernatant using a Pasteur pipette;

e) Place the cryotube in a CoolCell container and freeze overnight at -80 °C;

8.3.1.9 Plasma Cards (DBS/FTA cards)

Recommended number and volume of aliquots: 2 cards 50-100 µL.

Materials

- Plasma cards (certified collection paper)
- Disposable gloves
- Clean working area
- Zip-lock bags with desiccant packs
- Permanent marker or barcode labels

Methods

i) Apply 50–100 µL of plasma per circle on the plasma card, ensuring full saturation.

ii) Allow to dry at room temperature for at least 3 hours, away from direct light or humidity.

iii) Place the dried card into a labeled zip-lock bag with desiccant.

iv) Store at room temperature in a dry, dark location, or at -20 °C for long-term preservation.

8.3.1.10 Dry Blood Drops (DBS)

Recommended number and volume of aliquots: 2 cards 50-100 µL.

Materials

- Filter paper cards (Whatman or equivalent)
- Disposable gloves
- Zip-lock bags with desiccant packs
- Permanent marker or barcode labels

Methods

i) Spot 20–50 µL of whole blood onto designated circles of filter paper.

ii) Allow to dry at room temperature for a minimum of 3 hours.

iii) Insert each card into a labeled zip-lock bag with desiccant.

iv) Store at room temperature in a dry environment or at -20 °C for extended storage.

8.3.2 Processing of Cerebrospinal Fluid (CSF)

This type of sample is collected in a certified professional.

Materials

- Collection tubes (anticoagulant tubes recommended for collection).
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 6 aliquots, 250 µL each.

Methods

i) Ensure that the centrifuge is in proper working condition and that the tubes are securely closed and properly balanced to prevent breakage or leakage (collection in tubes without anticoagulant is recommended);

ii) Centrifuge at 2000 × g, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), for 10 minutes at room temperature;

iii) Carefully remove the tubes from the centrifuge;

iv) Recover the supernatant using a micropipette with sterile disposable tips;

v) Transfer the supernatant into pre-labelled 2 mL screw-cap cryotubes (six 250 µL aliquots are recommended);

vi) Tightly seal the cryotube and immediately freeze at -80 °C.

8.3.3 Processing of Saliva

If the sample is collected in insufficient volume (less than 2 mL) or presents macroscopic characteristics that may compromise its quality, it must be discarded.

Sample collection and processing may follow one of the two methods described below:

8.3.3.1 Saliva collection in a 50 mL Falcon tube

Materials

- 50 mL Falcon tubes (for saliva collection)
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 6 aliquots, 250 µL each.

Methods

i) Falcon tubes should be centrifuged at maximum speed (3000 × g), with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), for 30 minutes at 4 °C. If immediate centrifugation is not possible, samples must be kept at 4 °C and processed within 24 hours of collection.

ii) Aliquot the collected saliva into 250 µL aliquots in pre-labelled 2 mL screw-cap cryotubes.

iii) Close the tubes tightly and store them at -80 °C.

8.3.3.2 Saliva collection in a NEST tube

Materials

- NEST tubes (for saliva collection)
- Glycerol (used as bacterial cryoprotectant)
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 6 aliquots, 250 µL each.

Methods

i) Confirm that the volume of saliva does not exceed the limit indicated by the manufacturer.

ii) Add the appropriate amount of glycerol directly to the tube containing the saliva (used as a bacterial cryoprotectant in microbiology studies). As a general guideline, each NEST tube should contain 2 mL of saliva, requiring 320 µL of glycerol. If the saliva volume exceeds the specified limit, adjust the amount of glycerol accordingly.

iii) Seal the tube tightly and store directly at -80 °C.

8.3.3.3 Saliva – Salivette Collection

Recommended number and volume of aliquots: 6 aliquots, 250 µL each.

Materials

- Salivette collection device (cotton swab + tube)
- Micropipette (calibrated)

- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode system

Methods

i) Ensure swab has been chewed/kept in mouth as per manufacturer's instructions.
ii) Centrifuge Salivette tube at 1000 × g for 2 minutes at 4 °C.
iii) Aliquot supernatant into 250 µL fractions in cryovials.
iv) Store at -80 °C.

8.3.3.4 Dry Saliva Filter

Recommended number and volume of aliquots: 2 cards.

Materials

- Commercial saliva filter paper strips
- Zip-lock bags with desiccant
- Permanent marker or barcode labels

Methods

i) Collect saliva by chewing/soaking the strip.
ii) Air-dry the filter completely at room temperature.
iii) Insert into labeled zip-lock bag with desiccant.
iv) Store at room temperature in dry, dark environment, or at -20 °C.

8.3.4 Processing of Pleural Fluid

This type of sample is collected by a certified professional.

Materials

- 50 mL Falcon tubes
- RPMI 1640 Medium
- Fetal Bovine Serum (FBS)
- Penicillin-Streptomycin [10,000 U/mL] (Pen/Strep)
- Dulbecco's Phosphate Buffered Saline, no calcium, no magnesium 1x (DPBS)
- RBC Lysis Buffer
- Neubauer chamber or equivalent hemocytometer
- Trypan Blue dye (0.4%) or similar viability dye
- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Methods

Prepare in advance: cRPMI medium: 5 mL FBS + 500 µL Pen/Strep + 50 mL RPMI 1640. Label the medium with the date and time of preparation. It may be used for up to 2 weeks after preparation if stored at 4 °C.

Prior to sample processing: Warm the cRPMI to 37 °C in a water bath for 20 minutes.

i) Transfer the pleural fluid from the original container into 50 mL Falcon tubes.

ii) Centrifuge the tubes at 609 × g for 5 minutes at 22 °C, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum).

iii) Discard or aspirate the supernatant into a designated biological liquid waste container.

iv) Resuspend the cell pellet in 1 mL of cRPMI.

v) Repeat the previous step until the entire volume of pleural fluid has been processed.

vi) Centrifugation may be repeated, and the same tubes reused for the same sample, if needed, until a final cell pellet is obtained in a 50 mL Falcon tube.

vii) Resuspend the final pellet in 5 mL of RBC Lysis Buffer at 4 °C and keep on wet ice for 5 minutes.

viii) After 5 minutes of incubation, fill the tube with DPBS and centrifuge at 609 × g for 5 minutes at 22 °C with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum)

ix) Aspirate the supernatant and resuspend the pellet in 5 mL of cRPMI.

x) Perform a cell count using a Neubauer chamber or equivalent. Count the number of viable cells in suspension (cells/mL). Use Trypan Blue dye or a similar reagent to distinguish between viable and non-viable cells.

a) Resuspend the cell suspension by pipetting up and down.

b) Prepare four different dilutions:

- 1:1 – 50 µL of cell suspension + 0 µL of cRPMI
- 1:10 – 10 µL of cell suspension + 90 µL of cRPMI
- 1:100 – 10 µL of cell suspension + 990 µL of cRPMI
- 1:200 – 5 µL of cell suspension + 995 µL of cRPMI

c) For 50 µL of each dilution, add 50 µL of 0.4% Trypan Blue dye and mix thoroughly.

d) Perform cell counting within 3 minutes to avoid interference with cell viability.

e) Apply the Trypan Blue dilution mixture at the edge of the coverslip to allow the chamber to fill completely. Avoid air bubbles; if bubbles form, repeat the loading process.

f) Examine the chamber under a microscope and proceed with the cell count.

If red blood cell contamination prevents accurate counting, repeat lysis using RBC Lysis Buffer (step xii). Repeat the cell count and document the result.

If debris contamination prevents accurate counting, fill the tube with cRPMI and centrifuge again at 609 × g for 5 minutes at 22 °C with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum). Repeat the cell count and document the result.

8.3.5 Processing of Urine

8.3.5.1. Aseptic Urine Processing

Materials

- Micropipette (calibrated)
- 15 mL or 50 mL Falcon tubes
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 6 aliquots, 1 mL each.

Methods

i) Transfer the total urine volume into 15 mL or 50 mL Falcon tubes, depending on the received sample volume.

ii) Centrifuge the tubes at 2000 × g for 10 minutes at 22 °C with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum).

iii) Carefully remove the tubes from the centrifuge.

iv) Recover the supernatant using a micropipette with sterile disposable tips. Collect the supernatant from the surface, leaving at least a 1 cm margin above the urinary sediment. Transfer the supernatant into 2 mL screw-cap cryotubes, previously labeled.

v) Close the cryotubes tightly and store at -80 °C.

8.3.5.2. 24-Hour Urine Processing

Materials

- Micropipette (calibrated)
- 15 mL or 50 mL sterile Falcon tubes
- Sterile disposable pipette tips
- 5 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 3 aliquots, 5 mL each.

Methods

i) Thoroughly homogenize the contents of the urine collection container.

ii) Prepare 3 aliquots of 5 mL each in 5 mL screw-cap cryotubes.

iii) Close the cryotubes tightly and store at -80 °C.

8.3.5.3. Large Volume Urine (for stem cell isolation)

Materials

- Sterile urine collection container (100–400 mL)
- Centrifuge (swing-bucket rotor)

- 50 mL Falcon tubes
- DPBS
- Sterile micropipette tips
- Dulbecco's Modified Eagle Medium (DMEM)
- Dimethyl sulfoxide (DMSO)
- 2 mL Cryovials
- Cryobox

Recommended number and volume of aliquots: 3 aliquots, 1 mL each.

Methods

Prepare in advance: Cryopreservation medium: 10% DMSO in DMEM. Label the medium with the date and time of preparation. It may be used for up to 2 weeks after preparation if stored at 4 °C.

i) Centrifuge urine at 400 × g for 10 minutes at 22 °C with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum).

ii) Collect cell pellet and wash twice in DPBS.

iii) Resuspend pellet in appropriate cryopreservation medium.

iv) Transfer into cryovials (1 mL per vial).

v) Freeze gradually (CoolCell or Mr. Frosty) at -80 °C, then transfer to liquid nitrogen.

8.3.6 Processing of Cystic Fluid

This type of sample is collected by a certified professional, using a 15 mL or 50 mL Falcon tube.

Materials

- Micropipette (calibrated)
- Sterile disposable pipette tips
- Sterile 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 6 aliquots, 250 µL each and 6 aliquots with dry pellets.

Methods

i) Transfer 250 µL of cystic fluid into six 2 mL screw-cap cryotubes. Ensure the cryotubes are tightly sealed.

ii) Centrifuge at 2000 × g, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), for 10 minutes at room temperature.

iii) Carefully remove the tubes from the centrifuge.

iv) Recover the supernatant using a micropipette with sterile disposable tips and transfer it into new 2 mL screw-cap cryotubes—aiming to obtain six cryotubes with the resulting pellet and six cryotubes with the supernatant.

v) Tightly seal all cryotubes and proceed with gradual freezing (-20 °C for 48 hours, followed by storage at -80 °C).

8.3.7 Processing of Tears

Materials

- Schirmer strips (without anesthesia)
- Cryotubes
- Cryobox
- Dry ice
- PPE
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 1 aliquot with 1 strip each.

Methods

i) The collection is performed using sterile Schirmer strips applied to the lower conjunctival sac.

ii) The subject should remain with eyes closed (without squeezing) for 5 minutes or as defined by the study protocol.

iii) The length of the wetted portion of the strip corresponds to the tear volume: 1 mm = 1 µL.

iv) Upon removal, the strip must not be touched in the wetted area.

v) Immediately after collection, each Schirmer strip should be inserted into a pre-labeled cryotube and identified with:

   - The eye from which the tear was collected (right or left).
   - The volume collected, corresponding to the length (in mm) of the wetted portion of the strip (1 mm = 1 µL)

vi) The cryotube is placed on dry ice for temporary stabilization.

vii) Once all samples are collected, they must be transferred to -80 °C.

8.3.8. Stool Sample Processing (Aliquoting)

Materials

- Micropipette (calibrated)
- Sterile disposable pipette tips
- 2 mL screw-cap cryovials
- Cryobox
- Permanent marker or barcode labeling system
- Sterile disposable spatulas
- Vortex mixer

Recommended number and volume of aliquots: 5 aliquots with 200-300 mg or 5 aliquots with 1 mL.

Methods

i) Under aseptic conditions, homogenize the sample if necessary and aliquot into sterile 1.5 mL or 2.0 mL cryovials.(recommend 5 vials of 2 mL)

ii) Use sterile disposable spatulas to transfer approximately 200–500 mg of stool if stool is collected without medium.

iii) Vortex the tube with medium 15sec and aliquot 1 mL for a 2 mL screw cap tube.

iv) Store aliquots at -80 °C.

8.3.9. Processing of Skin Tissue

8.3.9.1. Cryopreservation for Histopathological Analysis (Snap-Freezing)

Materials

- 2 mL screw-cap cryovials
- Sterile forceps
- Sterile scissors or scalpels
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 2 aliquots with 2 fragments.

Methods

i) Handle skin biopsies using sterile forceps.

ii) Cut tissue into ~0.5 cm fragments using sterile scissors or scalpels.

iii) Place fragments into pre-labeled 2 mL cryovials.

iv) Immediately immerse cryovials in liquid nitrogen for a minimum of 2 minutes to ensure rapid freezing (snap-freezing).

v) Store at -80 °C or in liquid nitrogen (-196 °C) for long-term preservation.

8.3.9.2. Cryopreservation for Molecular Studies

Materials

- 2 mL screw-cap cryovials
- Sterile forceps
- RNAlater solution
- Sterile scissors or scalpels
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 2 aliquots with 2 fragments in 1 mL of RNA Later.

Methods

i) Handle skin biopsies using sterile forceps.

ii) Cut tissue into ~0.5 cm fragments using sterile scissors or scalpels.

iii) Place fragments into pre-labeled 2 mL cryovials or suitable RNase-free tubes.

iv) Add sufficient RNAlater solution to completely submerge the tissue (typically 5–10 volumes of RNAlater per tissue volume).

v) Incubate samples at 4 °C for at least 24 hours to allow penetration of RNAlater.

vi) Store samples at -80 °C for long-term preservation of RNA integrity.

8.3.9.3 Cryopreservation for Cell Culture

Materials

- 2 mL screw-cap cryovials
- Sterile forceps

- DPBS
- DMEM
- Pen/Strep
- DMSO
- Controlled-rate freezing container (e.g., Mr. Frosty)
- Sterile scissors or scalpels
- Cryobox
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 2 aliquots with 2 fragments in 1 mL of cryopreservation medium

Methods

Prepare in advance: DPBS supplemented with Pen/Step: 500 µL Pen/Strep + 50 mL DPBS. Cryopreservation medium: 10% DMSO in DMEM. Label the media with the date and time of preparation. It may be used for up to 2 weeks after preparation if stored at 4 °C.

i) Decontaminate the external surface of the tissue using DPBS supplemented supplemented with Pen/Step.

ii) Dissect small tissue fragments (~1–2 mm).

iii) Transfer fragments into cryovials containing cryopreservation medium.

iv) Ensure vials are properly labeled.

v) Perform controlled-rate freezing at approximately -1 °C/minute, using a freezing container (e.g., Mr. Frosty) placed at -80 °C for at least 24 hours.

vi) After initial freezing, transfer vials to liquid nitrogen for long-term storage.

8.3.9.4 Skin Patches (surface samples)

Materials

- Sterile adhesive skin patches (pre-labelled)
- 2 mL sterile cryovials
- Cryobox

Recommended number and volume of aliquots: 2 aliquots with 1 patch per aliquot

Methods

i) Apply patch to defined skin area for specified time (per study protocol).

ii) Remove using sterile technique and place into labeled cryovial.

iii) Store at -80 °C.

8.3.10 Processing of Hair Samples

8.3.10.1 For DNA Extraction from Hair Follicles

Materials

- Sterile forceps
- Sterile, dry microtubes (pre-labeled)
- Permanent marker or barcode labeling system

Recommended number and volume of aliquots: 2 aliquots with 2 fragments.

Methods

i) Collect hair strands with intact follicles using sterile forceps; avoid touching the root directly.

ii) Place 5–10 strands with visible follicles into sterile, dry, labeled microtubes.

iii) Do not add buffer or liquid unless otherwise specified by downstream application (as RNA extraction with RNA Later).

iv) Ensure tubes are tightly sealed and store at room temperature or 4 °C in a dry, dark environment protected from humidity.

8.3.10.2 Keratinocytes from Hair Follicles

Materials

- Sterile forceps and scissors
- DPBS
- Trypsin-EDTA solution
- DMEM
- FBS
- Pen/Strep
- DMSO
- Cryovials
- Controlled-rate freezing container

Recommended number and volume of aliquots: 4 aliquots with 1 mL.

Methods

Prepare in advance: Culture medium: 5 mL FBS + 500 µL Pen/Strep + 50 mL DMEM. Cryopreservation medium: 10% DMSO in DMEM. Label the media with the date and time of preparation. It may be used for up to 2 weeks after preparation if stored at 4 °C.

i) Isolate intact hair follicles under sterile conditions.

ii) Wash follicles in DPBS.

iii) Digest follicles in trypsin-EDTA for 30 minutes at 37 °C to release keratinocytes.

iv) Transfer cells into culture medium and centrifuge at 300 × g for 5 minutes.

v) Resuspend pellet in cryopreservation medium.

vi) Freeze gradually at -80 °C (CoolCell/Mr. Frosty), then transfer to liquid nitrogen.

8.3.11 Non-Human Samples

8.3.11.1 Water Samples

Materials

- Sterile collection tubes or bottles
- Cryovials

Recommended number and volume of aliquots: 10 aliquots with 1 mL.

Methods

i) Collect 10–50 mL of water sample (or N/A if no grey-water system).

ii) Aliquot into 2 mL cryovials.

iii) Store at -80 °C.

8.3.11.2 Air Samples

Materials

- Filter cassettes or settle plates
- Sterile forceps
- 5 mL Cryovials

Recommended number and volume of aliquots: 6 aliquots with 1 filter.

Methods

i) Collect air particulate matter using filters or settle plates per study protocol.

ii) Transfer filters/plates into sterile cryovials.

iii) Store at -80 °C.

8.3.11.3 Surface Swabs

Materials

- Sterile swabs
- 2 mL sterile cryovials with transport medium (if required)

Recommended number and volume of aliquots: 5 aliquots with 1 swab.

Methods

i) Swab designated surfaces/vents following study protocol.

ii) Place swab into cryovial (with or without medium).

iii) Store at -80 °C.

8.3.11.4 Plant-Derived Samples (Leaves, Roots, Seeds)

Materials

- Sterile scissors or scalpel
- Sterile forceps
- Sterile cryovials (2–5 mL) or Whirl-Pak bags
- Silica gel (for dry preservation) or RNA/DNA stabilization solution (if required)
- Labels and waterproof marker

Recommended number and volume of aliquots:

Leaves: 3–5 aliquots (~0.5–1 g per aliquot)

Roots: 3 aliquots (~0.5 g per aliquot)

Seeds: 5–10 seeds per aliquot (depending on size)

Methods

i) For DNA/RNA analysis: Immediately place tissue in cryovial with stabilization solution or flash-freeze in liquid nitrogen.

ii) For dry preservation: Place material in a bag with silica gel (ensure rapid desiccation).

iii) Frozen samples: Store at -80 °C (or in liquid nitrogen for long-term RNA integrity).

iv) Dry samples: Store at room temperature in airtight containers with desiccant.

8.3.12 Indirect Human Samples

Indirect human biological materials represent analytes or molecular derivatives obtained from human specimens rather than the primary biological matrix. Proper handling and storage are critical to maintain molecular integrity and analytical reproducibility

8.3.12.1 Soluble Proteins

Materials

- Plasma, serum, or cell culture supernatant (as source)
- Protease inhibitor cocktail (1:10 dilution from 1× solution)
- 2 mL cryovials, micropipette, sterile tips

Recommended number and volume of aliquots: 4 aliquots with 100 µL.

Methods

i) Centrifuge biological fluids at 2000 × g for 10 minutes at 4 °C, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), to remove debris and cells.

ii) Add protease inhibitors immediately after centrifugation.

iii) Aliquot supernatant (500 µL–1 mL per vial) into pre-labelled cryovials.

iv) Freeze gradually and store at -80 °C. Avoid repeated freeze–thaw cycles.

8.3.12.2 DNA (Genomic or Cell-Free)

Materials

- Extracted DNA (from tissue, blood, saliva, or cfDNA plasma)
- Low-EDTA TE buffer or nuclease-free water

Recommended number and volume of aliquots: 4 aliquots of 100 µL.

Methods

i) Quantify DNA concentration and purity (A260/280 ~ 1.8; A260/230 > 1.5).

ii) Aliquot ≥ 20 ng/µL DNA in 50–200 µL per vial.

iii) Store at -80 °C.

Note: Avoid repeated freeze–thaw cycles. DNA can also be stored at -20 °C for short-term use.

8.3.12.3 RNA (mRNA, miRNA)

Materials

- Extracted total RNA or miRNA fractions
- RNase-free tubes, RNase inhibitor, and low-TE buffer

Recommended number and volume of aliquots: 4 aliquots with 100 µL.

Methods

i) Quantify RNA and assess integrity (RIN ≥ 7).

ii) Aliquot RNA in small volumes (≤ 20 µL) to minimize freeze–thaw.

iii) Store at -80 °C in RNase-free conditions.

Note: For long-term preservation, use RNA stabilizing reagents (e.g., RNAlater).

8.3.12.4 Secretome (Extracellular Vesicles, Exosomes, Metabolites, Lipids)

Materials

- Cell culture supernatant or plasma
- Ultracentrifuge or filtration system (0.22 µm filters)
- Cryovials

Recommended number and volume of aliquots: 4 aliquots of 200 µL.

Methods

i) Centrifuge samples at 2000 × g for 10 minutes at 4 °C, with acceleration and deceleration set to 9 (acceleration and deceleration on and maximum), to remove cells.

ii) Perform EV isolation (e.g., ultracentrifugation, precipitation, or size-exclusion chromatography) according to analytical needs.

iii) Aliquot purified EV fraction or metabolite-containing supernatant.

iv) Store at -80 °C.

8.3.12.5 Lipids (from Cell Lysates or Fluids)

Materials

- Organic solvents (chloroform, methanol, or isopropanol, depending on protocol)
- Antioxidants (e.g., BHT)

Recommended number and volume of aliquots: 4 aliquots of 250 µL.

Methods

i) Perform lipid extraction using validated methods (e.g., Folch or Bligh & Dyer).

ii) Evaporate solvent under inert gas (nitrogen or argon).

iii) Store lipid extracts under inert atmosphere at -80 °C, protected from light.

8.3.12.6 Microorganisms (Bacteria, Viruses, and Fungal Spores)

Materials

- Sterile swabs or liquid media
- Cryovials or biocontainment tubes
- Appropriate biosafety equipment (BSL-2 minimum)

Recommended number and volume of aliquots: 4 aliquots of 2 mL

Methods

i) Collect and handle samples in biosafety cabinet under aseptic conditions.

ii) For culture isolates: centrifuge at low speed, resuspend pellet in cryomedium (10% glycerol in broth).

iii) For nucleic acid extraction: process immediately or store at -80 °C.

9. RECORDS GENERATED BY THIS PROCEDURE

Records' Identification	Indexation	Archive Responsible
Sample Information Form (SIF)	GIMM - ESA SharePoint	Ana Rita Cruz, GIMM Project Manager

10. ANNEXES

- Sample Information Form (SIF).xlsx

Table 2. Summary of sample processing and storage requirements.

Protocol Section	Sample Type	Recommended Aliquots & Volume	Centrifugation Conditions	Special Additives / Notes	Storage Conditions
8.3.1.1	Whole Blood	2–4 × 1 mL aliquots	N/A	K2EDTA or heparin tubes as per study	-80 °C
8.3.1.2	Serum	4–8 × 0.5–1 mL	2000 × g, 10 min, 4 °C	Use serum separator tube	-80 °C
8.3.1.2.1	Serum (Biomarkers)	6–10 × 0.25–0.5 mL	2000 × g, 10 min, 4 °C	Protect from repeated freeze–thaw	-80 °C
8.3.1.3	Plasma	4–8 × 0.5–1 mL	2000 × g, 10 min, 4 °C	Collect in K2EDTA tubes	-80 °C
8.3.1.4	Blood for RNA	2–3 × 2.5 mL (PAXgene tubes)	N/A	Dedicated RNA tubes	-80 °C
8.3.1.5	Blood for DNA	2–3 × 2 mL	N/A	K2EDTA preferred	-20 °C (short) / -80 °C (long)
8.3.1.6	Plasma for cfDNA	4–6 × 0.5–1 mL	1600 × g, 10 min, 4 °C, then 16,000 × g, 10 min	Process rapidly after collection	-80 °C
8.3.1.7	Erythrocytes	2–4 × 1 mL	2000 × g, 10 min, 4 °C	Pellet after plasma removal	-80 °C
8.3.1.8	Platelet-Rich Plasma (PRP)	2–4 × 0.5–1 mL	200 × g, 10 min, RT	Process promptly	-80 °C
8.3.1.9	Plasma Cards (DBS/FTA)	50–100 µL per spot	N/A	Dry completely, store with desiccant	RT (dry, dark) / -20 °C
8.3.1.10	Dry Blood Drops (DBS)	20–50 µL per spot	N/A	Use certified filter paper, dry with desiccant	RT / -20 °C
8.3.2	CSF	4–6 × 0.5–1 mL	2000 × g, 10 min, 4 °C	Handle under sterile conditions	-80 °C
8.3.3.1	Saliva (Falcon)	4–8 × 0.5–1 mL	2000 × g, 10 min, 4 °C	Avoid food/drink before collection	-80 °C
8.3.3.2	Saliva (NEST)	2–4 × 1 mL	2000 × g, 10 min, 4 °C	Use stabilization buffer if provided	-80 °C
8.3.3.3	Saliva (Salivette)	2–4 × 0.25 mL	1000 × g, 2 min, 4 °C	Centrifuge swab in device	-80 °C
8.3.3.4	Dry Saliva Filter	N/A	N/A	Dry completely, store with desiccant	RT / -20 °C
8.3.4	Pleural Fluid	2–6 × 1 mL	2000 × g, 10 min, 4 °C	Sterile handling required	-80 °C

This table continues in the next page.

Protocol Section	Sample Type	Recommended Aliquots & Volume	Centrifugation Conditions	Special Additives / Notes	Storage Conditions
8.3.5.1	Urine (aseptic)	4–8 × 1 mL	2000 × g, 10 min, 4 °C	First morning urine preferred	-80 °C
8.3.5.2	24h Urine	10–20 × 1 mL	2000 × g, 10 min, 4 °C	Record total volume	-80 °C
8.3.5.3	Large Volume Urine (stem cells)	100–400 mL, pellet resuspended in cryomedium	400 × g, 10 min, RT	Cryomedium: 10% DMSO in DMEM	-80 °C → LN_2
8.3.6	Cystic Fluid	2–4 × 1 mL	2000 × g, 10 min, 4 °C	Sterile handling required	-80 °C
8.3.7	Tears	2–4 × 50–100 µL	N/A	Collect with sterile capillaries	-80 °C
8.3.8	Stool	2–6 × 200 mg	N/A	Collect into sterile fecal container	-80 °C
8.3.9.1	Skin (snap-frozen)	1–2 fragments (2–5 mm)	N/A	Use sterile scalpel	-80 °C
8.3.9.2	Skin (molecular)	2–4 × fragments	N/A	Store in RNAlater if required	-80 °C
8.3.9.3	Skin (cell culture)	2–4 × fragments	N/A	Process into cryomedium	-80 °C → LN_2
8.3.9.4	Skin Patches	1 per site	N/A	Sterile adhesive patches	-80 °C
8.3.10.1	Hair (DNA)	5–10 strands	N/A	Store dry, labeled	RT / -20 °C
8.3.10.2	Hair (keratinocytes)	2–4 × 1 mL cryovials (cells)	300 × g, 5 min, RT	Cryomedium: 10% DMSO in DMEM	-80 °C → LN_2
8.3.11.1	Water Samples	10–50 mL	N/A	Aliquot into cryovials	-80 °C
8.3.11.2	Air Samples	N/A	N/A	Collect on filters/plates	-80 °C
8.3.11.3	Surface/Vent Swabs	1 swab per site	N/A	With/without transport medium	-80 °C
8.3.11.4	Plant-Derived Samples (Leaves, Roots, Seeds)	Leaves: 3–5 × 0.5–1 gRoots: 3 × 0.5 g Seeds: 5–10 seeds	N/A	For DNA/RNA: stabilization solution or flash-freeze For dry: silica gel	Frozen: -80 °C / LN_2 Dry: RT with desiccant

This table continues in the next page.

Protocol Section	Sample Type	Recommended Aliquots & Volume	Centrifugation Conditions	Special Additives / Notes	Storage Conditions
8.3.12.1	Soluble Proteins	4 × 100 µL	2000 × g, 10 min, 4 °C	Add protease inhibitors immediately	-80 °C
8.3.12.2	DNA (Genomic or cfDNA)	4 × 100 µL	N/A	Low-EDTA TE buffer or nuclease-free water	-80 °C
8.3.12.3	RNA (mRNA, miRNA)	4 × 100 µL	N/A	RNase-free tubes, RNase inhibitor	-80 °C
8.3.12.4	Secretome (EVs, Metabolites, Lipids)	4 × 200 µL	2000 × g, 10 min, 4 °C	Isolation via ultracentrifugation or filtration	-80 °C
8.3.12.5	Lipids	4 × 250 µL	N/A	Store under inert gas, protect from light	-80 °C
8.3.12.6	Bacteria, Viruses, Fungal Spores	4 × 2 mL	Low speed centrifugation for isolates	Cryomedium: 10% glycerol in broth	-80 °C

Table 3. Summary of recommended reagents, consumables, and materials.

Reagent / Material	Purpose / Use	Suggested Brand / Reference	Section(s)
K_2EDTA Blood Collection Tubes	Anticoagulant tubes for plasma, whole blood, CBC	BD Vacutainer® K_2EDTA, Ref. 367525	8.3.1.1, 8.3.1.3, 8.3.1.6–8
K_3EDTA Blood Collection Tubes	Alternative anticoagulant for hematology	Greiner Bio-One Vacuette®, Ref. 454021	8.3.1.3
SST (Serum Separator Tubes, Red/Gold Cap)	Serum collection after clotting	BD Vacutainer® SST II Advance, Ref. 367955	8.3.1.2, 8.3.1.2.1
PAXgene® RNA Tubes	Whole blood RNA stabilization	Qiagen/PreAnalytiX, Ref. 762165	8.3.1.4
PAXgene® DNA Tubes	Whole blood DNA stabilization	Qiagen/PreAnalytiX, Ref. 761115	8.3.1.5
Fluoride-Oxalate Tubes (Gray Cap)	Glycolysis inhibition for glucose measurement	BD Vacutainer® Fluoride/Oxalate, Ref. 368920	8.3.1.3
Protease Inhibitor Cocktail (1×)	Protection of protein integrity in serum/plasma	Sigma-Aldrich®, P8340 or Roche Complete™	8.3.1.2.1, 8.4.1
Cryovials (2 mL Screw-Cap)	Sample aliquoting and storage	Sarstedt®, Ref. 72.694.406 or Nunc® CryoTube™	All sections
Cryoboxes	Organized cryostorage	Fisher Scientific™, Ref. 03-337-25	All sections
CoolCell® or Mr. Frosty Freezing Container	Controlled-rate freezing (–1 °C/min)	BioCision CoolCell® LX, Ref. BCS-405 or Nalgene® Mr. Frosty	8.3.1.8, 8.3.9.3
Physiological Saline (0.9% NaCl)	Washing red blood cells	Gibco™, Ref. 11533-027	8.3.1.7
Phosphate-Buffered Saline (PBS)	Washing and cell suspension	Gibco™, Ref. 10010-015	8.3.1.8, 8.3.9.3
Prostaglandin E1 (PGE1)	Inhibits platelet activation	Sigma-Aldrich®, Ref. P5515	8.3.1.8
RPMI 1640 Medium	Culture medium for pleural cells	Gibco™, Ref. 11875-093	8.3.4
Fetal Bovine Serum (FBS)	Supplement for cell culture	Gibco™, Ref. 10270-106	8.3.4, 8.3.9.3
Penicillin-Streptomycin (10,000 U/mL)	Antibiotic supplement	Gibco™, Ref. 15140-122	8.3.4, 8.3.9.3
RBC Lysis Buffer	Red blood cell removal	BioLegend®, Ref. 420301	8.3.4

This table continues in the next page.

Reagent / Material	Purpose / Use	Suggested Brand / Reference	Section(s)
Trypan Blue (0.4%)	Cell viability staining	Invitrogen™, Ref. T10282	8.3.4
RNAlater® Solution	RNA stabilization in tissues	Invitrogen™, Ref. AM7020	8.3.9.2, 8.4.3
DMEM (Dulbecco's Modified Eagle Medium)	Culture medium for tissue and fibroblasts	Gibco™, Ref. 41965-039	8.3.9.3, 8.3.10.2
Dimethyl Sulfoxide (DMSO)	Cryoprotectant for cells/tissue	Sigma-Aldrich®, Ref. D2650	8.3.9.3, 8.3.5.3
Schirmer Strips	Tear collection	Whatman®, Ref. WHA10548222	8.3.7
Desiccant Packs & Zip-Lock Bags	Dry storage of cards/filters	Fisherbrand™, Ref. 04-515-52	8.3.1.9–8.3.10.1
Filter Paper Cards (DBS/FTA)	Dried blood/plasma spots	Whatman® FTA Card, Ref. WB120205	8.3.1.9–8.3.1.10
Glycerol (≥99%)	Cryoprotectant for microbial/saliva samples	Sigma-Aldrich®, Ref. G5516	8.3.3.2
Trypsin-EDTA Solution	Cell detachment (keratinocytes)	Gibco™, Ref. 25300-054	8.3.10.2
Neubauer Chamber / Hemocytometer	Cell counting	BRAND®, Ref. 717805	8.3.4
Bioanalyzer / TapeStation Reagents	DNA/RNA integrity check	Agilent Technologies®	8.4.2–8.4.3
BHT (Butylated Hydroxytoluene)	Antioxidant for lipid extraction	Sigma-Aldrich®, Ref. W218405	8.4.5
Chloroform, Methanol, Isopropanol	Lipid extraction solvents	Sigma-Aldrich® HPLC grade	8.4.5
Ultracentrifuge Tubes (Polypropylene)	Extracellular vesicle isolation	Beckman Coulter®, Ref. 344057	8.4.4
Sterile Swabs (Flocked or Cotton)	Environmental or microbial collection	Copan FLOQSwabs®, Ref. 520C	8.3.11.3, 8.4.6
Biosafety Cabinet (Class II)	Microbial sample handling	Thermo Scientific™ 1300 Series A2	8.4.6
qPCR / Sequencing Kits (for microbes, DNA/RNA)	Molecular downstream analysis	Qiagen®, Illumina®, Thermo Fisher™	8.4.2–8.4.6
Sterile Scissors or Scalpel	Cutting plant tissues (leaves, roots, seeds)	Fisher Scientific™ or VWR	8.3.11.4

This table continues in the next page.

Reagent / Material	Purpose / Use	Suggested Brand / Reference	Section(s)
Sterile Forceps	Handling plant samples	Fisher Scientific™	8.3.11.4
Whirl-Pak Bags	Dry preservation of plant samples	Nasco Whirl-Pak®	8.3.11.4
Silica Gel	Rapid desiccation for dry storage	Sigma-Aldrich®	8.3.11.4
RNA/DNA Stabilization Solution	Preserve nucleic acids in plant tissues	Invitrogen™ RNAlater®	8.3.11.4, 8.3.12.3
Labels and Waterproof Marker	Sample identification	Brady® or Fisherbrand™	8.3.11.4
RNase-Free Tubes	RNA handling for indirect samples	Ambion® or Thermo Fisher™	8.3.12.3
Ultracentrifuge	Isolation of extracellular vesicles	Beckman Coulter® Optima™	8.3.12.4
Organic Solvents (Chloroform, Methanol, Isopropanol)	Lipid extraction	Sigma-Aldrich® HPLC grade	8.3.12.5
BHT (Butylated Hydroxytoluene)	Antioxidant for lipid preservation	Sigma-Aldrich®, Ref. W218405	8.3.12.5
Biosafety Cabinet (Class II)	Safe handling of microbes	Thermo Scientific™ 1300 Series A2	8.3.12.6

HRE Exploration Biobank

Deliverables [D-01] and [D-02]

[D-01]: Mandatory standardised protocols and SOPs for ESA-directed research samples.

[D-02]: Recommended protocols for enabled research samples.

SOP

- SOP.BIO/ESA.001 – Collection and Transportation of Biological Samples
- SOP.BIO/ESA.002 – Processing and Storage of Biological Samples
- SOP.BIO/ESA.004 – Shipment of Biological Samples to GIMM Biobank in European Space
- SOP.BIO/ESA.005 – DNA Extraction from Biological Samples
- SOP.BIO/ESA.006 – RNA Extraction from Biological Samples
- SOP.BIO/ESA.007 – Peripheral Blood Mononuclear Cells Isolation
- SOP.BIO/ESA.008 – Establishment of Primary Fibroblast Cell Culture from Skin Biopsies

SOP.BIO/ESA.004.V2 – SHIPMENT OF BIOLOGICAL SAMPLES TO GIMM BIOBANK IN EUROPEAN SPACE

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DATE: 12-12-2025

APPROVAL DATE:

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VERSION CONTROL

Number of Version	Date of Version	Summary of Changes
V1	19-09-2025	Original version
V2	12-12-2025	Added guidelines for sending institutions to share cryobox listing per transportation box; minor text updates

This document will be reviewed and updated if any quality control issues are identified during operations, or if the company contracted for the shipment of the samples is changed.

SOP.BIO/ESA.004.V2 – SHIPMENT OF BIOLOGICAL SAMPLES TO GIMM BIOBANK IN EUROPEAN SPACE

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7. ANNEXES **8**

1. SCOPE

This Standard Operating Procedure (SOP) provides guidance for the shipment of human-direct, human-indirect and non-human biological samples from the ESA sites in European space to GIMM Biobank. It ensures that all participating research centres prepare, pack, document, and ship human-direct biological materials (e.g., blood, urine, saliva, stool, hair, skin patches), human-indirect biological samples (e.g. *in vitro* cultured samples, microbiology samples), and non-human biological samples (e.g., environmental or habitat samples such as plants, water, air and surface swabs) under standardized, safe, and traceable conditions, using temperature control systems and the services of the company contracted for the shipment of the samples Cencora, Inc. (from now on referred as World Courier). This SOP applies exclusively to the shipment of samples that fall within the categories: category B Biological Substances (UN3373) and/or dry ice (UN1845). If additional sample types or classifications are to be included in the future, this SOP must be revised accordingly, or a separate SOP shall be created.

This SOP should be read in conjunction with the SOP for *Process Documentation for Ingestion and Dissemination of Samples* (SOP.BIO/ESA.003), which provides essential context for the application of the present procedures.

2. SAFETY INFORMATION

All biological samples must be considered potentially infectious. Appropriate personal protective equipment (PPE) must be used during sample handling and packaging. Shipping must comply with international regulations for Biological Substance Category B (UN3373) and, when applicable, Dry Ice (UN1845). Adequate training in sample handling and transport regulations is mandatory.

3. RESPONSIBILITIES

Sending Institution

- Submit required documents for the Ingestion request within the deadline (see SOP.BIO/ESA.003) and correctly report samples to be shipped to GIMM Biobank.
- Correctly prepare samples for shipment: primary labelling, primary packaging, and sealing with the Site tamper-proof seal.
- Ensure that samples are delivered to the correct courier and confirm that all documents have the correct destination and recipient (see Section 4.6).
- Notify the GIMM Biobank that samples have been shipped and provide a file specifying which sample cryoboxes were included in each transportation box.

GIMM Biobank

- Book incoming shipments.
- Confirm receipt of the shipment to the Sending Institution.
- Verify shipment integrity.
- Notify the Sending Institution and the company contracted for the shipment of samples of any discrepancies, damage, or other issues identified (with description and photographs), upon receipt, within 24 hours.
- Ensure appropriate storage upon arrival according to all applicable protocols, temperature requirements and established documentation.

World Courier (Company contracted for the shipment of the samples)

- Provide appropriate secondary and tertiary packaging, external labelling and temperature controlling materials (dry ice or refrigeration units).
- Ensure continuous maintenance of the specified temperature throughout transit.
- Equip shipment with necessary temperature monitoring devices and ensure accurate temperature records are logged every hour.

- Generate and share a tracking number, to ensure full traceability of the shipment.
- Ensure that samples are collected on the agreed date and delivered to the designated recipient within the agreed timeframe.
- Ensures full compliance with Privacy and Data Protection rules applicable worldwide.
- Promptly report any delays or changes in the pickup or delivery schedule to both the Sending Institution and GIMM Biobank.

4. PROCEDURE

4.1 Shipment Scheduling

- The sending institution must submit the ingestion request at least 15 calendar days prior to the expected shipment of samples, via email or the online form (see SOP.BIO/ESA.003).
- The sending institution must transfer the completed Sample Information Form (SIF) no later than 5 working days prior to the expected shipment of samples (see SOP.BIO/ESA.003).
- The GIMM Biobank schedules the pickup of the samples at the ESA site for a Monday at 15:00. If Monday is a public holiday in the country of the sending institution, the pickup is schedelued for the next working day.

4.2 Shipping Method

4.2.1 Courier Service

World Courier has been selected by the GIMM Biobank as the courier service under the HRE Exploration Biobank. World Courier is a certified international courier service with demonstrated compliance with international regulations and conventions applicable to any mode of transport for the transport of Category B Biological Substances (UN3373) and dry ice (UN1845).

All shipments of samples that are classified under UN3373 and/or UN1845 shall be conducted via World Courier. Given World Courier's global infrastructure and regulatory compliance, no formal backup courier service is deemed necessary at this time.

4.2.2 Regulatory Classifications

UN3373 – Biological Substance, Category B

Definition: Biological substances that contain or are reasonably expected to contain pathogens, including microorganisms (including bacteria, viruses, rickettsiae, parasites, fungi) or other agents such as prions, that can cause disease in humans or animals but do not meet the criteria for inclusion in Category A (Infectious Substances).

Packaging Requirements: Must be packaged in compliance with Packing Instruction P650 (ADR/IATA), which specifies triple packaging (primary receptacle, secondary packaging, outer packaging) with appropriate absorbent material and labeling. Refer to section 4.3.1 for detailed information.

Labeling: Packages must bear the diamond-shaped "Biological Substance, Category B" mark with "UN3373". Refer to section 4.3.3 for detailed information.

UN1845 – Carbon Dioxide, Solid (Dry Ice)

Definition: Dry ice used as a refrigerant for the shipment of temperature-sensitive biological materials.

Packaging Requirements: Dry ice must be placed outside the secondary packaging but within the outer packaging to allow the release of gas and prevent pressure build-up. The packaging must permit the venting of carbon dioxide gas.

Labeling: The outer package must be marked with "UN1845" the proper shipping name "Carbon dioxide, solid" or "Dry Ice," and the net weight of dry ice contained. A Class 9 Miscellaneous Dangerous Goods hazard label is required. Refer to section 4.3.3 for detailed information.

4.2.3 Temperature Control

Shipments may be transported under the following temperature conditions as required:

- Ultra-low Temperature (Dry ice) (<-60 ºC)
- Frozen Controlled Temperature (-35 ºC/-20 ºC)
- Refrigerated Controlled Temperature (+2 ºC/+8 ºC)
- Ambient Controlled Temperature (+15 ºC/+25 ºC)
- Ambient Non-Controlled Temperature (when appropriate for the sample type)

4.3 Packaging Requirements

4.3.1 Triple packaging complying with UN3373 Classification

- Primary container: leak-proof tubes (*e.g.*, cryotubes) inside cryoboxes.
    - Supplied by: Sending institution
    - Packaging by: Sending institution
    - Sealed with site-sender tamper-proof seal by: Sending institution
- Secondary container: sealed biohazard specimen bags with absorbent material.
    - Supplied by: World Courier, when the samples are to be picked up.
    - Packaging by: Sending institution
- Tertiary container: the outermost container, selected according to the transport temperature requirements and the quantity of samples.
    - Supplied by: World Courier, when the samples are to be picked up.
    - Packaging by: Sending institution. World Courier personnel may assist with dry ice replenishment or handling of temperature loggers, but they do not handle or manipulate the samples directly.

4.3.2 Controlled Temperature Packaging

The appropriate shipping containers (secondary and tertiary), will be provided by World Courier as follows:

- For frozen samples requiring the use of dry ice: insulated polystyrene boxes (GDI System) will be provided, as well as dry ice. The quantity of dry ice will depend on the size of the box used.
- For frozen/refrigerated/ambient controlled temperatures: containers made of polypropylene copolymer with vacuum insulated panels (GTC System) will be provided.

4.3.2 Temperature Monitoring and Registry

Temperature Monitoring and registry are supplied by World Courier. Temperature data loggers, used in controlled temperature shipment, record continuously at configurable intervals, which can be set to

one hour or less. These time-stamped acquisitions ensure full traceability and verification of temperature compliance throughout the transport.

4.3.3 Labels

Labels are supplied and placed on the tertiary container by World Courier.

UN3373 - "Biological Substance, Category B", as shown in **Fig.1**.

Figure 1. UN3373 Label.

UN1845 - "Dry Ice", including net weight and hazard symbol, as shown in **Fig.2**.

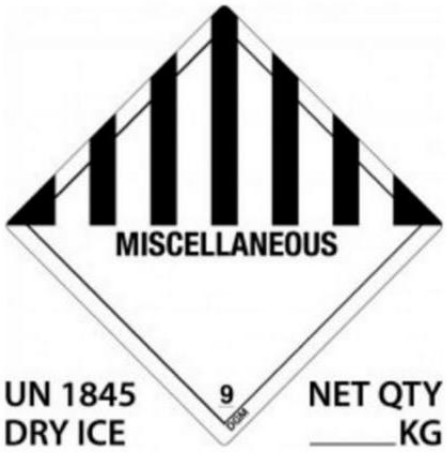

Figure 2. UN1845 Label

4.5 Required Documentation

Include with the shipment a duplicate of the "ESA Sample Ingestion Form" (annexed to this SOP)

4.6 Address of the GIMM Biobank

Confirm samples are addressed to the GIMM Biobank:

Institution: Fundação GIMM – Gulbenkian Institute for Molecular Medicine

Biobanco GIMM

Address: Edifício Egas Moniz

Avenida Professor Egas Moniz, Nº 28

1649-028 Lisboa, Portugal

Email: biobank@gimm.pt/ logistics@gimm.pt

Phone: 00351217999411/00351217999409

Emergency Phone: 00351965152588

Contact person: Ângela Afonso

5. QUALITY CONTROL

In case of spillage, delay or temperature deviation, the shipping company is responsible for:

- Informing GIMM Biobank without undue delay (and not later than 24 hours) after identification.
- Upon client request, the shipping company (Service Provider) shall provide a complete investigation report and corrective action plan within twenty-eight (28) calendar days

Note: All losses, thefts or destructions are risks to be noted and should be reported without undue delay.

Any deviation to this SOP must be documented in the Laboratory information management system (LIMS) of GIMM Biobank with appropriate annotations to maintain traceability and protocol compliance.

6. RECORDS GENERATED BY THIS PROCEDURE

Records Identification	Indexation	Archive Responsible
ESA Sample Ingestion Form	GIMM - ESA SharePoint	Ana Rita Cruz, GIMM Project Manager
Certificates from World Courier	GIMM - ESA SharePoint	Ana Rita Cruz, GIMM Project Manager
Complete Investigation Report from World Courier (if applicable)	GIMM - ESA SharePoint	Ana Rita Cruz, GIMM Project Manager
Deviations to the SOP	LIMS	José Maximino, GIMM Biobank Data Manager

7. ANNEXES

- ESA Sample Ingestion Form

ESA Sample Ingestion Form

Filled by GIMM Biobank	
Request entry date:	
Request reference:	
Date of evaluation by the scientific committee:	
Request submission date:	
Transport Temperature	
Temperature Monitor	

1. STUDY OR PROJECT IDENTIFICATION

Study/Project Identifier (e.g., VIVALDI III study): _____

2. SENDING INSTITUTION / ORIGINATING SITE

Institution Name: _____

3. RESPONSIBLE CONTACT PERSON

Name: _____

Email: _____

Phone: _____

4. SHIPMENT INFORMATION

Requested Shipment Date*: ____ / ____ / _____

** It must be a Monday, unless it is a holiday in the country of the Sending Institution*

5. SAMPLE INFORMATION

Sample Description: _____

Sample Category: ☐ Human Biological Samples ☐ Indirect-Human Biological Samples ☐ Non-Human Biological Samples ☐ Other: _____

Sample Type: ☐ Blood ☐ Urine ☐ Stool ☐ Saliva ☐ Hair ☐ Cells☐ Organoids ☐ DNA ☐ RNA ☐Other _____

Transport Temperature: ☐ Dry Ice ☐ Refrigerated ☐ Room Temperature

Quantity / Volume: _____

6. ASSOCIATED MANDATORY DOCUMENTATION

- Sample Information Form (SIF): ☐ Yes ☐ No
- Blank Copy of the Consent Form Template: ☐ Yes ☐ No
- Statement confirming that informed consent was obtained in accordance with legal and ethical regulations: ☐ Yes ☐ No

Please make sure the listed documents are attached to this form

7. ASSOCIATED OPTIONAL DOCUMENTATION

- Material Transfer Agreement (MTA): ☐ Not applicable ☐ Included ☐ In preparation
- Other documents: _____

Please make sure the listed documents are attached to this form.

Signature and Date

_____

Signature of Sending Responsible Person