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Molecular Medicine Ireland Guidelines for Standardized Biobanking

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Background to Molecular Medicine Ireland

Molecular Medicine Ireland (MMI) is a research partnership in molecular medicine formed by the National University of Ireland Galway, the Royal College of Surgeons in Ireland, University College Cork, University College Dublin, and Trinity College Dublin and their associated academic hospitals to build a sustainable national system to coordinate, support, and promote translational and clinical research. Molecular Medicine Ireland was formally established as a not-for-profit company in April 2008, through funding from the Irish Government's Higher Education Authority's (HEA) Programme for Research in Third Level Institutions (PRTLI) Cycle 4.

Introduction

Progress in efforts to develop novel treatments for complex disease is driven by the availability of large sets of well-documented, up-to-date, epidemiological, clinical, biological and molecular information and corresponding material from large numbers of patients and healthy persons. Biobanks of human biological material are recognized increasingly as major assets in disease research. As medical research moves from the study of simple monogenetic disorders to the investigation of complex diseases, the availability of biological materials will determine our ability to dissect out the interplay of genetic and other factors that contribute to complex polygenic diseases.[1] In order to investigate the complex and often subtle effects of genes on health and disease, there is a need to study information and samples from a large cohort of people.[2–5] Typically, researchers will study patient groups with the same disease or trait of interest. The data generated are then compared with samples from a group of "normal/healthy" people. By comparing information from each of these groups, we can discover more about the genes involved in disease.

The availability of large, well phenotyped, standardized biobanks is an increasingly important driver of translational and clinical research programs as it:

- Facilitates cutting edge disease research.
- Creates the critical infrastructure, to partner with genetics and biotechnology industries.
- Provides valuable datasets for additional health research.
- Assists investigators in leveraging additional research funding.
- Avoids duplication of resources and effort.
- Provides significant opportunities for international collaboration to enhance research program outputs

Molecular Medicine Ireland (MMI) has recognized the importance of a strategic approach to standardized biobanking as a key pillar for clinical and translational research in Ireland. To this end MMI, in association with Queen's University Belfast and University of Ulster put together the design phase of GeneLibrary Ireland, an all-island control bioresource, to be developed jointly in two jurisdictions—Ireland and Northern Ireland. GeneLibrary Ireland, as proposed would be an all-island reference library of biological specimens from healthy volunteers with linked health and lifestyle information to provide a shared control group for a wide range of studies by health and biomedical scientists.[6] A number of European countries have already established well-organized population-based, disease-oriented and/or case-control bioresources. These biobanks, include HUNT in Norway,[7] the UK Biobank,[8] the Estonian Population Biobank,[9] Icelandic deCode biobank,[10] and Generation Scotland.[11] The UK DNA Banking Network undertakes ISO 9001–2000 management on behalf of over 30 large disease-specific collections.[12] There are 123 large population-based cohorts described in the catalogue of the global biobanking project, the P3G Consortium.[13] Typically these biobanks contain data on health, environmental risk factors, nutrition, demographic, socioeconomic, and lifestyle variables pertaining to corresponding biospecimens from healthy and disease populations. While these biocollections have tremendous value at a national level, the exchange of data and material across national boundaries has proved difficult due to variations in legislation and ethical issues. This has contributed, in Europe for example, to the fragmentation

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of its biobanking activities.[14] There is no doubt that these currently established biobanks and biomolecular resources are a unique European strength, but the ethical and legislative barriers to amalgamating these national bioresources, which is required for statistically powered studies, has been a significant limiting factor.

If Europe is to realize the full research potential from human biobanks, there is a need for convergence, coordination and harmonization of the biobanking and biomolecular resources infrastructure across Europe. In an effort to address this challenge, the European Commission, through Framework Program 7 (FP7), has funded the preparatory phase of the Biobanking and Biomolecular Resources Infrastructure (BBMRI) project. BBMRI is being funded to establish a pan-European biobanking network through the coordination and harmonization of the biomolecular resource infrastructure, including population-based cohorts, disease-based cohorts, twin registries and clinical case/control studies. Networking and harmonization of biobanking across Europe will increase the success of coordinated, large-scale biomarker discovery and validation; facilitate the identification of susceptibility genes and their association with environment and lifestyle factors; elucidate etiological pathways for multifactorial diseases and facilitate discovery of new drugs and therapies.[15,16]

A major challenge in the convergence of biobanks established in different countries and institutions and by different groups is to promote the harmonization of collection and processing methodologies. This harmonization is critical if samples from different collections are to be analyzed and the data generated compared. Once biobanks in different jurisdictions are harmonized, they will be able to address the question of which procedures require standardization. In this publication, a set of integrated procedures for sample collection and processing to address this critical issue is proposed.

In order to harness the immense potential and power of well-annotated biological materials, a strong focus is needed on the harmonization and quality of biological samples across biobanks internationally. A number of international organizations including ISBER, WHO-IARC and OECD have developed guidelines based on best practices that address key elements for the establishment, management, organization and governance of bio-repositories which have significantly advanced biobanking.[17–21] The adoption of standardized management procedures will ensure the consistency, integrity and quality of biological materials across individual biobanks so that the molecular changes identified in subsequent adequately powered research studies reflect biology and not process or sample variability particularly as increasingly sensitive diagnostic tests and experimental platforms become available. This technological progress means there is an increasing need for careful preparation and maintenance of biological specimens. As a first step towards the development of detailed interoperable procedures to ensure harmonization of biological materials across jurisdictions, MMI has developed guidelines for the collection, processing and storage of biological specimens that stem from the design phase of GeneLibrary Ireland. The use of standardized protocols for sample collection, processing, and storage will help to provide the proper safeguards and assurances required for sample quality, consistency, and integrity among biocollections at different sites. This harmonization will facilitate the global movement of biological materials across research sites and the aggregation of samples for research studies. This will expedite high-quality research and reduce its costs.

These guidelines do not specifically address the ethical and legal issues that are fundamental to the collection and storage of biological materials from human subjects for research purposes and their international exchange across jurisdictions with differing legislative frameworks. These issues have been addressed in detail in international best practice guidelines, in the report of the design phase of GeneLibrary Ireland, and are the focus of BBMRI's Ethical, Legal and Societal Issues Working Group.[6,18–22] These guidelines provide general principles for obtaining informed consent for use of human biological samples for research purposes that is at the cornerstone of research involving human subjects. All studies involving human biological materials require approval from an ethics committee. While these guidelines do not address the process required for ethical and regulatory approval, it is necessary that appropriate ethics and regulatory approvals be in place, based on national regulations prior to initiation of any study involving human biological materials.

The MMI Guidelines have been drafted with reference to international best practice guidelines in biobanking, including validation studies conducted by the UK Biobank and reflect the collective experience of MMI partners engaged in biobanking.[17–21,23,24] The adoption of these guidelines by the biobanking community will serve as an important first step towards a more structured framework to standardize the collection, processing and storage of biological specimens. Adoption will ensure the consistency of high-quality samples harmonized across sites, creating one of the pillars of BBMRI's vision of a pan-European network of biobanks, which in turn will allow free movement of knowledge and biological materials between scientists across borders.

These guidelines are intended to be evolutionary in nature and will be reviewed in a timely fashion and revised in light of experience gained and developments in best practice for biobanking.

Scope

The scope of these guidelines is to provide guidance to standardize procedures for the collection, processing, and storage of biological materials (including blood, urine, DNA, RNA, cells, and tissue) that can be used across different clinical and research centers.

These guidelines are divided into three parts pre-clinical, clinical, and laboratory standard operating procedures.

PART I: PRE-CLINICAL STANDARD OPERATING PROCEDURES

1.1 Principles of Informed Consent

1. Obtaining informed consent from research participants for all research and clinical studies will be conducted in compliance with Good Clinical Practice, the Declaration of Helsinki, and relevant Irish and European legislation.
2. Suitably qualified research personnel should obtain informed consent from research participants.
3. The research participant must be deemed competent to give true written informed consent prior to initial involvement in any research or clinical study conducted. A provision for a parent/legal guardian to provide consent may be necessary dependant on the participant's age or in the case of an incapacitated participant and in accordance with the relevant legislation.
4. The research participant will not be coerced in any way by research personnel to participate in a research project or clinical trial and it will be made clear that he/she may decline to take part in and have the right to withdraw at any time from the research project or clinical trial. It will also be made clear that the research participant will have the right to withdraw their consent to the use of his/her samples and data at any time. The research participant will be advised that it is not possible to withdraw data that has already been accrued and analyzed on him/her but that no new data will be generated and that his/her remaining samples will be destroyed provided that samples and data have not been annonymized.
5. If the research participant is a hospital inpatient or outpatient, it will be stressed that if he/she declines to participate in or withdraw from a research project or clinical trial, it will not affect any future treatment or care that he/she receives.
6. Research personnel will allow the research participant sufficient time to reflect on the implications of participating in the research project or clinical trial.
7. Research personnel will allow potential research participants to give informed consent by ensuring that they understand the following:

   - the research has been approved by a recognized research ethics committee
   - the purpose of the research
   - the practicalities and procedures involved in participating in the study
   - the benefits and risks of participation and the alternative therapies
   - their role if they agree to participate in the research
   - that their participation is voluntary
   - have the right to withdraw from the study at any time, without giving any reason and without compromising their future treatment
   - the informed consent form
   - the patient information leaflet
   - the amount and nature of tissues, organs, or body fluids which will be taken
   - that permission for access to their medical records may be required
   - how data about them will be stored and published
   - how information will be provided to them throughout the study
   - how clinically relevant results will be fed back to them
   - how individual research results will be not be communicated to participant but rather as cumulative group research findings
   - that their samples and data will be protected by a unique study identification code and information stored in a password protected database
   - that records identifying the research participant will be kept confidential
   - the insurance indemnity arrangements for the conduct of the research is in place where appropriate
   - that their data will be stored in accordance with the Data Protection legislation
   - who will have access to their samples and data and how access will be managed
   - whether there is any potential for commercialization from this research
   - the sponsor and funding body of the research
   - contact details, should they have further questions or want to withdraw

8. The scientific potential of samples and data can often only be fully realized if their use is not confined to individual research projects specifiable in advance. Consideration should be given to the use of the model of broad consent that is used in a number of international biobanks where research participants will be asked to give generalized consent to the use of their samples and data for the purposes of medical, including genetic research and including future, unspecified use. This model of consent recognizes the significant pace at which research develops and avoids research participants having to be re-contacted for each research study involving their biological material. These research studies will have ethical oversight. The use of a standardized consent form across difference sites would be of considerable value.
9. A patient information sheet about the research or clinical study will be given to the research participant to keep. Research personnel will ensure that the research participant has the opportunity and appropriate time to read and consider the patient information leaflet.
10. The research participant will be given every opportunity to ask questions prior to providing written informed consent.
11. Research personnel will ensure that all fields on the research or clinical study informed consent form have been completed. All signatures will be accompanied by the printed name of the signatory and the date will also be recorded.
12. The appropriate responsible person, for example the principal investigator will review and sign all informed consent forms at his or her convenience.
13. A copy of the signed informed consent form will be made available for the research participant to take away with him/her or will be forwarded to the research participant when available.
14. The name and signature of the person obtaining informed consent will also be recorded in the participants file along with a copy of the signed informed consent form.

1.2 Guiding Principles for Quality Assurance in Biological Repositories

A biological repository should be established, governed and managed in accordance with the highest scientific, ethical and legal standards to protect participant's privacy and confidentiality. The recommended principles and best practices are covered in detail in a number of International Best Practice Guidelines, including the OECD, ISBER, NCI and IARC.[17–21] This section provides an overview of guiding principles for quality assurance taken from these International Best Practice Guidelines for consideration by those managing biological repositories.

1. Quality Management System: International Best Practice recommends biorepositories to establish, document, and implement a quality management system and commit to a quality assurance program. A quality improvement system should be in place to continuously improve the efficiency and effectiveness of the quality management system. The appointment of a Quality Manager is recommended to develop, implement, and maintain the quality management system. However if it is not possible to have a formal quality management system with dedicated staff, a process should be in place to document and review procedures and assess the quality and efficiency of the operation of the biorepository to ensure that all biospecimens are handled uniformly.
2. Standard Operating Procedures (SOPs): A biorepository should develop and implement standardized operating procedures to provide written detail for all processes relating to sample handling to ensure uniformity, quality, and reproducibility. SOPs should be reviewed accordingly to defined timelines to ensure that the procedure is current. A system should be in place to update, approve, and adopt SOPs and to ensure that the current version is in use.
3. Infrastructure, Storage Facilities and Environment: The ISBER International Best Practice Guidelines provides significant detail in relation to the appropriate facilities and the storage equipment and environment for the establishment and operation of a biorepository. Policies, procedures and schedules should be developed for equipment inspection, maintenance, repair and calibration according to the manufacturers' instructions for use. A system should also be in place to record daily operations and incidents either using logbooks or an electronic system.
4. Tracking Informed Consent: A system should be in place to link the informed consent to the biospecimen to ensure that its future use is consistent with the original consent. In addition a mechanism should be in place to facilitate the identification and destruction of all unused biospecimen when consent is withdrawn.
5. Choice of Method: Consideration should be given to the analytes under investigation and downstream analysis when choosing the collection, processing, and storage methods. It may also be important to consider which anticoagulant and/or stabilizing agents are acceptable given the particular analyte under investigation. The methods chosen should aim to preserve the greatest number of analytes.
6. Recording of Methods: The SOPs used should be recorded in the study specific documentation or the data management system and any deviations or nonconformances that occur should be recorded.
7. Recording of Data: The data to be recorded for each specimen type and study protocol should be identified and the procedure for recording this data defined in the study specific documentation or data management system.
8. Pilot Study: Biorepositories should consider conducting a small-scale pilot study/proof of performance study when implementing new protocols, validating new processing equipment or laboratory procedures for sample collection, processing, and storage.
9. Personnel Training: The competency of all staff to perform tasks according to SOPs should be verified on a regular basis (e.g., annually) and in accordance with local policy and procedures. Personnel should also be trained in the possible

biohazards for working with potentially pathological specimens. A system should be in place to document training records for all staff.

10. Recording of Time: The time of sample collection, processing, and storage should be recorded for all specimens in a clear legible format in the study specific documentation or data management system in accordance with local policy and procedures. If tissue samples are to be collected, the time of ischemia should also be recorded. This will allow confirmation that the sample(s) has been collected, processed, and stored within the timelines defined in the SOP or alternatively to record the variation in time outside that stipulated in the SOP as it may affect the results in downstream analysis.
11. Identification of Biospecimens: Each biospecimen container should have a unique identifier and/or combination of identifiers affixed that can withstand the storage conditions and facilitate efficient retrieval. These should be documented in the inventory tracking system.
12. Data Management System: The development of a centralized, well-planned integrated, secure, interoperable and compliant information management platform is integral to the efficient operation of a biorepository. The information management system should support all aspects of the bioresource including required levels of security; participant enrollment and informed consent; collection and storage of phenotypic data; biospecimen collection, processing, storing, tracking and dissemination; quality control and quality assurance; documentation management; reporting and return of data, including "omics" data.
13. Biospecimen Tracking: A system either paper or electronic, such as a Laboratory Information Management System should be in place to facilitate the tracking of biospecimens from collection to processing, storage, retrieval and shipment from a biorepository.
14. Biospecimen Storage: SOPs should be applied consistently to ensure biospecimens are stored uniformly. Appropriate sample size aliquots should be used to avoid thawing and refreezing of biospecimens. The use of inventory tracking methods will minimize the disruption of the stabilized storage environment during sample retrieval. Care should also be taken to use storage containers and labels that have been validated to withstand the required storage temperature and duration.
15. Storage System Monitoring: Automated security systems are available that continually monitor storage equipment and produce an alarm in the event of freezer failure. An alarm response procedure should be in place and tested on a regular basis. Detailed debriefing should be held after any incident to identify possible preventive actions and to improve emergency responses. A back-up system should be in place such as an alternative power supply that is automatically activated when necessary.
16. Shipping Conditions: Packing and shipping of biospecimens should conform to all governing regulations. The ISBER Best Practice Guidelines and WHO Guidance on regulations for the Transport of Infectious Substances 2009–2010 contain detailed guidelines with regard to shipment of biospecimens including, regulation of sample temperature during shipping, verification of packaging, material transfer agreements, documentation required, shipment log, labeling and regulatory requirements. The International Air Transport Association regulations 2006 should be consulted for guidelines with regard to classification and shipment of samples by air internationally.[20,25,26] Personnel should be trained appropriately in handling specimen shipment and training should be documented.
17. Quality Control: Points for critical checks in the process should be identified and the quality control measure to be performed should be defined in a SOP.
18. Quality Checks: International Best Practice recommends that a representative sample of a biorepository be checked annually including assessment of specimen quality, electronic/paper records and storage location. This check should include:

    - Physical verification of the specimen location and of the durability of the storage container
    - Verification of annotation of specimens and data records
    - Formalized quality control check should be developed to verify sample quality, biological activity and integrity, for example extraction and analysis of DNA, RNA and other biomolecules should be conducted. The Canadian Tumour Repository Network has developed standardized procedures to evaluate the quality of nucleic acids such as DNA and RNA.[27]
    - Confirmation of use of SOPs for sample collection, processing, and storage processes and verification that they have been adhered to.
    - Quality control results should be recorded, feedback obtained and inputted into the continuous improvement process and made available for examination upon request for audit.

19. Access to Samples and Return of Data: A biorepository should have clearly defined policies and procedures with regard to access to biospecimens and return of data which is in keeping with its core objectives and overall ethics approval.
20. Certification of Biorepositories: ISO9001:2000 *Requirements of Quality Management Systems* is the recognized international quality standard that biorepositories are working to implement.

SOP 1.3 Assessment of the Research Participant

SOP Number: 1.3
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for assessment of the research participant.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. The research personnel will greet the research participant in the waiting area, identify themselves, and then escort the research participant to the interview room.
2. The research participant will be correctly identified by their name and date of birth.
3. Assessment of research participants will be conducted as per the relevant study protocol.
4. The research personnel will explain the assessment procedure to the research participant.
5. Current information with regard to the research participant's medical history and medications will be documented at appropriate visits in accordance with the study specific protocol.
6. The research personnel will decide following assessment, the research participant's suitability for enrolment in a particular research or clinical study.
7. The research personnel will then discuss the informed consent process with research participants (see section 1.1 Guiding Principles of Informed Consent)

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 1.4 Safety Guidelines
SOP 1.4.1 Safety Guidelines for Blood Collection

SOP Number: 1.4.1
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for safe blood collection.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. Research personnel will have completed the appropriate training and be deemed competent in the procedure of venipuncture prior to any blood collection in accordance with local policy and procedures.
2. Research personnel will have been deemed competent to respond in the event of fainting or any other adverse event during or after the blood collection procedure.
3. Research personnel will greet the research participant, identify themselves, and then explain the blood collection procedure to the research participant.
4. The research participant will be approached in a friendly calm manner and their cooperation will be gained prior to blood collection.
5. The research participant will be correctly identified prior to blood collection by asking them to give their name and date of birth.
6. The research participant will be positioned safely and comfortably in the chair/couch provided for venipuncture, ensuring that the protective arm is in the correct position to support the research participant in the event of fainting or any other adverse event.
7. The research participant's mouth will be free from food or gum prior to venipuncture.
8. All sample containers and equipment needed to competently and efficiently carry out the venipuncture will be assembled prior to the procedure.
9. Research personnel will wear gloves at all times during venipuncture.
10. Research personnel will use appropriate barrier protection, such as gloves, gowns, masks, and protective eyewear to prevent exposure to skin and mucus membranes when working with known infectious research participants.
11. Research personnel will ensure that needles should never be broken, bent or recapped.
12. Research personnel will take care to prevent needle stick injuries when using and disposing of needles. Local policy and procedures should be followed in the event of a needle stick injury.
13. Blood collection tubes will not be labelled in advance of venipuncture.
14. A unique study identification number and/or bar-coded label will be applied to all blood samples immediately after venipuncture. The time of collection will also be recorded in the study specific documentation or data management system.
15. A puncture-resistant incineration container will be placed as close to the use-area as practical.
16. Using gloves, blood spillages will be covered with Milton or "Presept" granules, mopped up with paper towels and discarded into puncture-resistant incineration containers.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 1.4.2 Safety Guidelines for Urine and Feces Collection

SOP Number: 1.4.2
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for safe collection of urine and feces.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. Research personnel will have been deemed competent in the procedure of urine and feces collection.
2. Research personnel will wear gloves at all times during specimen collection.
3. Research personnel will use appropriate barrier protection, such as gloves, gowns, masks, and protective eyewear to prevent exposure to skin and mucus membranes when working with known infectious research participants.
4. Specimen containers will not be labelled in advance of urine/feces collection.
5. A unique study identification number and/or bar-coded label will be applied to all specimens immediately after collection. The time of collection will also be recorded in the study specific documentation or data management system.
6. Research personnel will ensure that the lids of the specimen containers are securely replaced so that leakage does not occur during transport.
7. Using gloves, all spillages will be covered with Milton or "Presept" granules, cleaned up with paper towels and discarded into puncture-resistant incineration containers.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 1.4.3 Safety Guidelines for Handling of Biological Materials

SOP Number: 1.4.3
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for safe handling of biological materials.

Responsibility
It is the responsibility of all research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. Research personnel will be aware of the correct procedures for the handling of biological materials.
2. All research personnel will have received the appropriate immunizations as per local policy and procedures prior to working with potentially infectious materials. Regular controls of immunization status will be performed as per local policy and procedures.
3. All biological materials collected will be treated as being potentially infectious for blood-borne diseases regardless of their known infectious status.
4. Universal precautions will be applied to all blood, body fluids, and tissue specimen collections, regardless of their infectious status even when they do not contain visible blood.
5. Research personnel will use appropriate barrier protection, such as gloves, gowns, masks, and protective eyewear to prevent exposure to skin and mucus membranes when working with biological materials.

6. Gloves will be changed after the handling of each biological material or when contaminated and will be disposed of correctly in the appropriate waste disposal bins provided.
7. Hands will be washed immediately after removing gloves, using a hand-washing technique defined by local standardized procedures.
8. Specimens of biological materials (e.g., blood) will be placed in a secure secondary container to prevent breakage and leakage during transport and transported in accordance with the relevant regulations.[25,26]

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 1.4.4 Safety for Disposal of Sharps

SOP Number: 1.4.4
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for safe disposal of used sharps.

Responsibility
It is the responsibility of all research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. Research personnel will be responsible for the safety and disposal of used sharps.
2. Sharps will be placed in the sharps container as soon as possible after use and will not be left lying around.
3. Sharps containers will be placed as near as possible to the site of use.
4. Sharps containers will not be overfilled and will be securely closed. Used and sealed sharps containers will be stored in a location which will prevent risk of injury to staff, research participants and other personnel, while awaiting collection by appropriate personnel.
5. Research personnel will never discard needles or other sharps, including plastic pipette tips into polythene bags.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 1.4.5 Safety Guidelines for Handling Chemical Hazards

SOP Number: 1.4.5
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for safe handling of chemical hazards.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. All research laboratory personnel will receive the appropriate training and education to develop and implement work practices to minimize personal and co-worker exposure to the chemicals in the laboratory. Based on the realization that all chemicals inherently present hazards in certain conditions, exposure to all chemicals shall be minimized.
2. Research laboratory personnel will be required to read and understand the Material Safety Data Sheet (MSDS) and SOP for the chemical and/or process that they are working with. This will allow research laboratory personnel to be familiar with the symptoms of exposure for the chemicals with which they work and the precautions necessary to prevent exposure.
3. General precautions to be followed for the handling and use of all chemicals are as follows:

   - Skin contact with all chemicals shall be avoided.
   - Research personnel will wash all areas of exposed skin prior to leaving the laboratory.
   - Mouth suction for pipetting or starting a siphon is prohibited.
   - Eating, drinking, smoking, gum chewing or application of cosmetics in areas where laboratory chemicals are present shall not be permitted. Hands shall be thoroughly washed prior to performing these activities.
   - Storage, handling and consumption of food or beverages shall not occur in chemical storage areas, laboratories or refrigerators, nor shall any glassware or utensils used for laboratory operations be used in the handling of food or beverages.
   - Any chemical mixture shall be assumed to be as toxic as its most toxic component.
   - Substances of unknown toxicity shall be assumed to be toxic.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 1.4.6 Safety Guidelines for Handling Dry Ice

SOP Number: 1.4.6
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for safe handling of dry ice.

Responsibility
It is the responsibility of all research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. Avoid contact with skin and eyes when handling dry ice as it can cause severe burning and frostbite within seconds. NEVER handle dry ice with bare hands. Dry ice should not be put in the mouth or ingested.
2. When handling dry ice wear insulated gloves, safety glasses or goggles, long sleeves, long pants and shoes. Tongs may be used to handle large blocks of dry ice as required.
3. Never store dry ice in glass or other sealed/air-tight containers as it would be liable to cause an explosion. Do not store dry ice in a confined space, only store in a well ventilated area. Do not store dry ice in a freezer/fridge.
4. Do not use dry ice in a confined area. Do not place on a tilted surface or on laminated counter tops as it may destroy bonding agents. Only work with dry ice on a solid wooden board.
5. Do not dump dry ice; allow it to sublime in a well-ventilated area where there is no opportunity for the gas to build up. Do not dispose of dry ice in sewers, sinks, or toilets—the extreme cold may damage pipes.
6. If a dry ice spillage occurs isolate the area. Put on personal protective equipment, i.e. gloves, safety goggles and long sleeves. Using a dust pan and brush, carefully collect the spilt dry ice and dispose of in the usual disposal area.
7. Carbon dioxide monitors that alarm when levels are too high should be used in areas where dry ice will be stored or used.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 1.4.7 Safety Guidelines for Handling Liquid Nitrogen

SOP Number: 1.4.7
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for safe handling of liquid nitrogen.

Responsibility
It is the responsibility of all research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. Liquid nitrogen is very cold (−196°C) and can quickly freeze the skin. Only persons trained in the safe handling should be allowed use liquid nitrogen.
2. Users must first read the relevant Material Safety Data Sheet for liquid nitrogen.
3. Personal protective equipment must be worn, including protective gloves specifically designed for cryogenic handling, a closed lab coat, a face shield, and shoes when working with liquid nitrogen.
4. Pour liquid nitrogen slowly and carefully to minimize splashing and rapid cooling of the receiving container. Never overfill containers.

5. Use tongs when placing in or removing items from liquid nitrogen.
6. Use dip sticks to check liquid depth in dewars. Do not use fingers.
7. The area for use should be well ventilated.
8. Oxygen sensors and alarms should be put in place to detect a drop in ambient oxygen if a spill occurs.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 1.5 Specimen Identification and Labeling

SOP Number: 1.5
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for specimen identification and labeling.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. Specimen containers will not be labelled in advance of specimen collection.
2. The appropriate allocated unique study identification number and/or bar-coded label will be applied to the specimen container immediately following collection from the research participant thereby ensuring correct labelling.
3. Each specimen will be labelled with labels that have been previously tested and proven to survive potential storage conditions, for example −80°C and liquid nitrogen and to the conditions to which the vial will be exposed in downstream processes (e.g., heat blocks). The labels should also be tested to withstand exposure to common chemicals used in the laboratory.
4. Where barcode labels are not in use, research personnel will ensure that the research unique study identification number is written legibly in permanent marker on the specimen container immediately following collection.
5. Research personnel will ensure that the correct unique study identification number and/or bar code label appropriate to the research study and written informed consent form is applied to the specimen.
6. Research personnel will record the time of collection in the study-specific documentation or data management system.
7. Research personnel will ensure that the lid of the specimen container is securely replaced to avoid potential leakage. The label should be tested to ensure that exposure of material will not render it illegible.
8. Where research personnel are in doubt as to the identity of a particular specimen it will be destroyed appropriately according to local policy and procedures.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

PART II: CLINICAL STANDARD OPERATING PROCEDURES

2.1 Overview Table for Sample Collection Based on Analyte

See page 16 for the Overview Table

2.2 General Principles for Blood Collection

1. Follow the standard procedures outlined in Part I: Pre-Clinical SOPs, in particular the SOPs for safety, handling of biological materials, disposal of sharps and sample identification and labeling (SOPs 1.4.1–1.4.7).
2. Transfer of specimens collected using a syringe and needle to a blood collection tube is not recommended as this additional manipulation of sharps such as hollow bore needles increases the potential for needle-stick injury.
3. Transfer of specimens from a syringe to an evacuated tube using a non-sharps device should be performed with caution for the reasons outlined below:

   - Depressing the syringe plunger during transfer can create a positive pressure, forcefully displacing the stopper and sample, causing splatter and potential blood exposure.

2.1 Overview Table for Sample Collection Based on Analyte

Sample Type	Blood	Blood	Blood	Blood	Blood	Blood	Blood	Blood	Blood	Saliva	Urine	Feces
Derivative	Plasma	Serum	DNA	RNA	Proteomics	Biochemistry	Hematology	Metabolomics	PBMC Lymphocyte immortalization	DNA	DNA, RNA Protein	DNA
Tube type	EDTA	SST	EDTA	Paxgene (Qiagen)	P100, PST	PST with LH	EDTA	PST with LH SST, plain tube	ACD BD CPT LeukoSep	Sterile container Collection kit	Sterile container	Sterile container
	PST with LH	Plain tube		Tempus RNA tubes (Applied Biosystems)	SST, plain tube	Plain tube with LH SST						
Order of collection	3	1	2	8	6	7	9	5	4	NA	NA	NA
Local processing	Yes SOP 2.5.1	Yes SOP 2.5.2	No SOP 2.5.3	No SOP 2.5.4	Yes SOP 2.5.5 &2.5.6	No SOP 2.5.7	No SOP 2.5.8	Yes SOP 2.5.9	Yes/No SOP 2.5.10	No SOP 2.6	No SOP 2.7	No SOP 2.8
Transport to Lab*: Temp Timing	4°C ASAP/ within 24 hrs	4°C ASAP/ within 24 hrs	4°C ASAP/ within 48 hrs	4°C ASAP/ within 24 hrs	4°C ASAP/ within 24 hrs	4°C Within 24 hrs	RT (18–22°C) Within 24 hrs	4°C ASAP/ within 24 hrs	18–22°C ASAP/ within 24 hrs	18–22°C ASAP/ within 48 hrs	18–22°C ASAP/ within 24 hrs	18–22°C ASAP/ within 24 hrs
Storage Temp^	−80°C	−80°C	−80°C	−80°C	NA	NA	NA	−80°C	−80°C for short-term or LN_2 for long-term storage	−80°C	−80°C	−80°C
Time to storage from collection*	ASAP/ within 48 hrs	ASAP/ within 48 hrs	ASAP/ within 48 hrs	ASAP/ within 24 hrs	ASAP/ within 48 hrs	NA	NA	ASAP/ within 48 hrs	ASAP/ within 48 hrs	ASAP/ within 48 hrs	ASAP/ within 48 hrs	ASAP/ within 48 hrs

*Samples should be processed and reach the appropriate storage conditions as soon as is practicable (ASAP). The time limits proposed are guidelines and should be read in association with a study specific protocol and cognisant of the analyte of interest.

EDTA = ethylenediaminetetraacetic acid; PST = plasma separator tube; SST = serum separator tube; LH = lithium heparin; ACD = acid citrate dextrose; LN = liquid nitrogen; NA = not applicable

^Storage temperature: −80°C and/or liquid nitrogen for long-term storage as appropriate.

Equipment/reagent requirements

- Blood collection system. Personal protective equipment; gloves, laboratory coat, protective glasses
- A polystyrene container with ice to maintain temperature at 4°C for processing and/or transport to processing laboratory, or alternatively use a water-bath (plus a thermometer) with iced water to maintain temperature at 4°C or a pre-cooled ice pack.
- Centrifuge capable of 1,100–1,600 g at the bottom of the tube
- Refrigerator (2–4°C), if overnight sample storage is required. Freezer −20°C/−80°C if short-term storage is required

- Using a syringe for blood transfer may also cause overfilling or underfilling of tubes, resulting in an incorrect blood-to-additive ratio and potentially incorrect analytical results.
- Evacuated tubes are designed to draw the volume indicated. Filling is complete when vacuum no longer continues to draw, though some tubes may partially fill due to plunger resistance when filled from a syringe. The laboratory should be consulted regarding the use of these samples.

4. If blood is collected through an intravenous (I.V.) line, ensure that the line has been cleared of I.V. solution before beginning to fill the blood collection tubes. This is critical to avoid erroneous laboratory/analytical results from I.V. fluid contamination.
5. Overfilling or underfilling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect laboratory/analytical results or poor product performance.
6. During collection it is important to avoid possible backflow from blood collection tubes that contain chemical additives which may result in the possibility of an adverse patient reaction.

2.3 Equipment and Reagents for Blood Collection and Immediate Processing

1. General blood taking equipment, correct evacuated tubes dependent on sample type, appropriate gauge needle, blood collection set, tourniquet, alcohol wipes, cotton wool, adhesive bandage, and sharps disposal system will be required prior to blood collection.
2. Personal protective equipment including, gloves, eye protection glasses, and laboratory coat will be worn as necessary for protection from exposure to blood borne pathogens.
3. Centrifuge capable of generating a G force of 1,100–3,000 g at the bottom of the tube. Counter balanced test tubes filled with water/saline for use to balance blood collection tubes during centrifugation. Disposable transfer plastic/Pasteur pipettes.
4. A refrigerator (4°C) and/or freezer (−20°C/−80°C) as necessary dependent on immediate processing requirements and whether overnight and/or short-term storage of samples is required. It may also be important to have access to dry ice supplies as appropriate dependent on sample type and transport requirements.
5. A polystyrene container with ice to maintain temperature at 4°C for immediate processing and/or transport to the processing laboratory, or alternatively use a water-bath (plus a thermometer) with iced water to bring the temperature to 4°C or a pre-conditioned gel pack at 4°C.

SOP 2.4 Recommended Order for Blood Draw

The recommended order for blood draw based on blood collection tube type is outlined as follows:

1. Blood collection tubes for sterile samples
2. Blood collection tubes without additives
3. Blood collection tubes for coagulation studies (e.g. with citrate additive)
4. Blood collection tubes with other lyophilised additives (vacutainers, heparin, EDTA, plasma, BD P100, or serum separator tubes)
5. Blood collection tubes with other liquid additives (e.g. PAXgene™ and BD vacutainer CPT)

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5 Blood Collection—Venipuncture

SOP Number: 2.5
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection (venipuncture) from research participants.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. The research participant's arm will be hyperextended and positioned comfortably on the armrest of the venipuncture chair/couch as appropriate.
2. The tourniquet will be applied 3–4 inches above the selected puncture site and will not be left in position for longer than two minutes.
3. The research participant will be asked to make a fist without pumping the hand.
4. A vacuum collection system (for example Monovette) will be used for venipuncture where possible. Syringes and needles will be used in place of the vacuum collection system in special circumstances.
5. The puncture site will be cleansed using a Sterets pre-injection swab in a circular motion from the center to the periphery.
6. The cleansed site will be allowed to air dry prior to venipuncture.
7. The research participant's vein will be anchored and the needle will then be inserted through the skin, bevel edge uppermost, into the lumen of the vein.
8. The tourniquet will be released when the last collection tube to be drawn is filling.
9. Tubes containing anticoagulants must be properly mixed immediately after each is drawn by inverting the tube. See manufacturer instructions for number of inversions required.
10. Clean dry gauze or cotton wool will be placed on the venipuncture site and the needle will be removed in a swift backward motion using a needle protector.
11. The research personnel will press down on the gauze/cotton wool once the needle has been drawn out of the vein applying adequate pressure to avoid formation of a haematoma.
12. The research participants arm will not be placed in a bent position at any time following venipuncture.
13. The research participants arm will be inspected to ensure bleeding has stopped and a band-aid strip will be applied.
14. The research personnel will ensure that the research participant has not experienced any adverse events from the venipuncture and will then assist them from the chair.
15. All contaminated materials/supplies will be disposed of in the designated containers.
16. All blood collection tubes will be labelled immediately following collection with the appropriate research study labels, for example a unique study identification number and/or bar code label. The time of collection will also be recorded in the study specific documentation and/or data management system.
17. The research personnel will arrange for the blood specimens to be transported to the research laboratory as applicable.

Safety precaution: During collection it is important to avoid possible backflow from blood collection tubes that contain chemical additives, for example BD Vacutainer CPT which may result in the possibility of an adverse patient reaction. The following precautions should be observed:

- Use a blood collection set with a safety lock for example; a BD Vacutainer® Safety-Lok™ Blood Collection Set.
- Place arm in a downward position
- Hold tube with stopper upper-most
- Release tourniquet as soon as blood starts to flow into the tube
- Ensure that tube additives do not touch the stopper or the end of the needle during venipuncture

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.1 Blood Collection for Plasma

SOP Number: 2.5.1
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for plasma.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: EDTA, plasma separator tube (PST) with lithium heparin*
- A polystyrene container with ice to maintain temperature at 4°C for processing and/or transport to processing laboratory, or alternatively use a water-bath (plus a thermometer) with iced water to maintain the temperature at 4°C or a pre-conditioned gel pack at 4°C
- Centrifuge capable of generating 1,100–1,300 g at the bottom of the tube
- Refrigerator (2–4°C) if overnight sample storage is required
- Freezer −20°C/−80°C if short-term storage is required

Procedure

1. Draw blood directly into the evacuated tube. Filling the blood collection tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube may affect laboratory results due to the incorrect blood/additive ratio.
2. The blood collection tube is appropriately labeled either with a unique study identification number and/or a bar code label generated electronically.
3. Record the time that the sample was taken in the study specific documentation or data management system.
4. Invert the tube 8–10 times immediately after collection. This helps to prevent the formation of fibrin which may affect subsequent analysis.
5. Maintain tubes at 4°C at all times following collection and during processing. Centrifuge tubes within *2 hours* of collection to separate plasma from cells. Place the blood collection tubes in a centrifuge and spin at 1,300 g for 10 minutes at 4°C. Record the time processing was initiated in the study specific documentation or data management system.
6. Avoid mixing/agitation of PST tubes between centrifugation and separation or transport to the laboratory as this may lead to mixing and/or re-suspension of cells and platelets that were previously on or near the gel surface.
7. Using a plastic Pasteur/transfer pipette collect plasma, being sure to stay above the gel/cell layer so that no cells or portions of the gel are collected. Distribute the plasma (clear liquid) among cryostorage tube(s) maintained at 4°C which have been labeled as per point 2 above. Record the volume in each tube in the study specific documentation or data management system.
8. Transfer tubes to a −80°C freezer for storage. If there is not a −80°C freezer on site store at −20°C. If neither is available transport to the processing laboratory at 4°C in a polystyrene container on ice. The specimen should reach the −80°C freezer within 48 hours of collection. Record the time of storage in the study specific documentation or data management system. **Note:** As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.2 Blood Collection for Serum

SOP Number: 2.5.2
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for serum.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: serum separator tube (SST) or plain tube*
- A polystyrene container with ice to maintain temperature at 4°C for processing and/or transport to processing laboratory, or alternatively use a water-bath (plus a thermometer) with iced water to maintain the temperature at 4°C or a pre-conditioned gel pack at 4°C
- Centrifuge capable of generating 1,100–1,600 g at the bottom of the tube
- Refrigerator (2–4°C) if overnight sample storage is required
- Freezer −20°C/−80°C if short-term storage is required

Procedure

1. Draw blood directly into the evacuated tube. Filling up the blood collection tube to the black mark on the tube label indicates that the correct amount of blood has been drawn.
2. The blood collection tube is appropriately labeled either with a unique study identification number and/or a barcode label generated electronically.
3. Note the time that the sample was taken in the study specific documentation or data management system.
4. Allow the blood to clot for 15 to 30 minutes at room temperature (RT) (18–22°C). The time for clotting is dependent on tube type so refer to the manufacturer's instructions for use for recommended time for specific tube types.
5. Place tubes in the centrifuge and spin at 1,600 g at RT (18–22°C) for 10 minutes. This speed, time and temperature will minimize platelet contamination of the specimen which may affect sample analysis. Record the time processing was initiated in the study specific documentation or data management system.
6. Using a plastic transfer/Pasteur pipette collect the serum being careful not to disrupt the clot or to collect any of the gel. Transfer the serum (straw coloured liquid) into 0.5 mL cryostorage tubes maintained at 4°C which have been labeled as per point 2 above.
7. Transfer tubes to a −80°C freezer for storage. If there is not a −80°C freezer on site store at −20°C. If neither is available transport tubes to the processing laboratory at 4°C in a polystyrene container on ice. The specimen should reach the −80°C freezer within 48 hours of collection. Record the time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.3 Blood Collection for DNA Extraction

SOP Number: 2.5.3
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for extraction of DNA.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: EDTA.*
- **Note:** Lithium heparin is not recommended for blood collection for DNA extraction as the heparin co-purifies with the DNA and can interfere with enzymatic reactions.
- A polystyrene container with ice to maintain temperature at 4°C for processing and/or transport to processing laboratory, or alternatively use a water-bath with iced water to maintain the temperature at 4°C (plus a thermometer) or a pre-conditioned gel pack at 4°C
- Refrigerator (2–4°C) if overnight sample storage is required
- Freezer −20°C/−80°C if short-term storage is required

Procedure

1. Draw blood directly into the evacuated tube. Filling up the blood collection tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube can affect results due to the incorrect blood/additive ratio.
2. The blood collection tube is appropriately labeled either with a unique study identification number and/or a bar code label generated electronically.
3. Invert the tube 8–10 times to avoid the formation of microclots.
4. Record the time that the sample was taken in the study specific documentation or data management system.
5. Maintain the tubes at 4°C in a refrigerator/polystyrene container with ice. Transport tubes to the processing laboratory as soon as is practicable or within a maximum of 48 hours for immediate processing of DNA or for direct storage at −80°C. Tubes should be transported at 4°C in a polystyrene container on ice. Record date and time of processing of DNA and the data/time that DNA is frozen in the study specific documentation or data management system.
6. If a sample for DNA is frozen locally at −20°C then the sample should be transported frozen, using dry ice to the processing laboratory. Vacutainers should be tested to ensure that they can withstand storage temperature and re-thaw. If a sample is thawed DNAse enzymes break down the DNA rapidly.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.4 Blood Collection for RNA isolation

SOP Number: 2.5.4
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for extraction of RNA.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: ACD tube, Tempus™ Blood RNA Tubes (Applied Biosystems) or PaxGene (Qiagen)*
- A polystyrene container with ice to maintain temperature at 4°C for processing and/or transport to processing laboratory, or alternatively use a water-bath (plus a thermometer) with iced water to maintain the temperature at 4°C or a pre-conditioned gel pack at 4°C
- Refrigerator (2–4°C) if overnight sample storage is required
- Freezer −20°C/−80°C if short-term storage is required
- Vortex for sample mixing

Procedure
Using ACD tubes

1. Draw blood directly into the evacuated tube. Filling the tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube can affect laboratory results.
2. The blood collection tube is labeled appropriately either with a unique identification study number and/or a bar code label generated electronically.
3. Record the time that the sample was taken in the study specific documentation or data management system as available.
4. Maintain tubes at RT (18–22°C) and transport to the processing laboratory within 24 hours at RT (18–22°C) for processing.
5. If immediate transfer is not possible, samples can be maintained at RT (18–22°C) and transferred to the processing laboratory for RNA isolation as soon as is practicable or within a maximum of 24 hours. Record the time of processing in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Using Tempus Blood RNA Tubes

1. Draw blood directly into the evacuated Tempus Blood RNA Tube. Filling the blood collection tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube can affect laboratory results due to the incorrect blood/additive ratio.
2. Immediately after the Tempus tube is filled, stabilize the blood by shaking the tube vigorously or vortexing the contents for 10 seconds to ensure that the stabilizing reagent makes uniform contact with the sample.
   IMPORTANT: Failure to mix the stabilizing reagent with the blood leads to inadequate stabilization of the gene expression profile and the formation of microclots that can potentially clog the purification filter.
3. The Tempus Blood RNA tube is appropriately labeled either with a unique study identification number and/or a bar code label generated electronically.
4. Record the time that the sample was taken in the study specific documentation or data management system.
5. Maintain the tubes at 4°C using a refrigerator/polystyrene container with ice. Transport tubes to the processing laboratory as soon as is practicable or within a maximum of 24 hours for immediate processing of RNA or for direct storage at −80°C. Tubes should be transported at 4°C in a polystyrene container on ice. Record the time of processing in the study specific documentation or data management system.

Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Using Paxgene tubes

1. Draw blood directly into the evacuated Paxgene tube. Filling the blood collection tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube can affect laboratory results due to the incorrect blood/additive ratio.
2. The tube is gently inverted 8–10 times.
   IMPORTANT: It is critical to RNA quality and yield that tubes are thoroughly mixed by inversion at the time of collection, that a full tube of blood be taken and that nothing is placed over the black fill mark on the manufacturer's label of the tube.
3. The Paxgene tube is appropriately labeled either with a unique study identification number and/or a bar code label generated electronically.
4. Record the time that the sample was taken in the study specific documentation or data management system.
5. Maintain the tubes at 4°C in a refrigerator/polystyrene container with ice. Transport tubes to the processing laboratory as soon as is practicable or within a maximum of 24 hours for immediate processing of RNA or for direct storage at −80°C. Tubes should be transported at 4°C in a polystyrene container on ice. Record the time of processing in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.5 Blood Collection for Proteomics using Plasma

SOP Number: 2.5.5
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for plasma isolation for proteomic studies.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: plasma separator tube, BD P100 proteomics tube*
- A polystyrene container with ice to maintain temperature at 4°C for processing and/or transport to processing laboratory, or alternatively use a water-bath (plus a thermometer) with iced water to maintain the temperature at 4°C or a pre-conditioned gel pack at 4°C
- Refrigerator (2–4°C) if overnight sample storage is required
- Freezer −20°C/−80°C if short-term storage is required

- Centrifuge capable of generating a G force of 1,100–1,300 g at the bottom of the tube

SAFETY PRECAUTION: The BD P100 evacuated blood collection tube contains chemical additives. It is important to avoid possible backflow from the tube, which may lead to the possibility of adverse patient reaction. To guard against backflow observe the following precautions:

- Use a blood collection set with a safety lock, for example a BD Vacutainer® Safety- Lok™ Blood Collection Set.
- Place the arm in a downward position.
- Hold tube with stopper upper-most.
- Release tourniquet as soon as blood starts to flow into the tube.
- Ensure that tube additives do not touch the stopper or the end of the needle during venipuncture.

Procedure

1. Draw blood directly into the evacuated tube. Filling the blood collection tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube may affect laboratory results.
2. Invert the tube 8–10 times to avoid the formation of microclots.
3. The blood collection tube is labeled appropriately either with a unique study identification number and/or a bar code label generated electronically
4. Record the time that the sample was taken in the study specific documentation or data management system.
5. Centrifuge tubes within *2 hours* of collection to separate plasma from cells. Maintain tubes at 4°C during processing. Place the blood collection tubes in a centrifuge and spin at 1,300 g for 10 minutes at 4°C. Record the time processing was initiated in the study specific documentation or data management system.
6. Using a plastic Pasteur/transfer pipette collect plasma being sure not to get too close to the cell layer or gel. Distribute the plasma (clear liquid) among 0.5 mL cryostorage tube(s) maintained at 4°C which have been labeled as per point 3 above.
7. Transfer tubes to a −80°C freezer for storage. If there is not a −80°C freezer on site store at −20°C. If neither is available transport to the processing laboratory at 4°C in a polystyrene container on ice. The specimen should reach the −80°C freezer as soon as is practicable or within a maximum of 48 hours following collection. Record the time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.6 Blood Collection for Proteomics Using Serum

SOP Number: 2.5.6
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for serum isolation for proteomic studies.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: SST or plain tube*
- Plain plastic 15 mL conical centrifuge tube
- Protease Inhibitor Cocktail (for example P3840 Sigma Aldrich)
- A polystyrene container with ice to maintain temperature at 4°C for immediate processing and/or transport to processing laboratory, or alternatively use a water-bath with iced water to maintain the temperature at 4°C (plus a thermometer) or a pre-conditioned gel pack at 4°C
- Refrigerator (2–4°C) if overnight sample storage is required
- Freezer −20°C/−80°C if short-term storage is required
- Centrifuge capable of generating a G force of 1,100–1,600 g at the bottom of the tube

Procedure

1. Draw blood directly into the evacuated tube. Filling the tube to the black mark on the tube label indicates that the correct amount of blood has been drawn.
2. The blood collection tube is labeled appropriately either with a unique study identification number and/or a bar code label generated electronically.
3. Record the time that the sample was taken in the study specific documentation or data management system.
4. Allow the blood to clot for 15 to 30 minutes on ice (4°C). Note the clotting time in the study specific documentation or data management system.
5. Centrifuge tubes within *2 hours* of collection to separate serum from cells. Maintain tubes at 4°C during processing. Place the blood collection tubes in a centrifuge and spin at 1,600 g for 10 minutes at 4°C. Record the time processing was initiated in the study specific documentation or data management system.
6. Using a plastic transfer/Pasteur pipette collect serum being sure not to disrupt the clot or to collect any of the gel. Transfer the serum (straw colored liquid) to a plain plastic 15 mL conical centrifuge tube.
7. Add 100× protease inhibitor solution, for example 10 ul of protease inhibitor solution to 1 mL of serum and mix by inversion at least 8–10 times.
8. Using a plastic transfer/Pasteur pipette transfer serum into each 0.5 mL cryostorage tube maintained at 4°C which have been labeled as per point 2 above.
9. Transfer tubes to a −80°C freezer for storage. If there is not a −80°C freezer on site store at −20°C. If neither is available transport to the processing laboratory at 4°C in a polystyrene container on ice. The specimen should reach the −80°C freezer as soon as is practicable or within 48 hours following collection. Record the time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.7 Blood Collection for Biochemistry

SOP Number: 2.5.7
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for biochemical analysis.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: PST with lithium heparin or a plain tube with lithium heparin. SST or plain tube.*
- A polystyrene container with ice to maintain temperature at 4°C for processing and/or transport to processing laboratory, or alternatively use a water-bath with iced water to maintain the temperature at 4°C (plus a thermometer) or a pre-conditioned gel pack at 4°C
- Refrigerator (2–4°C), if overnight sample storage is required
- Centrifuge capable of generating a G force of 1,100–1,300 g at the bottom of the tube

Procedure

1. Draw blood directly into the evacuated tube. Filling the tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube can affect laboratory results due to the incorrect blood/additive ratio.
2. Invert the tube 8–10 times immediately after collection, this helps to prevent the formation of fibrin which may affect the laboratory result.
3. The blood collection tube is labeled appropriately with a unique study identification number generated and/or a bar code label generated electronically.
4. Record the time that the sample was taken in the study specific documentation or data management system.
5. Transport directly (within 4 hours) to the Biochemistry laboratory for processing. If transport to the Biochemistry laboratory within 4 hours is not possible separate the plasma from the blood samples by following the procedure outlined from points 6–8 below.
6. Centrifuge tubes within *2 hours* of collection to separate plasma from cells. Maintain tubes at 4°C during processing. Place the blood collection tubes in a centrifuge and spin at 1,300 g for 10 minutes at 4°C. Record the time processing was initiated in the study specific documentation or data management system.
7. Using a plastic Pasteur/transfer pipette collect plasma, being sure not to disrupt the cell layer or gel. Transfer the plasma (clear liquid) to a 0.5 mL cryostorage tube maintained at 4°C which have been labeled as per point 3 above.
8. Maintain the plasma sample at 4°C and transfer to the biochemistry laboratory within 24 hours for processing.
   Note: Immediate separation of plasma/serum from cells provides optimal analyte stability at RT. Storage of un-centrifuged specimens after 24 hours has resulted in clinically significant changes in measured analytes. When prolonged contact of plasma/serum with cells is unavoidable use of serum is recommended because of the higher instability of plasma analytes.[28]

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.8 Blood Collection for Hematology

SOP Number: 2.5.8
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for hematological analysis.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: EDTA*

Procedure

1. Draw blood directly into the evacuated EDTA tube. Filling up the tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube can affect laboratory results due to the incorrect blood/additive ratio.
2. The blood collection tube is labeled appropriately with a unique study identification number generated and/or a bar code label generated electronically.
3. Invert the tube 8–10 times to avoid the formation of microclots.
4. Record the time that the sample was taken in the study specific documentation or data management system.
5. Transport to the Hematology laboratory within 4 hours at RT (18–22°C) for processing.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.9 Blood Collection for Metabolomics Using Serum

SOP Number: 2.5.9
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for serum isolation for metabolomic studies.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: SST or plain tube*
- A polystyrene container with ice to maintain temperature at 4°C for processing and/or transport to processing laboratory, or alternatively use a water-bath (plus a thermometer) with iced water to maintain the temperature at 4°C or a pre-conditioned gel pack at 4°C
- Refrigerator (2–4°C) if overnight sample storage is required
- Centrifuge capable of generating a G force of 1,100–1,600 g at the bottom of the tube

Procedure

1. Draw blood directly into the evacuated tube. Filling the tube to the black mark on the tube label indicates that the correct amount of blood has been drawn.
2. The blood collection tube is labeled appropriately either with a unique study identification number and/or a bar code label generated electronically.
3. Record the time that the sample was taken in the study specific documentation or data management system.
4. Allow the blood to clot for 15–30 minutes at RT (18–22°C). Record clotting time in the study specific documentation or data management system.
5. Centrifuge tubes within *2 hours* of collection to separate serum from cells. Maintain tubes at 4°C during processing. Place the blood collection tubes in a centrifuge and spin at 1,600 g for 10 mins at 4°C. Record the time processing initiated.
6. Using a plastic transfer/Pasteur pipette collect serum being sure not to disrupt the clot or gel. Transfer the serum into 0.5 mL cryostorage tube(s) maintained at 4°C which have been labeled as per point 2 above.
7. Transfer tubes to a −80°C freezer for storage. If there is not a −80°C freezer on site transport to the processing laboratory at 4°C in a polystyrene container on ice. The specimen should reach the −80°C as soon as is practicable or within 48 hours of collection. Record the time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.10 Blood Collection for Isolation of Peripheral Blood Mononuclear Cells

SOP Number: 2.5.10
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for blood collection for isolation of peripheral blood mononuclear cells.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- *Blood collection tube: Acid Citrate Dextrose (ACD) tubes or BD CPT tube*
- If using a BD CPT tube a centrifuge capable of generating a G force of 1,100– 1,300 g at the bottom of the tube is required.

Procedure
Using ACD tubes

1. Draw blood directly into the evacuated tube. Filling the tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. Underfilling or overfilling of the tube can affect laboratory results.
2. The blood collection tube is labeled appropriately either with a unique identification study number and/or a bar code label generated electronically.
3. Record the time that the sample was taken in the study specific documentation or data management system.
4. Maintain tubes at RT (18–22°C) and transport to the processing laboratory within 24 hours at RT (18–22°C) for processing.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

   Note: For BD CPT follow the manufacturer's instructions outlined in the information for use.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.6 Saliva Collection

SOP Number: 2.6
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for saliva collection from research participants.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment

- Personal protective equipment: gloves (optional)
- Specimen container or saliva collection kit (for example Oragene DNA Emergo Europe)
- Sterile container with warm water

Procedure

1. Research personnel will greet the research participant, identify themselves, and then explain the saliva collection procedure to the research participant.
2. The research participant will be approached in a friendly calm manner and their co-operation will be gained prior to saliva collection.
3. The research participant will be correctly identified prior to saliva collection, by asking them to give their name and date of birth.
4. All sample containers and equipment needed to competently and efficiently carry out the saliva collection will be assembled prior to the procedure. For specific saliva collection kits follow the manufacturer's instruction for sample collection as outlined in the information for use, for example Oragene DNA saliva collection kit (Emergo Europe).
5. The research participant will rinse mouth out with warm water. Avoid use of mouthwashes or fluoride rinses. In addition, the research participant should not drink, smoke or chew gum for 30 minutes before giving a saliva sample.
6. The research participant will be asked to fill the specimen container to a certain marked level with saliva.
7. The inside of the specimen container will not be touched and the outer surfaces of the specimen container will not be contaminated with saliva.
8. A unique study identification number or bar code label will be applied to the sample immediately after saliva collection. Record the time of saliva collection in the study specific documentation or data management system.
9. Transport to the processing laboratory as soon as is practicable or within a maximum of 48 hours for immediate processing of DNA or for direct storage at −80°C. Saliva collection tubes should be transported at 4°C in a polystyrene container on ice. Alternatively when using commercial collection kits such as Oragene DNA the sample may be stored at RT for up to 18 months in the cell lysis buffer in the container prior to DNA processing. The saliva collection kit should be transported to the processing laboratory at RT. Follow the manufacturer's instructions for use in the information for use/package insert in all cases. Record date/time processing was initiated and date/time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.7 Urine Collection

SOP 2.7.1 Mid-Stream Urine Specimen

SOP Number: 2.7.1
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for mid-stream urine collection from research participants.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment

- Personal protective equipment: gloves
- Sterile urine container

Procedure

1. Research personnel will greet the research participant, identify themselves, and then explain the urine collection procedure to the research participant.
2. The research participant will be approached in a friendly calm manner and their co-operation will be gained prior to urine collection.
3. The research participant will be correctly identified prior to urine collection, by asking them to give their name and date of birth.
4. All sample containers and equipment needed to competently and efficiently carry out the urine collection will be assembled prior to the procedure.
5. The research participant will be asked to provide a urine sample by instructing them to discard the first part of the voided urine and without disrupting the flow to collect approximately 10 mls of urine in the sterile container.
6. A unique study identification number and/or bar code label will be applied to the sample immediately after urine collection. Record the time of urine collection in the study-specific documentation or data management system.
7. Transport tubes to the processing laboratory as soon as is practicable or within a maximum of 24 hours for RNA isolation or 48 hours for DNA and/or protein extraction or for direct storage at -80°C. Tubes should be transported at 4°C in a polystyrene container on ice. Record the time processing was initiated and time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.7.2 24-Hour Urine Collection

SOP Number: 2.7.2
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for 24-hour urine collection from research participants.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment

- Personal protective equipment: gloves
- 24-hour sterile urine collection container

Procedure

1. Research personnel will greet the research participant, identify themselves, and then explain the urine collection procedure to the research participant.
2. The research participant will be approached in a friendly calm manner and their co-operation will be gained prior to urine collection.
3. The research participant will be correctly identified prior to urine collection, by asking them to give their name and date of birth.
4. All sample containers and equipment needed to competently and efficiently carry out the urine collection will be assembled prior to the procedure.
5. The research participant will be asked to empty his/her bladder and discard the first morning urine void.
6. The 24-hour urine collection will commence with the empty bladder and the research participant will note the time of emptying.
7. All urine over the next 24 hours including the last specimen at the same time the next day will be saved and collected in the container provided.
8. Research personnel will ensure that an accurate 24-hour urine collection has been made by the research participant prior to labeling and transport of the specimen to the processing laboratory.
9. Transport tubes to the processing laboratory as soon as is practicable or within a maximum of 24 hours for RNA isolation and 48 hours for DNA and/or protein extraction or for direct storage at −80°C. Tubes should be transported at 4°C in a polystyrene container on ice. Record the time processing was initiated and time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.7.3 Catheter Specimen of Urine

SOP Number: 2.7.3
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for collection of a catheter specimen of urine from research participants.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment

- Personal protective equipment: gloves
- Sterile urine container

Procedure

1. Research personnel will greet the research participant, identify themselves, and then explain the urine collection procedure to the research participant.
2. The research participant will be approached in a friendly calm manner and their co-operation will be gained prior to urine collection.
3. The research participant will be correctly identified prior to urine collection, by asking them to give their name and date of birth.
4. All sample containers and equipment needed to competently and efficiently carry out the urine collection will be assembled prior to the procedure.
5. If the catheter specimen of urine is to be collected from an indwelling catheter the specimen will be obtained aseptically from a sample port in the catheter tubing. The sample will not be obtained from the collection bag.
6. If the catheter specimen of urine is to be collected from a transient catheter the specimen will be obtained via a catheter passed aseptically into the bladder.
7. A unique study identification number or bar code label will be applied to the sample immediately after urine collection.
8. Transport tubes to the processing laboratory as soon as is practicable or within a maximum of 24 hours for RNA isolation and 48 hours for DNA and/or protein extraction or for direct storage at −80°C. Tubes should be transported at 4°C in a polystyrene container on ice. Record the time processing was initiated and the time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.8 Feces Collection

SOP Number: 2.8
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for feces collection from research participants.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment:

- Personal protective equipment: gloves
- Sterile feces container

Procedure

1. Research personnel will greet the research participant, identify themselves, and then explain the feces collection procedure to the research participant.
2. The research participant will be approached in a friendly calm manner and their co-operation will be gained prior to feces collection
3. The research participant will be correctly identified prior to feces collection, by asking them to give their name and date of birth.
4. All sample containers and equipment needed, to competently and efficiently carry out the feces collection will be assembled prior to the procedure.
5. The research participant will be asked to provide a feces sample. The specimen will be passed into a clean dry, disposable bedpan or similar container. The research personnel will then transfer the feces specimen into a sample container using a disposable wooden spatula.
6. The inside of the specimen container will not be touched and the outer surfaces of the specimen will not be contaminated with feces.
7. A unique study identification number and/or bar code label will be applied to the sample immediately after feces collection.
8. Transport tubes to the processing laboratory as soon as is practicable or within a maximum of 24 hours for RNA and 48 hours for DNA/or protein extraction or for direct storage at −80°C. Tubes should be transported at 4°C in a polystyrene container on ice. Record the time processing was initiated and the time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.9 Buccal Collection

SOP Number: 2.9
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for buccal collection from research participants.

Responsibility
It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment

- Personal protective equipment: gloves (optional)
- Sterile single use cotton swab

Procedure:

1. Research personnel will greet the research participant identify themselves, and then explain the buccal collection procedure to the research participant.
2. The research participant will be approached in a friendly calm manner and their co-operation will be gained prior to buccal collection.
3. The research participant will be correctly identified prior to buccal collection, by asking them to give their name and date of birth.
4. All sample containers and equipment needed to competently and efficiently carry out the buccal collection will be assembled prior to the procedure.
5. A sterile single use cotton swab will be removed from its packaging.
6. Sterile technique will be observed as the cotton swab is brushed inside the mouth along the cheek.
7. The buccal swab will be returned to a sterile wrapping or will be wrapped inside sterile plastic wrap.
8. A unique study identification number and/or bar code label will be applied to the sample immediately after buccal collection. Record the time of collection in the study specific documentation or data management system.
9. Transport tubes to the processing laboratory as soon as is practicable or within a maximum of 24 hours for RNA isolation and 48 hours for DNA and/or protein extraction or for direct storage at $-80^{\circ}C$. Tubes should be transported at $4^{\circ}C$ in a polystyrene container on ice. Record the time processing was initiated and the time of storage in the study specific documentation or data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

2.10 Guiding Principles for Tissue Collection, Processing, and Storage

Solid tissues are collected by biopsy or during surgical procedures. Tissue collection should be carefully planned with surgeons, clinical staff, pathologists and research personnel.

The following are guiding principles for the collection processing and storage of tissue adapted from the Common Minimum Technical Standards and Protocols for Biological Resource Centers Dedicated to Cancer Research prepared by the IARC/WHO International Agency for Research on Cancer.[17] It is recommended that a detailed SOP be developed based on the type of tissue to be collected, processed and stored in accordance with individual study requirements.

- The collection of tissue samples for research should never compromise the diagnostic integrity of a specimen. Only tissue which is excess to diagnostic purposes should be collected for research purposes.
- All materials and instruments should be prepared in advance of tissue collection. Appropriate personal protective equipment should be worn and safety measures should be adhered to in accordance with local policy and procedures.
- All staff involved including the surgeon, pathologist, research nurse, laboratory technician and research personnel should be trained in the study specific SOP.
- All tissue should be treated as potentially infectious; the collection process should be carried out in accordance with an aseptic technique as possible.
- The surgical specimen or biopsy should be sent to pathology as soon as possible.
- It is recommended that surgical specimens or biopsy be preserved within 1 hour of excision; however, tissue subject to a delay up to 2 hours can still be collected.
- A detailed record of the timing of events from time of ischemia to fixation or freezing should be documented.
- Each specimen receptacle must be clearly labeled with a unique study number and/or barcode and the details of tissue as required by the study specific protocol.

- Transfer of specimens must be carried out as soon as possible in order to minimize the effect of hypoxia upon gene expression, degradation of RNA, proteins, and other tissue components.
- For transport from surgery to pathology, or to the biobank, fresh specimens should be placed in a closed, sterile container on ice at 4°C.
- It is recommended that a pathologist should supervise the preparation/dissection of the tissue and ensure that adequate tissue is taken for diagnosis before sampling for research.
- Research personnel must be available to freeze or fix the tissue as quickly as possible and in accordance with a study specific SOP.
- Formalin fixation of tissue is standard practice in routine histopathology laboratories and should be conducted in accordance with a study specific SOP.
- Label cryostorage vials with a unique study identification number/barcode, local pathology number. Record the date and time of ischemia, excision and storage in the study specific documentation or data management system.
- Tissues must be snap frozen either directly or enclosed in a container immersed in the freezing medium (e.g. pre-cooled isopentane). Snap frozen samples should be placed in the −80°C freezer. Liquid nitrogen is not recommended as a suitable freezing medium for direct snap freezing due to the potential formation of cryo-artefacts. RNA can be protected in fresh tissue by the addition of Allprotect/RNAlater prior to storage at −20°C.

PART III: LABORATORY STANDARD OPERATING PROCEDURES

SOP 3.1 Personal Protective Equipment

SOP Number: 3.1
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for the correct personal protective equipment for laboratory personnel.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. The appropriate personal protective equipment (PPE) assessments required for each workspace will be defined and an appropriate person appointed with responsibility for enforcement and training on the use of the appropriate PPE. Training will include the selection, use, decontamination of and deterioration of PPE. Research personnel should wear laboratory coats, safety eye protection and gloves.
2. PPE should never be worn in public areas. Hazards must be contained in public areas.
3. Safety glasses are required for research personnel and visitors to the laboratory and will be worn at all times when in the laboratory. Contact lenses shall be permitted in the laboratory.
4. Chemical goggles and/or a full-face shield will be worn during chemical transfer and handling operations as procedures dictate or when in a chemical storage area.
5. Sandals and bare feet are prohibited. Safety shoes are required where personnel routinely lift objects over 65 lbs.
6. Laboratory coats or aprons will be worn in the laboratory. Laboratory coats should be removed immediately upon discovery of significant contamination.
7. Appropriate chemical-resistant gloves will be worn when using chemicals and the manufacturer's instructions for use followed. Used chemical-resistant gloves should be inspected and washed prior to re-use. Damaged or deteriorated chemical-resistant gloves will be immediately replaced. Gloves should be washed prior to removal from the hands. Disposable gloves should not be reused.
8. Thermal-resistant gloves will be worn for operations involving the handling of cryogenic, heated materials and exothermic reaction vessels. Thermal-resistant gloves should be non-asbestos and should be replaced when damaged or deteriorated.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.2 Specimen Receipt

SOP Number: 3.2
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for specimen receipt.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure

1. Research laboratory personnel will be trained in the receipt and inspection of samples on arrival at the laboratory. All specimens will be inspected upon arrival, verifying that the correct specimen was received and that the information on the sample form, specimen collection container and sample identification, match each other. During this process of verification, appropriate handling temperature will be maintained.

2. If the integrity of the sample container is compromised, the proper amount of sample is not present, or the sample containers are not adequate this will be documented.
3. When the sample arrives in the research laboratory/biobank it must be registered and recorded in accordance with local laboratory procedure. The date and time that the specimen is received will be recorded in the study specific documentation or data management system.
4. All samples to be stored in a biobank facility must be labelled in accordance with local procedures. The following information is recommended:

   - Investigator: the name of the principal investigator who will take responsibility for that sample.
   - Research Study Name: the actual project or study to which the sample is assigned
   - Unique study identification number
   - Sample type: serum, DNA, urine etc.
   - Storage temperature: the temperature at which that sample is to be stored
   - Date and time of storage

   This information should also be recorded in the study specific documentation or data management system.

5. The following SOPs outline how different specimens should be processed and stored. All samples should be processing according to the temperatures specified in the individual SOPs.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.3 Preparation of Serum and Plasma from Blood

SOP Number: 3.3
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for preparation of serum and plasma from whole blood.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- A refrigerated centrifuge capable of generating 1,300 g
- −80°C Freezer

Procedure

1. Centrifuge tubes within *2 hours* of collection to separate plasma from cells. Maintain tubes at 4°C during processing. Place the blood collection tubes in a centrifuge and spin at 1,300 g for 10 min at 4°C. Record the time processing initiated in the study specific documentation or data management system.

2. Avoid mixing/agitation of tubes between centrifugation and aliquoting as this may lead to mixing and/or re-suspension of cells and platelets on or near the gel surface.
3. Using a plastic Pasteur/transfer pipette collect plasma or serum being sure to avoid collection of cells or the gel. Distribute the plasma among 0.5 mL cryostorage tube(s) maintained at 4°C which have been labeled with a unique study identification number and/or bar code label.
4. Transfer tubes to a −80°C freezer and/or liquid nitrogen for storage as soon as is practicable or within 48 hours of collection. Record the time of storage in the study specific documentation or the data management system.
   Note: As a general rule samples should be processed and reach the appropriate storage conditions as soon as is practicable. The maximum time limits proposed are guidelines and should be read in association with a study specific protocol.

Storage considerations

- All refrigerators and freezers used for samples should have controlled access. A system should be in place to record temperature either using a log book or electronically using an automated temperature recording device. A system should also be in place for sample tracking to facilitate sample retrieval/withdrawal etc.
- Samples should be stored in refrigerators and freezers, separate from standards and reagents.
- Freezing and thawing should be avoided. It is therefore important to prepare sample aliquots in volumes to avoid freeze/thaw cycles. Where this can not be avoided, the number and duration of thaws should be recorded.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.4 DNA Extraction from Blood

SOP Number: 3.4
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for DNA extraction from whole blood.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

General precautions

- Prior to commencing, read and understand the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (for example, isopropanol).
- Protective clothing, including laboratory coat, gloves and protective glasses, must be worn at all times when performing this procedure.

Considerations

- A number of different methods are available for the isolation of DNA from whole blood, including salting out/salt precipitation, phenol/chloroform extraction, silica gel extraction, proteinase K extraction and anion exchange. The choice of method depends on many factors including the required quantity, purity required for downstream application, time, molecular weight of DNA and expense. These guidelines propose the salting out method for DNA extraction from whole blood which appears to be the method of choice for use in molecular biology laboratories and is also used by the majority of biobankers within the P3G Consortium as highlighted in the following link: http://www.p3gobservatory.org/dna/comparisonTable.htm. The salting out method proposed is based on the method of Ciulla et al.[29]

- DNA can be isolated from whole blood (EDTA) or a cell pellet following plasma separation from an EDTA sample.
- DNA should be processed as soon as is practicable but a specimen can be stored at 4°C for 48 hours prior to processing or alternatively can be stored directly at −80°C for DNA processing at a later date.

Equipment/reagent requirements

- Red blood cell lysis buffer
- White blood cell lysis buffer
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- A centrifuge capable of generating 3,000 g
- A vortex mixer
- −80°C Freezer
- Incubator/water-bath capable of temperatures up to 65°C
- A spectrophotometer capable of reading 260 and 280 nm/Nanodrop

Procedure

Cell Lysis

1. Dispense 30 mL of red blood cell lysis buffer (NH_4CL, $NAHCO_3$, EDTA) into a 50 mL centrifuge tubes containing 5–10 mL whole blood. Incubate at RT for 5 min, inverting occasionally to mix.
2. Centrifuge the samples at 3,000 g for 10 min to pellet the white blood cells. Pour the supernatant to waste.
3. Add 10 mL white cell lysis buffer (SDS, EDTA) to white blood cell pellet and vortex vigorously for 10 sec. (RNAase may be added at this stage to remove RNA from the preparation). Incubate at 37°C for 15 min.

Protein Precipitation

4. Add 3.3 mL of ammonium acetate protein precipitation solution, and vortex vigorously for 20 sec at high speed.
5. Centrifuge for 5 min at 3,000 g. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.

DNA Precipitation

6. Dispense 10 mL isopropanol into a clean 50 mL centrifuge tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.
7. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge for 5 min at 3,000 g. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care not to dislodge the pellet.
8. Wash the DNA, by adding 10 mL of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge for 5 min at 3,000 g and drain to remove ethanol, ensure that the DNA pellet is not disturbed.

DNA Hydration

9. Add 0.3–1.0 mL of DNA hydration buffer (e.g. TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly.
10. The absorbance of the DNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260 nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50 ug/mL/ Nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at −80°C.
11. An aliquot of the DNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100 kbp and preferably exceeds 200 kbp.
    Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for DNA isolation should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.5 RNA Extraction from Blood

SOP Number: 3.5
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for RNA extraction from whole blood.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

General precautions

- Prior to commencing read and understand the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (chloroform, 2-mercaptoethanol, CTAB, isopropanol and isoamyl alcohol).
- Protective clothing, including laboratory coat, gloves and protective glasses, must be worn at all times when performing this procedure.
- Always use chloroform and 2-mercaptoethanol in a fume hood. When working in the fume hood, ensure the fan is on and the sash is lowered to the correct level as indicated by the arrows on the frame and sash.
- Extraction of good quality total RNA is vital to the production of high-quality expression data. Follow the procedure outlined and all necessary precautions for the preparation of the RNA to prevent degradation and/or contamination.

Considerations

- RNA should be extracted as soon as is practicable after sample collection. For best results, use either fresh samples or samples that have been quickly frozen and stored at -80°C.
- RNase inhibitors can be used to protect RNA from degradation both during isolation and purification and also in downstream applications such as reverse transcription into cDNA by RT-PCR.

Equipment/reagent requirements

- Hanks balanced salt solution
- Ficoll density gradient solution
- TRI reagent
- Chloroform
- Isopropanol
- Ethanol
- RNAse free water
- A refrigerated centrifuge capable of 12,000 g
- A vortex mixer
- -80°C Freezer
- A spectrophotometer capable of reading 260 nm and 280 nm Nanodrop
- BioRad Experion/Agilent Bioanalyser 2100

Procedure: The Trizol or Tri-reagent method (Sigma Aldrich/Ambion)

1. Homogenization. Cells are isolated as follows;
   a. Dilute 10 mL of blood/cell pellet with 10 mL of 1× Hanks balanced salt solution (HBSS)
   b. Layer this solution over 5 mL of a ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) and centrifuge in a 15 mL disposable plastic centrifuge tube for 15 min at RT at 2,000 g.
   c. A white band containing peripheral lymphocytes should be visible in each tube.

d. Remove and discard the sample above this and transfer the white band to a fresh 15 mL conical centrifuge tube.
e. Wash the cells by adding 10 mL of HBSS, mix thoroughly, and recover the cells by centrifugation for 10 min at RT at 2,000 g
f. Discard the supernatant and resuspend the cell pellet in 20 mL 1× TRI reagent. Store the lysate for 5 min at RT (18–22°C).

2. RNA extraction: Add 0.1 mL bromochloropropane or 0.2 mL of chloroform to the mixture and mix vigorously. Store sample for 2–15 min at RT (18–22°C). Centrifuge at 12,000 g for 15 min at 4°C.
3. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5 mL of Isopropanol and mix, then store at RT. Centrifuge at 12,000 g for 8 min at 4–25°C.
4. RNA wash: Mix RNA pellet with 1 mL of 75% ethanol. Centrifuge at 7,500 g for 5 min at 4–25°C.
5. Solubilization: Air dry the RNA pellet for 5–10. Dissolve by pipetting in 50–200 µl of DEPC treated RNA free water and incubate at 55–60°C for 10 min.
6. The absorbance of the RNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodrop method to assess purity. The 260/280 ratio should be >1.8. An A_{260} of 1.0 in a 1-cm light path is equivalent to a RNA concentration of 40 µg/ml. The RNA sample is aliquoted in RNase-free water and stored at −80°C.
7. An aliquot of the RNA from a representative sample for each batch may be also analysed by electrophoresis on a 0.3% a denaturing agarose gel. Briefly, the RNA solution should be diluted with RNA loading buffer 1:2 at 65°C for 10 min and loaded into the wells and electrophoresed for 40 mins at 60 V. The presence of two strongly staining bands, 28S and 18S ribosomal RNAs indicate intact RNA. Degradation is observed by a smear running down the length of the gel.
8. Total and/or mRNA quality and quantity may be assessed using automated electrophoresis systems such as the BioRad Experion or Agilent Bioanalyser 2100. Determination of RIN may also be used as a measure of quality.
Note: There are a number of commercially available kits for RNA extraction from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for RNA isolation should be recorded in the study specific documentation or the data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.6 Protein Extraction from Blood

SOP Number: 3.6

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Version Number 1.0

Purpose
This SOP describes the procedure for protein extraction from whole blood.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

General precautions

- Prior to commencing read and understand the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (chloroform, 2-mercaptoethanol, CTAB, isopropanol and isoamyl alcohol).

- Protective clothing, including laboratory coat, gloves and protective glasses, must be worn at all times when performing this procedure.
- Always use chloroform and 2-mercaptoethanol in a fume hood. When working in the fume hood, ensure the fan is on and the sash is lowered to the correct level as indicated by the arrows on the frame and sash.

Considerations

- 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 otherwise inert surfaces such as glass and plastic. If high concentrations of the native protein are unrealistic, addition of a second inert protein at high concentration will help prevent losses of protein on inert surfaces. Rinsing with EDTA solution prior to deionized water removes any possibility of contamination by metal ions.
- Vigorous shaking or stirring (e.g., vortex) can generate shear forces that in certain instances can destroy biological activity.
- When storing proteins antibacterial agents such as sodium azide can be added to inhibit bacterial growth. The addition of stabilizers such as glycerol helps prevent damage to the protein during freezing and thawing. Typical concentrations for glycerol are 10% to 50%. Although stable while frozen, repeated thawing and freezing of a sample can lead to degradation and loss of activity.

Equipment/reagent requirements

- Hanks balanced salt solution
- Ficoll density gradient solution
- Cell lysis solution
- Protease inhibitor solution
- A refrigerated centrifuge capable of 12,000 g
- A vortex mixer
- −80°C Freezer
- A spectrophotometer capable of reading 260 nm and 280 nm/Nanodrop

Procedure

1. Cells are isolated as follows;
   a) Dilute 10 mL of blood/cell pellet with 10 mL of 1× Hanks balanced salt solution (HBSS)
   b) Layer this solution over 10 mL of a ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) and centrifuge in a 50 mL disposable plastic centrifuge tube for 15 min at RT at 2,000 g with BRAKE OFF.
   c) A white band containing peripheral lymphocytes should be visible in each tube.
   d) Remove and discard the sample above this and transfer the white band to a fresh 15 mL centrifuge tube.
   e) Wash the cells by adding 10 mL of HBSS, mix thoroughly, and recover the cells by centrifugation for 10 min at RT at 2,000 g
2. Discard the supernatant and resuspend the cell pellet in Cell Lysis Buffer. The volume of CelLytic™-M lysis/extraction reagent (Sigma Aldrich) to be added to the cells varies according to cell size and protein concentration required. In general: 125 µl CelLytic™-M is recommended for 10^6–10^7 cells.
3. A commercial protease inhibitor solution (for example P3840 Sigma-Aldrich) may be added to the CelLytic™-M reagent to reach a final concentration of 1× in the buffer.
4. Centrifuge the lysed cells for 15 min at 12,000–20,000 g at 4°C to pellet the cellular debris.
5. Remove the protein-containing supernatant to a 0.5 mL cryostorage tube chilled to 4°C and store at −80°C.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.7 Peripheral Blood Mononuclear Cell Isolation from Blood

SOP Number: 3.7
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for preparation of peripheral blood mononuclear cells (PBMCs) from whole blood.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Hanks balanced salt solution
- Ficoll density gradient centrifugation solution
- 10% FCS RPMI (500 mL RPMI, 10 mL HEPES, 5 mL Penstrep, 4 mLl Fungizone, 50 mL FCS)
- RPMI (500 mL RPMI, 10 mL HEPES, 5 mL Penstrep)
- Trypan blue
- DMSO/Fetal calf serum
- Hemocytometer counting chamber
- Light microscope

Procedure
Using Standard Ficoll Density Gradient Centrifugation

1. Dilute 10 mL of blood with 10 mL of 1× Hanks balanced salt solution (HBSS)
2. Place 10 mL ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) in a 50 mL disposable plastic centrifuge tube (X2 per patient).
3. Using a sterile Pasteur pipette, carefully layer 10 mL of the diluted blood onto the Ficoll density gradient centrifugation solution so that two distinct layers are formed (i.e. the diluted blood should not penetrate the ficoll density gradient centrifugation solution and should remain floating on top of it).
4. Centrifuge the solution at 400 g for 30 min at RT. Ensure centrifuge BRAKE IS OFF (sudden drop in G force would cause the layers to mix at the end of spin).
5. Carefully remove the interface "Buffy" layer (containing T and B lymphocytes, monocytes and NK cells), using a sterile Pasteur pipette, ensure that no red blood cells are removed and that minimum amounts of the ficoll density gradient centrifugation solution is removed.
6. Wash cells in 10% FCS RPMI solution by resuspending in 20 mL and centrifuge at 500 g for 15 mins at 4°C (BRAKE ON).
7. Carefully pour off the supernatant. Rewash cells in 20 mL of 10% FCS RPMI solution at 500 g for 10 mins at 4°C (BRAKE ON).
8. Resuspend the pellet in 10% RPMI solution to a final volume of 1–10 mL.
9. Perform cell count as described in the "*cell count*" section within this SOP.
   Note: For BD CPT follow the manufacturer's instructions outlined in the information for use.

Cell count

1. A hemocytometer slide is used to perform cell counts using trypan blue exclusion dye stain to count viable cells.
2. Add 20 µl trypan blue to 100 µl cell suspension, mix by gently vortexing or by aspirating the full volume of the suspension at least twice and incubate for 2 min at RT.
3. A 20 µl sample of the mixture is applied to the haemocytometer counting chamber and the cells are visualized by light microscopy.

4. Viable cells exclude the dye and remain clear while dead cells stain blue. PBMCs in the four outer quadrants are counted and an average obtained.
5. Count PBMCs consistently on the borders (i.e., if including cells that fall on the upper or left line of the chamber then exclude cells that fall on the lower or right line of the chamber).
6. The number of cells is determined as follows: total count/4 (average number of viable cells)$\times$1.2 (dilution factor)$\times 1\times 10^4$ (area under coverslip) = viable cells/mL.

Cell freezing

1. Following counting cells are centrifuged at 2,000 g for 10 min at RT with the brake on.
2. Decant supernatant and resuspend the cells carefully and slowly in 1 mL of DMSO/FCS (1:9 mix) per 10×10^6 cells, maintained at 4°C.
3. Swiftly aliquot the sample into labeled cryovials maintained at 4°C and place in a control rate freezer or in a −80°C freezer overnight within a Styrofoam container with a lid, cells should reach a −80°C freezer within 24 hours of collection.
4. Transfer to liquid nitrogen vapour phase for long term storage, cells should reach liquid nitrogen within 48 hours of collection. Record the time of storage in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.8 Processing of Urine

SOP Number: 3.8
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for processing of urine for storage.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Phosphate Buffered Saline
- A refrigerated centrifuge capable of 1,500 g
- −80°C freezer

Procedure

1. Urine samples should be centrifuged to remove cells prior to storage.
2. The cells shall be separated by centrifugation at 1,500 g at 4°C for 10 min.

3. Collect the supernatant and aliquot into cryostorage tubes for storage at −80°C.
4. The remaining cells at the bottom of the tube are washed by the addition of 5 mL of 1× PBS and centrifuged at 1,500 g at 4°C for 5 min.
5. Prepare cells in aliquots using 0.5 mL cryostorage tubes and store at −80°C.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.8.1 DNA Extraction from Cells in Urine

SOP Number: 3.8.1
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for DNA extraction from cells in urine.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- DNA extraction buffer
- Proteinase K
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- An incubator/water-bath capable of 30°C
- A refrigerated centrifuge capable of 12,000 g
- −80°C Freezer
- A spectrophotometer capable of reading absorbance at 260 nm and 280 nm/Nanodrop
- Vortex mixer

Procedure

1. Resuspend the cell pellet from step 4 in SOP 3.8 in 20 mLl DNA extraction buffer (0.1 M EDTA, 0.2 M NaCl, 0.05 M Tris-HCl, pH 8.0, 0.5% SDS, 50 μg/mL DNAse free RNAse).
2. Add proteinase K to a final concentration of 100 μg/mL and gently swirl the beaker to mix the components. Incubate the beaker at 37°C for at least 3 hours, preferably overnight, with gentle agitation. This can be achieved using a shaking water bath or by occasional swirling by hand. The solution should be reasonably clear and viscous at the end of the incubation.
3. Add 100 μL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.
4. Centrifuge at 12,000 g for 5 min. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.
5. Dispense 300 μL isopropanol into a clean 1.5 mL Eppendorf tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.

6. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge at 12,000 g for 3 min. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
7. Wash the DNA, by adding 300 µL of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge at 2,000 g at 4°C for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.
8. Add 50 µL of DNA hydration buffer (TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly.
9. The absorbance of the DNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260 nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50 ug/mL/ Nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at −80°C.
10. An aliquot of the DNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100 kbp and preferably exceeds 200 kbp.
    Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit.
    The method used for DNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.8.2 RNA Extraction from Cells in Urine

SOP Number: 3.8.2
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for RNA extraction from cells in urine.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Hanks balanced salt solution
- Ficoll density gradient solution
- TRI reagent
- Chloroform

- Isopropanol
- Ethanol
- RNAse free water
- An incubator/water-bath capable of generating a temperature of 65°C
- A refrigerated centrifuge capable of 12,000 g
- A vortex mixer
- −80°C Freezer
- A spectrophotometer capable of reading 260 and 280 nm/Nanodrop
- BioRad Experion/Agilent Bioanalyser 2100

Procedure

1. Resuspend the cell pellet from step 4 in SOP 3.8 in 20 mL 1× TRI reagent. Store the homogenate for 5 min at RT (18–22°C).
2. RNA extraction: Add 0.1 mL bromochloropropane or 0.2 mL of chloroform to the mixture and mix vigorously. Store sample for 2–15 min at RT (18–22°C). Centrifuge at 12,000 g for 15 min at 4°C.
3. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5 mL of isopropanol and mix, then store for 5–10 min at RT. Centrifuge at 12,000 g for 8 min at 4–25°C.
4. RNA wash: Mix RNA pellet with 1 mL of 75% ethanol. Centrifuge at 7,500 g for 5 min at 4–25°C.
5. Solubilization: Air dry the RNA pellet for 5–10 min. Dissolve by pipetting in 50–200 μl of FORMAzol, 0.5%SDS, or DEPC treated RNAse free water and incubate at 55–60°C for 10 min.
6. The absorbance of the RNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodrop method to assess purity. The 260/280 ratio should be >1.8. An A_{260} of 1.0 in a 1-cm light path is equivalent to a RNA concentration of 40 μg/mL. The RNA sample is aliquoted and stored at −80°C.
7. An aliquot of the RNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel in denaturing conditions. Briefly the RNA solution should be diluted with RNA loading buffer at a ratio of 1:2 and heated at 65°C for 10 min and loaded into the wells in the gel and electrophoresed for 40 min at 60 V. The presence of two strongly staining bands, 28S and 18S ribosomal RNAs indicate intact RNA. Degradation is observed by a smear running down the length of the gel.
8. Total and/or mRNA quality and quantity can also be assessed using automated electrophoresis systems such as the BioRad Experion or Agilent Bioanalyser 2100. Determination of RIN may also be used as a measure of quality.
   Note: There are a number of commercially available kits for RNA extraction from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for RNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.8.3 Protein Extraction from Cells in Urine

SOP Number: 3.8.3
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for protein extraction from cells in urine.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Cell lysis solution
- Protease inhibitor solution
- A refrigerated centrifuge capable of 12,000 g
- A vortex mixer
- −80°C Freezer

Procedure

1. Resuspend the cell pellet from step 4 in SOP 3.8 in Cell Lysis Buffer. The volume of CelLytic™-M lysis/extraction reagent (Sigma Aldrich) to be added to the cells varies according to cell size and protein concentration required. In general: 125 µl CelLytic™-M is recommended for 10^6–10^7 cells.
2. A commercial protease inhibitor solution (for example P3840 Sigma-Aldrich) may be added to the CelLytic™-M reagent to reach a final concentration of 1× in the buffer.
3. Centrifuge the lysed cells for 15 minutes at 12,000 g at 4°C to pellet the cellular debris.
4. Remove the protein-containing supernatant to a test tube chilled to 4°C and store at −80°C in aliquots using 0.5 mL cryostorage tubes.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.9 Processing of Buccal Swabs for DNA Extraction

SOP Number: 3.9
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for DNA extraction from buccal swabs.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Hanks balanced salt solution
- Ficoll density gradient centrifugation solution

- Proteinase K solution
- Cell Lysin Solution
- Protein precipitation solution
- DNA hydration solution
- Ethanol
- Isopropanol
- A refrigerated centrifuge capable of 2,000 g
- A vortex mixer
- −80°C Freezer
- An incubator/water-bath capable of generating a temperature of 65°C
- A spectrophotometer capable of reading 260 and 280 nm/Nanodrop

Considerations

RNA and protein extraction is not recommended from cheek cells because of the small number of cells.

Procedure

1. Aliquot 450 µl of Extraction Solution to each labeled microcentrifuge tube.
2. Carefully unwrap cheek swab and place cotton tip into the Extraction Solution.
3. Incubate samples for 30 min at 65°C.
4. Add 100 µL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.
5. Centrifuge at 12,000 g for 5 min. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.
6. Dispense 300 µL isopropanol into a clean 1.5 mL Eppendorf tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.
7. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge at 12,000 g at 4°C for 3 min. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
8. Wash the DNA, by adding 10 mL of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge at 12,000 g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.
9. Add 50 µl of DNA hydration buffer (e.g. TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly.
10. The absorbance of the DNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260 nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50 ug/ml/ Nanodrop. The DNA sample is aliquoted into cryostorage tubes and stored at −80°C.
11. An aliquot of the DNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100 kbp and preferably exceeds 200 kbp.
    Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for DNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.10 Processing of Feces for DNA extraction

SOP Number: 3.10
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for DNA extraction from fecal cells.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

General considerations

- Fecal samples should be collected as fresh as possible within 24 hours of defecation otherwise the DNA will be severely degraded.
- DNA is isolated from the sloughed intestinal cells that line the feces, therefore it is important that the general shape of the feces is kept intact.

Equipment/reagent requirements

- DNA extraction buffer
- Proteinase K
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- An incubator/water-bath capable of 30°C
- A centrifuge capable of 3,000 g
- A spectrophotometer capable of reading absorbance at 260 and 280 nm/Nanodrop
- Vortex mixer.

Procedure

1. Resuspend feces in 20 mL DNA extraction buffer (0.1 M EDTA, 0.2 M NaCl, 0.05 M Tris-HCl, pH 8.0, 0.5% SDS, 50 µg/mL DNAse free RNAse).
2. Add proteinase K to a final concentration of 100 µg/mL and gently swirl the beaker to mix the components. Incubate the beaker at 37°C for at least 3 hrs, preferably overnight, with gentle agitation. This can be achieved using a shaking water bath or by occasional swirling by hand. The solution should be reasonably clear and viscous at the end of the incubation.
3. Add 3.3 mL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.
4. Centrifuge at 3,000 g for 5 min. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.

5. Dispense 10 mL isopropanol into a clean 50 mL centrifuge tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.
6. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge 3,000 g for 5 min. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
7. Wash the DNA, by adding 10 mL of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge at 3,000 g for 5 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.
8. Add 300 µl of DNA hydration buffer (TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly.
9. The absorbance of the DNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260 nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50 ug/mL/ Nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at −80°C
10. An aliquot of the DNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100 kbp and preferably exceeds 200 kbp.
    Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for DNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.11 Processing of Tissue Samples

SOP 3.11.1 DNA Extraction from Frozen Tissue

SOP Number: 3.11.1
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for DNA extraction from frozen tissue.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

General considerations

- There is a number of tissue types including paraffin-embedded, formalin fixed and frozen tissue and protocols for extraction of DNA, RNA and protein will vary accordingly to tissue type as outlined in the following SOPs.
- Lysis time will vary from sample to sample depending on the type and amount of tissue processed.

- Yields will depend both on the size and the age of the sample processed. Reduced yields would be expected from frozen tissue compared to fresh. Therefore eluting DNA in 50–100 µl TE (10 mM TRIS-HCL PH 8.0, 1 mM EDTA) buffer is recommended.

Equipment/reagent requirements

- Phosphate Buffered Saline
- DNA extraction buffer
- Proteinase K
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- An incubator/water-bath capable of 65°C
- A vortex mixer
- A refrigerated centrifuge capable of 14,000 g
- A spectrophotometer capable of reading absorbance at 260 and 280 nm/Nanodrop

Procedure

1. Homogenise 3–5 mg of tissue in sterile PBS using a mechanical homogeniser according to the manufacturer's instructions for use and centrifuge at 14,000 g for 10 min at 4°C. Discard supernatant and resuspend the tissue pellet in 180 ul of DNA extraction buffer (0.1 M EDTA, 0.2 M NaCl, 0.05 M Tris-HCl, pH 8.0, 0.5% SDS, 50 DNAse free RNAse).
2. Add proteinase K to a final concentration of 100 µg/mL and gently swirl the eppendorf tube to mix the components. Incubate the beaker at 37°C for at least 3 hours, preferably overnight, with gentle agitation. This can be achieved using a shaking water bath or by occasional swirling by hand. The solution should be reasonably clear and viscous at the end of the incubation.
3. Add 100 ul of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.
4. Centrifuge at 12,000 g for 5 min. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.
5. Dispense 300 µl isopropanol into a clean 1.5 mL Eppendorf tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.
6. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge at 12,000 g at 4°C for 3 min. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
7. Wash the DNA, by adding 300 µl of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge at 12,000 g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.
8. Add 50 µl of DNA hydration buffer (TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly at 12,000 g for 5 min.
9. The absorbance of the DNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260 nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50 ug/ml/ Nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at −80°C
10. An aliquot of the DNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100 kbp and preferably exceeds 200 kbp.
    Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for DNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.11.2 DNA Extraction Paraffin Embedded Tissue

SOP Number: 3.11.2
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for DNA extraction from paraffin embedded tissue.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Toluene/xylene
- DNA extraction buffer
- Proteinase K
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- An incubator/water-bath capable of 65°C
- A vortex mixer
- A refrigerated centrifuge capable of 20,000 g
- A spectrophotometer capable of reading absorbance at 260 and 280 nm/Nanodrop

Procedure

1. Place a small section (not more than 25 mg) of paraffin-embedded tissue in a 2 mL microcentrifuge tube.
2. Add 300 µL xylene or toluene. Vortex vigorously.
3. Centrifuge at 12,000–20,000 g for 5 min at RT.
4. Remove supernatant by pipetting. Do not remove any of the pellet.
5. Add 300 µL ethanol (96–100%) to the pellet to remove residual xylene or toluene and mix gently by vortexing.
6. Centrifuge at 12,000–20,000 g for 5 min at RT.
7. Carefully remove the ethanol by pipetting. Do not remove any of the pellet.
8. Repeat steps 5–7.
9. Incubate the open microcentrifuge tube at 37°C for 10–15 min until the ethanol has evaporated.
10. Resuspend the tissue pellet in 180 ul of extraction buffer (0.1 M EDTA, 0.2 M NaCl, 0.05 M Tris-HCl, pH 8.0, 0.5% SDS, 50 µg/mL DNAse free RNAse).
11. Add proteinase K to a final concentration of 100 µg/mL and gently swirl the tube to mix the components. Incubate the tube at 37°C for at least 3 hrs, preferably overnight, with gentle agitation. This can be achieved using a shaking water bath or by occasional swirling by hand. The solution should be reasonably clear and viscous at the end of the incubation.
12. Add 100 µl of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.
13. Centrifuge at 12,000 g for 5 min. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.
14. Dispense 300 µl isopropanol into a clean 1.5 mL eppendorf tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.
15. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge at 12,000 g for 3 min. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.

16. Wash the DNA, by adding 300 µl of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge at 2,000 g at 4°C for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.
17. Add 50 µl of DNA hydration buffer (TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly.
18. The absorbance of the DNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260 nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50 ug/mL/ Nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at −80°C.
19. An aliquot of the DNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100 kbp and preferably exceeds 200 kbp.
    Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for DNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.11.3 DNA Extraction from Formalin Fixed Tissue

SOP Number: 3.11.3
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for DNA extraction from formalin fixed tissue.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- DNA extraction buffer
- Proteinase K
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- An incubator/water-bath capable of 65°C
- A vortex mixer
- Homogenizer

- A refrigerated centrifuge capable of 14,000 g
- A spectrophotometer capable of reading absorbance at 260 and 280 nm/Nanodrop

Procedure

1. Place tissue sample (5–10 mg) in a centrifuge tube and wash twice by addition of 1× PBS and centrifuge at 2,000 g for 5 min at 4°C.
2. Homogenize tissue in sterile PBS. Centrifuge at 14,000 g for 10 min at 4°C. Discard supernatent and resuspend the cell pellet in 180 ul of DNA extraction buffer (0.1 M EDTA, 0.2 M NaCl, 0.05 M Tris-HCl, pH 8.0, 0.5% SDS, 50µg/mL DNAse free RNAse).
2. Add proteinase K to a final concentration of 100 µg/mL and gently swirl the beaker to mix the components. Incubate the beaker at 37°C for at least 3 hours, preferably overnight, with gentle agitation. This can be achieved using a shaking water bath or by occasional swirling by hand. The solution should be reasonably clear and viscous at the end of the incubation.
3. Add 100 µl of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.
4. Centrifuge at 12,000 g for 5 min. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.
5. Dispense 300 µl isopropanol into a clean 1.5 mL eppendorf tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.
6. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge at 12,000 g at 4°C for 3 min. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
7. Wash the DNA, by adding 300 µl of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge at 12,000 g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.
8. Add 50 µl of DNA hydration buffer (e.g. TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly.
9. The absorbance of the DNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260 nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50 ug/ml/ Nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at −80°C.
10. An aliquot of the DNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100 kbp and preferably exceeds 200 kbp.
    Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for DNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.11.4 RNA Extraction from Tissue

SOP Number: 3.11.4
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for RNA extraction from tissue.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

General considerations

- RNA can be extracted from formalin fixed and wax embedded tissue but the products are shorter and fragmented, so it is preferable to use the fresh tissue or stored frozen.
- RNAse inhibitors can be used to protect RNA from degradation both during isolation and purification and also in downstream applications such as reverse transcription into cDNA by RT-PCR.

Equipment/reagent requirements

- TRI reagent
- Chloroform
- Isopropanol
- Ethanol
- Homogenizer
- An incubator/water-bath capable of 55–60°C
- RNAse free water
- A refrigerated centrifuge capable of 12,000 g
- A vortex
- −80°C Freezer
- A spectrophotometer capable of reading 260 and 280 nm/Nanodrop
- BioRad Experion/Agilent Bioanalyser 2100

Procedure

1. Homogenisation: The tissue should be homogenized in 1 mL TRI reagent per 50–100 mg tissue.
2. Resuspend the cells in 20 m 1× TRI reagent. Store the homogenate for 5 min at RT (18–22°C).
3. RNA extraction: Add 0.1 ml bromochloropropane or 0.2 mL of chloroform to the mixture and mix vigorously. Store sample for 2–15 min at RT (18–22°C). Centrifuge at 12,000 g for 15 min at 4°C.
4. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5 mL of isopropanol and mix, then store for 5–10 min at RT. Centrifuge at 12,000 g for 8 min at 4–25°C.
5. RNA wash: Mix RNA pellet with 1 ml of 75% ethanol. Centrifuge at 7,500 g for 5 min at 4–25°C.
6. Solubilization: Air dry the RNA pellet for 5–10 min. Dissolve by pipetting in 50–20 ul of FORMAzol, 0.5%SDS, or DEPC treated RNAase free water and incubate at 55–60°C for 10 min.
7. The absorbance of the RNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodrop method to assess purity. The 260/280 ratio should be >1.8. An A_{260} of 1.0 in a 1-cm light path is equivalent to a RNA concentration of 40 μg/ml. The RNA sample is aliquoted and stored at −80°C.
8. An aliquot of the RNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel in denaturing conditions. Briefly the prepared RNA should be treated with RNA loading buffer 1:2 at 65°C for 10 min and loaded into the wells for electrophoresis. The presence of two strongly staining bands, 28S and 18S ribosomal RNAs indicate intact RNA. Degradation is observed by a smear running down the length of the gel.
9. Total and/or mRNA quality and quantity can also be assessed using automated electrophoresis systems such as the BioRad Experion or Agilent Bioanalyser 2100. Determination of RIN may also be used as a measure of quality.
   Note: There are a number of commercially available kits based for RNA extraction from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for RNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.11.5 Protein Extraction from Tissue

SOP Number: 3.11.5
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for protein extraction from tissue.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Cell lysis solution
- Proteinase inhibitor solution
- Homogeniser
- A refrigerated centrifuge capable of 12,000 g
- A vortex
- −80°C Freezer
- A spectrophotometer capable of reading 260 and 280 nm

Procedure

1. Homogenization: The tissue should be homogenised in 1 ml Cell Lysis Buffer (CelLytic™-M lysis/extraction reagent, Sigma Aldrich).
2. A commercial protease inhibitor solution (for example P3840 Sigma-Aldrich) may be added to the CelLytic™-M reagent to reach a final concentration of 1× in the buffer.
3. Centrifuge the lysed cells for 15 minutes at 12,000–20,000 g at 4°C to pellet the cellular debris.
4. Remove the protein-containing supernatant to a test tube chilled to 4°C and store at −80°C.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.12 Processing of Cultured Cells

SOP Number: 3.12
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for processing cultured cells prior to DNA, RNA or protein extraction.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Phosphate Buffered Saline
- Trypsin
- Cell Culture Media
- A refrigerated centrifuge capable of 2,000 g

Procedure

1. Cell cultures should be washed and split prior to DNA, RNA, or protein extraction.
2. Remove the media from the flask or dish and add 5 mL of 1× PBS.
3. Remove the 1× PBS and then add 1–2 mL of trypsin 0.25% (pre-warm at 37°C) for a few minutes, until the cells float in the medium.
4. Add 2–3 volumes of serum containing cell culture medium to inhibit the action of trypsin and transfer the medium into a centrifuge tube.
5. Centrifuge at 1,200 g at 4°C for 5 min, remove the supernatant and refer to the following SOPs based on the derivate required.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.12.1 DNA Extraction from Cultured Cells

SOP Number: 3.12.1
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for DNA extraction from cultured cells.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Cell lysis buffer
- Proteinase K
- Phosphate buffered saline
- Protein precipitation solution
- Isopropanol
- Ethanol
- DNA hydration buffer
- An incubator/water-bath capable of 65°C
- A vortex
- A refrigerated centrifuge capable of 12,000 g
- A spectrophotometer capable of reading absorbance at 260 and 280 nm/Nanodrop

Procedure

1. Resuspend 10^6 cells in 125 µl Cell Lysis Buffer (CelLytic™-M lysis/extraction reagent, Sigma Aldrich).
2. Add proteinase K to a final concentration of 100 µg/mL and gently swirl the eppendorf tube to mix the components. Incubate the Eppendorf tube at 37°C for at least 3 hours, preferably overnight, with gentle agitation. This can be achieved using a shaking water bath or by occasional swirling by hand. The solution should be reasonably clear and viscous at the end of the incubation.
3. Add 100 µl ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.
4. Centrifuge at 12,000 g for 5 min. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step.
5. Dispense 300 µl isopropanol into a clean 1.5 mL eppendorf tube and add the supernatant from the previous step. It is important to ensure that the protein pellet is not dislodged during pouring.
6. Mix by inverting gently 50 times until the DNA is visible as threads or a clump. Centrifuge at 12,000 g for 3 min. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
7. Wash the DNA, by adding 300 µl of ethanol (70% v/v) and vortex at medium speed for 5 sec to dislodge the pellet from the bottom of the tube. Centrifuge at 12,000 g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.
8. Add 50 µl of DNA hydration buffer (TE buffer) and vortex for 5 sec at medium speed to mix. Incubate at 65°C for 1 hour to dissolve DNA. Incubate at RT overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Centrifuge samples briefly.
9. The absorbance of the DNA at 260 nm and 280 nm should be measured using quartz cuvets or the nanodroplet method to assess purity. A 260/280 ratio between 1.8 and 2.0 is desirable. A 260/280 ratio greater than 2.0 may indicate solvent contamination and a ratio less than 1.8 may indicate protein contamination. DNA concentration can be measured using absorbance at 260 nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50 ug/ml/ Nanodrop. The DNA sample is aliquoted into cryostorage tubes and stored at −80°C
10. An aliquot of the DNA from a representative sample from each batch may be analysed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100 kbp and preferably exceeds 200 kbp.
    Note: There are a number of commercially available kits based on the salting out methods from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for DNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.12.2 RNA Extraction from Cultured Cells

SOP Number: 3.12.2
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for RNA extraction from cultured cells.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- TRI reagent
- Chloroform
- Isopropanol
- Ethanol
- RNAse free water
- Homogeniser
- An incubator/water-bath capable of 55–60°C
- A refrigerated centrifuge capable of 12,000 g
- A vortex mixer
- −80°C Freezer
- A spectrophotometer capable of reading 260 and 280 nm/Nanodrop
- BioRad Experion or Agilent Bioanalyser 2100

Procedure

1. Resuspend 5–10×10^6 cells per 1 mL TRI-reagent. Store the homogenate for 5 min at RT (18–22°C).
2. RNA extraction: Add 0.1 mL bromochloropropane or 0.2 mL of chloroform to the mixture and mix vigorously. Store sample for 2–15-min at RT (18–22°C). Centrifuge at 12,000 g for 15 min at 4°C.
3. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5 mL of isopropanol and mix, then store for 5–10 min at RT. Centrifuge at 12,000 g for 8 min at 4–25°C.
4. RNA wash: Mix RNA pellet with 1 mL of 75% ethanol. Centrifuge at 7,500 g for 5 min at 4–25°C.
5. Solubilization: Air dry the RNA pellet for 5–10 min. Dissolve by pipetting in 50–200-µl of FORMAzol, 0.5%SDS, or DEPC treated RNAse free water and incubate at 55–60°C for 10 min.
6. The absorbance of the RNA at 260 nm and 280 nm should be measured using quartz cuvets or nanodrop method to assess purity. The 260/280 ratio should be >1.8. An A_{260} of 1.0 in a 1-cm light path is equivalent to a RNA concentration of 40 µg/ml. The RNA sample is aliquoted and stored at −80°C.
7. An aliquot of RNA from a representative sample for each batch may be analysed by electrophoresis through a 0.3% agarose gel in denaturing conditions. Briefly the RNA solution should be diluted with RNA loading buffer 1:2, heated at 65°C for 10 min and loaded into the wells and electrophoresed for 40 min at 60 V. The presence of two strongly staining bands, 28S and 18S ribosomal RNAs indicate intact RNA. Degradation is observed by a smear running down the length of the gel.
8. Total and/or mRNA quality and quantity can also be assessed using automated electrophoresis systems such as the BioRad Experion or Agilent Bioanalyser 2100. Determination of RIN may also be used as a measure of quality. Determination of RIN may also be used as a measure of quality.
   Note: There are a number of commercially available kits based for RNA extraction from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/package insert included with the kit. The method used for RNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 3.12.3 Protein Extraction from Cultured Cells

SOP Number: 3.12.3
Version Number 1.0

	Name	Title	Date
Author			
Authorizer			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for protein extraction from cultured cells.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Cell lysis solution
- Protease inhibitor solution
- A refrigerated centrifuge capable of 20,000 g
- A vortex mixer
- −80°C Freezer

Procedure

1. Resuspend the cells in Cell Lysis Buffer. The volume of CelLytic™-M lysis/extraction reagent (Sigma Aldrich) to be added to the cells varies according to cell size and protein concentration required. In general: 125 µl CelLytic™-M is recommended for 10^6–10^7 cells.
2. A commercial protease inhibitor solution (for example P3840 Sigma-Aldrich) may be added to the CelLytic™-M reagent to reach a final concentration of 1× in the buffer.
3. Centrifuge the lysed cells for 15 minutes at 12,000–20,000 g at 4°C to pellet the cellular debris.
4. Remove the protein-containing supernatant to a cryostorage tube chilled to 4°C and store at −80°C.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

Acknowledgments

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