MMI Guidelines for Standardised Biobanking

First Edition 2010

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FOREWORD

Molecular Medicine Ireland (MMI) was established in April 2008 through funding from the Higher Education Authority's Programme for Research in Third Level Institutions with the vision of improved healthcare through the development of diagnostics and therapies from concept to realisation. The mission of MMI is to mobilise the strengths of the five partner institutions and their associated hospitals to build a sustainable national system to coordinate, support and promote translational and clinical research. MMI has identified biobanking and in particular the importance of a national strategic approach to standardised biobanking as a key pillar for clinical and translational research in Ireland. MMI's vision for biobanking is the creation of an all-island, standardised and carefully phenotyped repository of biological specimens and associated clinical data, accessible to academic and industry researchers and to start-up and spin-out companies to underpin biomedical research and to fuel innovation and commercialisation.

Biomedically-relevant, quality-assessed biological materials and data are essential if clinical, academic and commercially-driven research is to diagnose, treat and prevent common and rare human diseases. The demand for large collections of standardised biological materials (including, blood, DNA, RNA, proteins, cells, tissue, urine and other body fluids) has dramatically increased. This demand stems from significant scientific advancements including the elucidation of the human genome, the revolution in high throughput technologies and the development of advanced molecular biology and bioinformatics tools used to investigate the genetic effects on human health and disease. Biomedically-relevant and quality assured biological specimens also provide a powerful and valuable resource for the biotechnology and pharmaceutical industry to develop and validate new biomarkers for screening, monitoring and treating diseases. Such biological resources, together with information derived from the human genome, are playing a key role in defining more targeted therapies for patients based on understanding an individual's genetic make-up, a particular example being herceptin in HER2-overexpressing breast cancer. Standardised and carefully phenotyped biological samples with associated clinical and environmental information are needed to drive advances in pharmacogenetics, disease screening, biomarker discovery and validation and to ensure competitiveness in the era of personalised medicine.

The development of these Guidelines evolved from the work undertaken by MMI to prepare the Design Phase of GeneLibrary Ireland funded by the Health Research Board and the Research and Development Office in Belfast. GeneLibrary Ireland, as proposed, would be an all-island reference library of donor samples and health information, providing a common

control group for a wide range of studies by health and biomedical scientists. GeneLibrary Ireland will offer a powerful and valuable resource for original health research on the genetics of the Irish population. In anticipation of a more strategic approach to biobanking and from the work of the Design Phase of GeneLibrary Ireland, MMI has developed these guidelines to standardise the collection, processing and storage of biological materials to ensure a level of consistency and harmonisation across the different clinical and research centres in Ireland. The use of standardised protocols for sample collection, processing and storage will help to provide the proper safeguards and assurances required for sample quality, consistency and integrity among bio-collections at different sites. This harmonisation will allow for the universal interchange of biological materials across sites and the amalgamation of samples for research studies. The importance of '*a national approach to biobanking'* and in particular that *'all Irish research centres and hospitals adopt standardised biobanking practices as a matter of priority*' has been highlighted in a recent report published by Forfas, *Health Life Sciences in Ireland – An Enterprise Outlook* 2009. MMI believes that the endorsement of these guidelines will serve as an important step in addressing these issues.

These guidelines have been drafted with reference to international best practice in biobanking. We hope that the adoption of these guidelines will help to provide a structured framework to standardise the collection of biological specimens across disease areas in Ireland and to ensure the consistency of high quality samples. We hope that all those engaged in biobanking, including research funding agencies, clinical research centres and hospitals, clinical networks and disease groups and patient organisations will adopt these guidelines as a significant step towards a more strategic approach to biobanking in Ireland.

On behalf of MMI, I would like to thank Dr Peter Doran, Principal Investigator of the Design Phase of GeneLibrary Ireland who proposed the development of these guidelines and Dr Jan Guerin, Progamme Manager for Research for her work in compiling these guidelines. I would also like to acknowledge the members of the Drafting Group; Dr Peter Doran, Ms Mary McGrath and Dr David Murray, UCD CRC, Dr Joe McPartlin, Trinity Biobank and Dr Jan Guerin, MMI who gave their time generously and shared their expertise in drafting these Guidelines for Standardised Biobanking.

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Chair of Molecular Medicine Ireland

THE INTERNATIONAL CONTEXT

I warmly welcome the publication of these guidelines for standardised biobanking issued by Molecular Medicine Ireland. Their value for biomedical research is international as well as national.

Biobanking has emerged as a term describing the application of quality management procedures to the existing practises of management of resources - samples and their annotations - for biomedical research.

The need for these quality management procedures arises from the fact that research focuses on increasingly fine distinctions 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 human samples and the various annotations describing them.

Moreover, the size of the population of patients or subjects to which biomedical scientists need access is growing continuously as we seek to distinguish ever smaller signals over noise. Hence both collection sizes and datasets are increasing rapidly.

This issue of increased size makes it imperative that biomedical scientists work together across jurisdictions. Failure to work together will leave a jurisdiction - even the largest - on the fringes of the global biotechnological revolution. Given the OECD's assessment[1] of the importance of this revolution for 21st century economies, no jurisdiction can ignore it.

If we are to work together, then we are obliged to develop interoperable procedures for managing samples and their annotations. The procedures must be technically and ethically acceptable to different jurisdictions so that there can be appropriate free movement of knowledge and materials between scientists across borders.

This is the vision of the European biobanking network project, BBMRI (Biobanking and BioMolecular Resources Research Infrastructure). This project, funded by the European Commission, is currently preparing plans for construction of this vital network for the European Research Area.

[1] Biological Resource Centres: Underpinning the Future of Life Sciences and Biotechnology. Organisation for Economic Co-Operation and Development. Paris 2001.

The plans will need the support of EU Member States. Those plans require not only detailed legal and ethical work, but also a clear vision of how European scientists are to manage interoperably their biomedical resources.

Some elements of this vision are already in place, thanks to the work of the OECD and WHO-IARC. Practising biobankers then need also to agree on practical proposals on how to carry out the operations of collecting, processing, storing, distributing and sharing samples and their annotations.

The publication by MMI of laboratory protocols is a significant and helpful step in this direction. It is significant because it demonstrates that MMI is supporting the work of international organisations and is enthusiastic in taking this work forward. It is significant because it demonstrates MMI's intent to promote collaborations with scientists in the other jurisdictions covered by BBMRI – and beyond. It is helpful because it demonstrates that Standard Operating Procedures can be made openly accessible: they are not 'lab secrets' to be guarded as though they were a recipe for a proprietary fizzy drink. Finally it is helpful to the scientists of MMI themselves: it prepares the ground for speedy progress in the construction of an advanced biobanking system integrated to European and international norms and able to play its full part in underpinning a major global industry of the 21st century.

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TABLE OF CONTENTS

1.0 Introduction

Biomedical research involving biological materials (blood, DNA, RNA, proteins, cells, tissue, urine and other body fluids) has dramatically increased over the last five years and will continue to rise (1-5). The demand for biological materials stems from significant scientific advancements in recent times, which include the ongoing elucidation of the human genome, the revolution in high throughput "*omics*" technologies and the development of highly advanced molecular biology and bioinformatics tools (1-5). The increasing demand and recognised value of biological samples with associated clinical and environmental data has led to the establishment of biobanks (6). A number of population and clinical biobanks have been established internationally including the HUNT Norway, UK Biobank, Generation Scotland, Estonian Population Biobank and the Wales Cancer Biobank (7-12). There are 123 large population-based cohorts described in the catalogue of the global biobanking project, the P3G Consortium (13).

While Ireland currently does not have a national population and/or clinical biobank there are disease specific bio-collections in oncology, cardiovascular disease, neuropsychiatric disorders and HIV that have been funded by research funding bodies. The Prostate Cancer Research Consortium is an example of a bio-resource that has been established as a multi-disciplinary, trans-institutional collaboration with a view to sharing, for medical research, tissue, blood and DNA from prostate cancer patients across Dublin (14).

There is a concerted effort nationally to create a harmonised and world-class clinical research infrastructure that will allow Ireland to be competitive in the era of personalised medicine. This has been facilitated through the significant investment by the Irish Government and the Wellcome Trust in the construction and implementation of major, world-class clinical research centres (CRCs) in Dublin, Cork and Galway which will be networked. A key element of these translational and clinical research initiatives is the opportunity presented to assemble and store collections of standardised biological specimens in a harmonised manner. These bio-collections need to be assembled in a way that allows them to be linked with other similar bio-collections at an all-island level if increased sample size is required. MMI led the preparation of the Design Phase of GeneLibrary Ireland funded by the Health Research Board and the Research and Development Office in Belfast. GeneLibrary Ireland, as proposed, would be an all-island reference library of donor samples and health information, providing a common control group for a wide range of studies by health and biomedical scientists. If all or part of the bio-collection is genotyped, GeneLibrary Ireland would offer a powerful and valuable resource for original health research on the genetics of the Irish population. The Design

Phase of GeneLibrary Ireland has provided the blueprint for a bio-resource infrastructure for biobanking to support disease bio-collections along with providing a valuable control sample with linked carefully phenotyped medical data for disease research which would be harmonised nationally and internationally (15).

In anticipation of a strategic national approach to standardise biobanking and building on the Design Phase of GeneLibrary Ireland MMI in association with its partners, has recognised the need to develop guidelines to standardise the collection, processing and storage of biological materials across the different clinical and research centres in Ireland. It is hoped that the adoption of these guidelines will help to provide a structured framework to standardise the collection of biological specimens across disease areas in Ireland and to ensure high quality samples.

The use of standardised protocols for sample collection, processing and storage will help to provide the proper safeguards and assurances required for sample quality, consistency and integrity among bio-collections at different sites. This harmonisation should allow for the universal interchange of biological materials across sites and the amalgamation of samples for research studies. In addition, standardisation of bio-collections will ensure that molecular changes identified in subsequent research studies will reflect biology and not process or sample variability. These guidelines provide some general principles for quality assurance in biological repositories based on international best practice. These standard procedures should be read in association with a study specific research protocol which may have individual study requirements, for example with regard to sample tubes, volume and immediate processing.

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. These issues have been addressed in detail in the report of the design phase of GeneLibrary Ireland, in international best practice guidelines and are the focus of BBMRI's Ethical, Legal and Societal Issues Working Group (15-19). These guidelines provide general principles for obtaining informed consent for use of human biological samples for research purposes which is at the cornerstone of research involving human subjects. In addition, the proposed Human Tissue Bill 2009 will provide the much needed legal framework to regulate the use of donated tissue from living persons for the purpose of research.

These standard procedures have been drafted with reference to international best practice guidelines in biobanking and validation studies conducted by the UK Biobank and reflect the

collective experience of MMI partners engaged in biobanking (19-25). Molecular Medicine Ireland would like to acknowledge the significant contribution made to the development of these guidelines by the UCD CRC, the Trinity Biobank and the Prostate Cancer Research Consortium in sharing their procedures and to all those researchers engaged in biobanking in Ireland who provided their input to these guidelines.

These guidelines have undergone international peer review for publication in the ISBER Biopreservation and Biobanking Journal (*Guerin J, Murray D, McGrath M, Yuille M, McPartlin J, Doran P. Molecular Medicine Ireland Guidelines for Standardised Biobanking. ISBER Biopreservation and Biobanking 2010, 8 (1) In press*).

2.0 Scope

The scope of these guidelines is to provide general guidance to standardise procedures for the collection, processing and storage of biological materials (including blood, urine, DNA, RNA cells and tissue) which can be used across different clinical and research centres in Ireland to ensure sample uniformity, quality and integrity.

All studies involving human biological materials require approval from a Recognised 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 are in place prior to initiation of any study involving human biological materials.

It is recommended that these SOPs are read in association with study specific SOPs.

These SOPs are also available in electronic format and can be downloaded from the MMI website at www.molecularmedicineireland.ie

2.1 Review

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.

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 analysed 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 annonymised.

5. If the research participant is a hospital in-patient or out-patient, 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 recognised 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 commercialisation 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 realised 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 which is used in a number of international biobanks where research participants will be asked to give generalised 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 recognises 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 standardised 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 (19-23). 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 programme. 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: A biorepository should develop and implement standardised 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/ stabilising 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 non conformances 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 centralised, 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 bio-resource 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 stabilsed 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 which 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 which 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 (22, 26, 27). 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

- Formalised 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 standardised procedures to evaluate the quality of nucleic acids such as DNA and RNA (28).
- 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 recognised 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			
Authoriser			

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			
Authoriser			

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 Faeces Collection

SOP Number: 1.4.2
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

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

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 faeces 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/faeces 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			
Authoriser			

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 immunisations as per local policy and procedures prior to working with potentially infectious materials. Regular controls of immunisation 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 standardised procedures.

8. Specimens of biological materials (for example 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			
Authoriser			

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			
Authoriser			

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 minimise personal and co-worker exposure to the chemicals in the laboratory. Based on the realisation that all chemicals inherently present hazards in certain conditions, exposure to all chemicals shall be minimised.

2. Research laboratory personnel will be required to read and understand the Material Safety Data Sheet (MSDS) and standard operating procedure 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			
Authoriser			

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			
Authoriser			

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^{o}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 minimise splashing and rapid cooling of the receiving container. Never overfill containers. Use dip sticks to check liquid depth in dewars. Do not use fingers. Use tongs when placing in or removing items from liquid nitrogen.

5. The area for use should be well ventilated. 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			
Authoriser			

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 (eg. 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 participant's 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 thestudy 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

Sample Type	Blood	Blood	Blood	Blood	Blood	Blood
Derivative	Plasma	Serum	DNA	RNA	Proteomics	Biochemistry
Tube type	EDTA PST with LH	SST Plain tube	EDTA	Paxgene (Qiagen) Tempus RNA tubes (Applied Biosystems)	P100, PST SST, plain tube	PST with LH Plain tube with LH SST
Order of collection	3	1	2	8	6	7
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
Transport to Lab*:						
Temp	4°C	4°C	4°C	4°C	4°C	4°C
Timing	ASAP or within 24hrs	ASAP or within 24hrs	ASAP or within 48hrs	ASAP or within 24hrs	ASAP or within 24hrs	Within 24hrs
Storage Temp^	-80°C	-80°C	-80°C	-80°C	NA	NA
Time to storage from collection*	ASAP or within 48hrs	ASAP or within 48hrs	ASAP or within 48hrs	ASAP or within 24hrs	ASAP or within 48hrs	NA

***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 1100-1600g 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

2.1 Overview Table for Sample Collection based on Analyte (continued)

Sample Type	Blood	Blood	Blood	Saliva	Urine	Faeces
Derivative	Haematology	Metabolomics	PBMC Lymphocyte immortalization	DNA	DNA, RNA Protein	DNA
Tube type	EDTA	PST with LH SST, plain tube	ACD, BD CPT LeukoSep	Sterile container Collection kit	Sterile container	Sterile container
Order of collection	9	5	4	NA	NA	NA
Immediate processing	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	RT (18-22°C)	4°C	18-22°C	18-22°C	18-22°C	18-22°C
Timing	Within 24hrs	ASAP or within 24hrs	ASAP or within 24hrs	ASAP or within 48hrs	ASAP or within 24hrs	ASAP or within 24hrs
Storage Temp	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*	NA	ASAP or within 48hrs	ASAP or within 48hrs	ASAP or within 48hrs	ASAP or within 48hrs	ASAP or within 48hrs

***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 1100-1600g 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

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.
   - Using a syringe for blood transfer may also cause over- or under-filling 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 under-filling 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, 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 – 3,000g 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/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.

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)

SOP 2.5 Blood Collection - Venipuncture

SOP Number: 2.5
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

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 arm-rest 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 centre 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			
Authoriser			

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 1,100-1,300g 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. Under-filling 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 1,300g for 10 min 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			
Authoriser			

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 1,100-1,600g 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 min 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 1,600g at RT (18-22°C) for 10 min. This speed, time and temperature will minimise 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 0.5mL 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			
Authoriser			

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. Under-filling 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			
Authoriser			

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. Under-filling 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. Under-filling or overfilling of the tube can affect laboratory results due to the incorrect blood/additive ratio.

2. Immediately after the Tempus tube is filled, stabilise the blood by shaking the tube vigorously or vortexing the contents for 10 seconds to ensure that the stabilising reagent makes uniform contact with the sample.
   IMPORTANT: Failure to mix the stabilising reagent with the blood leads to inadequate stabilisation 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. Under-filling 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			
Authoriser			

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 1,100-1,300g 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. Under-filling 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 1300g for 10 min 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 0.5mL 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			
Authoriser			

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 15mL 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 1,100-1,600g 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 min on ice (4°C). Note the clotting time in the study specific documentation or data management system.

5. Place tubes in the centrifuge and spin at 1600g for 10 min at 4°C. Maintain tubes at 4°C during processing. 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 coloured liquid) to a plain plastic 15mL conical centrifuge tube.

7. Add 100X protease inhibitor solution, for example 10uL of protease inhibitor solution to 1mL of serum and mix by inversion at least 8-10 times.

8. Using a plastic transfer/Pasteur pipette transfer serum into each 0.5mL 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			
Authoriser			

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 1,100-1,300g 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. Under-filling 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 1,300g for 10 min 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 0.5mL 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 (29).

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

SOP 2.5.8 Blood Collection for Haematology

SOP Number: 2.5.8
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for blood collection for haematological 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. Under-filling 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 Haematology 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			
Authoriser			

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 1,100-1,600g 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 min 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 1,600g for 10mins 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 0.5mL 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			
Authoriser			

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 1,100-1,300g 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. Under-filling 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			
Authoriser			

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 min 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 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			
Authoriser			

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 10mLs 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			
Authoriser			

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.

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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			
Authoriser			

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 and/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 Faeces Collection

SOP Number: 2.8
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for faeces 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 faeces container

Procedure:

1. Research personnel will greet the research participant, identify themselves, and then explain the faeces 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 faeces collection

3. The research participant will be correctly identified prior to faeces 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 faeces collection will be assembled prior to the procedure.

5. The research participant will be asked to provide a faeces sample. The specimen will be passed into a clean dry, disposable bedpan or similar container. The research personnel will then transfer the faeces 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 faeces.

7. A unique study identification number and/or bar code label will be applied to the sample immediately after faeces 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			
Authoriser			

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°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 2.10 Bronchoalveolar Lavage Collection using Bronchoscopy

SOP Number: 2.10
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for the collection of a bronchoalveolar lavage sample using bronchoscopy.

Responsibility

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

Procedure:

1. Explain and discuss the procedure with the participant and obtain written informed consent.

2. The participant's bronchoscopy is booked with the Endoscopy Dept. / Theatre in accordance with local policy and procedures.

3. The staff should ensure the Endoscopy Dept. / Theatre is informed, in advance, of any transferable infections the participant may have (e.g. MRSA, Hepatitis, TB, HIV).

4. The participant must have a legible armband and/or ankle band with their identifiers.

5. The participant ideally should fast (nothing to eat or drink) for at least 4-6 hours.

6. A doctor should insert an intravenous cannula prior to the procedure.

7. Suitable premedication prescribed by the medical team will be given as per local protocol prior to procedure.
8. Bronchoalveolar lavage (BAL) should be performed after general inspection of all the brochopulmonary segments and prior to any biopsies or brushing of the airway.

9. Advance the bronchoscope until it is wedged into a subsegment of the middle lobe or the anterior segment of a lower lobe.

10. Maintaining wedge position, apply gentle suction (50-80mmHg), collecting the lavage. The suction is turned down or off to prevent collapse of the airways during suctioning.

11. Infuse 50mL of sterile 0.9% saline with a syringe, wait a few seconds to dwell and apply constant suction for several seconds (20 seconds typically) until return of the frothy surfactant-rich fluid stops. The next aliquot is instilled and suction applied, repeat for all three aliquots.

12. Collected fluid is placed in a sterile container and stored preferably on ice until processing. The trash trap or hook up directly to the wall suction is then reapplied and bronchoscopy is performed as needed for biopsies and other procedures.

13. Return on BAL is quite variable (usually 40-60% recovery of total volume instilled), ideally in excess of 30mL of BAL fluid should returned for interpretation of results.

14. Record the date and time the BAL sample was collected on the collection tube and /or study specific documentation

15. The specimen should be stored in the refrigerator at $4^{o}C$ and transferred to the processing laboratory on ice as soon as is practicable.

16. Once the procedure is complete the participant is returned to the recovery area accompanied by the Endoscopy or Theatre nurse and porter as appropriate.

17. The participant's airway, respirations, pulse oximetry, pulse and blood pressure should be observed. One set of vital signs are required unless otherwise directed by the medical research team or clinically indicated, and recorded in the participants nursing notes.

18. Any abnormalities are immediately reported to the medical research team looking after the participant.
19. The participant remains fasting until the gag reflex returns and is alert and orientated; this normally takes 2-3 hours.

20. Once fully awake and deemed fit for discharge by the medical research team, the participant should be made aware of any instructions they need to follow on discharge and advised when and if they are to return for results.

21. The participant should not be discharged home unaccompanied and should be supervised for 10 hours. He/she should be advised not to operate machinery, drive or make any legal or binding decisions for 24 hours.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

2.11 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 Centres 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			
Authoriser			

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			
Authoriser			

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(s) 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			
Authoriser			

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 1,300g
- -80^{o}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 1,300g 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 0.5mL 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 cannot 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			
Authoriser			

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*, 1988 (30).
- 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 3,000g
- A vortex mixer
- -80^{o}C Freezer
- Incubator/water-bath capable of temperatures up to 65^{o}C
- A spectrophotometer capable of reading 260 and 280nm/ Nanodrop

Procedure

Cell Lysis

1. Dispense 30mL of red blood cell lysis buffer (NH_4CL, $NAHCO_3$, EDTA) into a 50mL centrifuge tubes containing 5-10mL whole blood. Incubate at RT for 5 min, inverting occasionally to mix.

2. Centrifuge the samples at 3,000g for 10 min to pellet the white blood cells. Pour the supernatant to waste.

3. Add 10mL 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 15min.

Protein Precipitation

4. Add 3.3mL of ammonium acetate protein precipitation solution, and vortex vigorously for 20 sec at high speed.

5. Centrifuge for 5 min at 3,000g. 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 10mL isopropanol into a clean 50mL 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 3,000g. 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 10mL 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 3,000g and drain to remove ethanol, ensure that the DNA pellet is not disturbed.

DNA Hydration

9. Add 0.3-1.0mL 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.

10. The absorbance of the DNA at 260nm and 280nm 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 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50ug/mL / nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at -80^{o}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 100kbp and preferably exceeds 200kbp.

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			
Authoriser			

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 12,000g
- A vortex mixer
- -80^{o}C Freezer
- A spectrophotometer capable of reading 260 and 280nm/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 10mL of blood/ cell pellet with 10mL of 1X Hanks balanced salt solution (HBSS).
   b. Layer this solution over 5mL of a ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) and centrifuge in a 15mL disposable plastic centrifuge tube for 15 min at RT at 2,000g.
   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 15mL conical centrifuge tube.
   e. Wash the cells by adding 10mL of HBSS, mix thoroughly, and recover the cells by centrifugation for 10 min at RT at 2,000g.
   f. Discard the supernatant and resuspend the cell pellet in 20mL 1X TRI reagent. Store the lysate for 5min at RT (18-22°C).

2. RNA extraction: Add 0.1mL bromochloropropane or 0.2mL of chloroform to the mixture and mix vigorously. Store sample for 2-15 min at RT (18-22°C). Centrifuge at 12,000g for 15min at 4°C.

3. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5mL of Isopropanol and mix, then store for 5-10 min at RT. Centrifuge at 12,000g for 8min at 4-25°C.

4. RNA wash: Mix RNA pellet with 1mL of 75% ethanol. Centrifuge at 7,500g for 5 min at 4-25°C.

5. Solubilisation: Air dry the RNA pellet for 5-10 min. Dissolve by pipetting in 50-200µl of DEPC treated RNA free water and incubate at 55-60°C for 10min.

6. The absorbance of the RNA at 260nm and 280nm 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 40µg/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 10min and loaded into the wells and electrophoresed for 40mins at 60V. 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 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 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
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

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 1mg/mL or greater. The high concentration tends to stabilise 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 deionised 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 stabilisers 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 12,000g
- A vortex mixer
- -80^{o}C Freezer
- A spectrophotometer capable of reading 260 and 280nm /Nanodrop

Procedure

1. Cells are isolated as follows;
   a) Dilute 10mL of blood / cell pellet with 10mL of 1X Hanks balanced salt solution (HBSS)
   b) Layer this solution over 10mL of a ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) and centrifuge in a 50mL disposable plastic centrifuge tube for 15 min at RT at 2,000g 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 15mL centrifuge tube.
   e) Wash the cells by adding 10mL of HBSS, mix thoroughly, and recover the cells by centrifugation for 10min at RT at 2,000g

2. Discard the supernatant and resuspend the cell pellet in Cell Lysis Buffer. The volume of CelLytic^{TM}-M lysis/extraction reagent (Sigma Aldrich) to be added to the cells varies according to cell size and protein concentration required. In general: 125µl CelLytic^{TM}-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^{TM}-M reagent to reach a final concentration of 1X in the buffer.

4. Centrifuge the lysed cells for 15 min at 12,000-20,000g at 4°C to pellet the cellular debris.

5. Remove the protein-containing supernatant to a 0.5mL 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			
Authoriser			

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 (500mL RPMI, 10mL HEPES, 5mL Penstrep, 4mL Fungizone, 50mL FCS)
- RPMI (500mL RPMI, 10mL HEPES, 5mL Penstrep)
- Trypan blue
- DMSO / Fetal calf serum
- Haematocytometer counting chamber
- Light microscope

Procedure

Using Standard Ficoll Density Gradient Centrifugation

1. Dilute 10mL of blood with 10mL of 1X Hanks balanced salt solution (HBSS)

2. Place 10mL ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) in a 50mL disposable plastic centrifuge tube (X2 per patient).

3. Using a sterile Pasteur pipette, carefully layer 10mL 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 400g 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 20mLs and centrifuge at 500g for 15mins at 4°C (BRAKE ON).

7. Carefully pour off the supernatant. Rewash cells in 20mL of 10% FCS RPMI solution at 500g for 10 min at 4°C (BRAKE ON).

8. Resuspend the pellet in 10% RPMI solution to a final volume of 1-10mLs.

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 haemocytometer slide is used to perform cell counts using trypan blue exclusion dye stain to count viable cells.

2. Add 20µl trypan blue to 100µl 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 20µl sample of the mixture is applied to the haemocytometer counting chamber and the cells are visualised 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) x 1.2 (dilution factor) x 1 x 10^4 (area under coverslip) = viable cells/mL.

Cell freezing

1. Following counting cells are centrifuged at 2000g for 10min at RT with the brake on.

2. Decant supernatant and resuspend the cells carefully and slowly in 1mL of DMSO/FCS (1:9 mix) per 10 x 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 -80ºC freezer overnight within a Styrofoam container with a lid, cells should reach a -80ºC 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			
Authoriser			

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 1,500g
- -80^{o}C freezer

Procedure

1. Urine samples should be centrifuged to remove cells prior to storage.

2. The cells shall be separated by centrifugation at 1,500g 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 5mL of 1X PBS and centrifuged at 1,500g at 4°C for 5 min.

5. Prepare cells in aliquots using 0.5mL 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			
Authoriser			

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^{o}C
- A refrigerated centrifuge capable of 12,000g
- Vortex mixer
- -80^{o}C Freezer
- A spectrophotometer capable of reading absorbance at 260 and 280nm / Nanodrop

Procedure

1. Resuspend the cell pellet from step 4 in SOP 3.8 in 180µL DNA extraction buffer (0.1M EDTA, 0.2M NaCl, 0.05M Tris-HCl, pH 8.0, 0.5% SDS, 50µg/mL DNAse free RNAse).

2. Add proteinase K to a final concentration of 100µg/mL and gently swirl the tube to mix the components. Incubate the 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 100µL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

4. Centrifuge at 12,000g 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 300µL isopropanol into a clean 1.5mL 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 12,000g for 3 min. Discard supernatant carefully. 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 300µL 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 12,000g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

8. Add 50µL 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 260nm and 280nm 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 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50ug/mL / nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at -80^{o}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 100kbp and preferably exceeds 200kbp.

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			
Authoriser			

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 12,000g
- A vortex mixer
- -80^{o}C Freezer
- A spectrophotometer capable of reading 260 and 280nm/Nanodrop
- BioRad Experion/ Agilent Bioanalyser 2100

Procedure

1. Resuspend the cell pellet from step 4 in SOP 3.8 in 20mL 1X TRI reagent. Store the homogenate for 5min at RT (18-22°C).

2. RNA extraction: Add 0.1mL bromochloropropane or 0.2mL of chloroform to the mixture and mix vigorously. Store sample for 2-15 min at RT (18-22°C). Centrifuge at 12,000g for 15min at 4°C.

3. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5mL of isopropanol and mix, then store for 5-10 min at RT. Centrifuge at 12,000g for 8min at 4-25°C.

4. RNA wash: Mix RNA pellet with 1mL of 75% ethanol. Centrifuge at 7,500g for 5min at 4-25°C.

5. Solubilisation: Air dry the RNA pellet for 5-10 min. Dissolve by pipetting in 50-200µl of FORMAzol, 0.5%SDS, or DEPC treated RNAse free water and incubate at 55-60^{o}C for 10min.

6. The absorbance of the RNA at 260nm and 280nm 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 40µg/mL. The RNA sample is aliquoted and stored at -80^{o}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 10min and loaded into the wells in the gel and electrophoresed for 40min at 60V. 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			
Authoriser			

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 12,000g
- A vortex mixer
- -80^{o}C Freezer

Procedure
1. Resuspend the cell pellet from step 4 in SOP 3.8 in Cell Lysis Buffer. The volume of CelLytic^{TM}-M lysis/extraction reagent (Sigma Aldrich) to be added to the cells varies according to cell size and protein concentration required. In general: 125μl CelLytic^{TM}-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^{TM}-M reagent to reach a final concentration of 1X in the buffer.

3. Centrifuge the lysed cells for 15 min at 12,000g 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 0.5mL 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			
Authoriser			

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

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

Considerations

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

Procedure

1. Aliquot 450µL of cell lysis Solution to a labeled microcentrifuge tube.

2. Carefully unwrap cheek swab and place cotton tip into the cell lysis solution and add 20µL of proteinase K (100µg/mL stock) solution.

3. Incubate samples for 30 min at 65°C.

4. Add 100µL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

5. Centrifuge at 12,000g 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 300µL isopropanol into a clean 1.5mL 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 12,000g 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.

17. Wash the DNA, by adding 300µL 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 12,000g at 4°C for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

18. Add 50µl 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.

19. The absorbance of the DNA at 260nm and 280nm 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 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50ug/mL / nanodrop. The DNA sample is aliquoted into cryostorage tubes and stored at -80^{o}C.

20. 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 100kbp and preferably exceeds 200kbp.

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 Faeces for DNA extraction

SOP Number: 3.10
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for DNA extraction from faecal 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

- Faecal 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 faeces, therefore it is important that the general shape of the faeces 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^{o}C
- A centrifuge capable of 3,000g
- Vortex mixer
- A spectrophotometer capable of reading absorbance at 260 and 280nm/Nanodrop

Procedure

1. Resuspend faeces in 20mL DNA extraction buffer (0.1M EDTA, 0.2M NaCl, 0.05M Tris-HCl, pH 8.0, 0.5% SDS, 50µg/mL DNAse free RNAse).

2. Add proteinase K to a final concentration of 100µg/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 3.3mL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

4. Centrifuge at 3000g 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 10mL isopropanol into a clean 50mL 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 3,000g 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 10mL 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 3,000g for 5 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

8. Add 300μl 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 260nm and 280nm 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 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50ug/mL / nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at -80^{o}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 100kbp and preferably exceeds 200kbp.

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			
Authoriser			

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 compared fresh or frozen tissue, are expected. Therefore eluting DNA in 50-100μL TE (10mM TRIS-HCL PH 8.0, 1mM 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^{o}C
- A vortex mixer
- A refrigerated centrifuge capable of 14,000g
- A spectrophotometer capable of reading absorbance at 260 and 280nm/Nanodrop

Procedure

1. Homogenise 3-5mg of tissue in sterile PBS using a mechanical homogeniser according to the manufacturer's instructions for use and centrifuge at 14,000g for 10min at 4°C. Discard supernatant and resuspend the tissue pellet in 180µL of DNA extraction buffer (0.1M EDTA, 0.2M NaCl, 0.05M Tris-HCl, pH 8.0, 0.5% SDS, 50µg/mL DNAse free RNAse).

2. Add proteinase K to a final concentration of 100µg/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 100µL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

4. Centrifuge at 12,000g 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 300µL isopropanol into a clean 1.5mL 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 12,000g 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 300µL 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 12,000g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

8. Add 50µL 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 12,000g for 5 min.

9. The absorbance of the DNA at 260nm and 280nm 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 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50ug/Ml / nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at -80^{o}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 100kbp and preferably exceeds 200kbp.

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 from Paraffin Embedded Tissue

SOP Number: 3.11.2
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

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^{o}C
- A vortex mixer
- A refrigerated centrifuge capable of 20,000g
- A spectrophotometer capable of reading absorbance at 260 and 280nm/Nanodrop

Procedure

1. Place a small section (not more than 25mg) of paraffin-embedded tissue in a 2mL microcentrifuge tube.

2. Add 300µL xylene or toluene. Vortex vigorously.

3. Centrifuge at 12,000-20,000g for 5 min at RT.

4. Remove supernatant by pipetting. Do not remove any of the pellet.

5. Add 300µL ethanol (96-100%) to the pellet to remove residual xylene or toluene and mix gently by vortexing.

6. Centrifuge at 12,000-20,000g for 5 min at RT.

7. Carefully remove the ethanol by pipetting. Do not remove any of the pellets.

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 180µL of extraction buffer (0.1M EDTA, 0.2M NaCl, 0.05M Tris-HCl, pH 8.0, 0.5% SDS, 50µg/mL DNAse free RNAse).

11. Add proteinase K to a final concentration of 100µg/mL and gently swirl the tube to mix the components. Incubate the 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.

12. Add 100µL of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

13. Centrifuge at 12,000g 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 300µL isopropanol into a clean 1.5mL 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 12,000g for 3 min. Discard the supernatant carefully. 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 300μL 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 12,000g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

17. Add 50μL 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 260nm and 280nm 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 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50μg/mL / nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at -80^{o}C.

19. 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 100kbp and preferably exceeds 200kbp.

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			
Authoriser			

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^{o}C
- A vortex mixer
- Homogeniser
- A refrigerated centrifuge capable of 20,000g
- A spectrophotometer capable of reading absorbance at 260 and 280nm/Nanodrop

Procedure

1. Place tissue sample (5-10mg) in a centrifuge tube and wash twice by addition of 1X PBS and centrifuge at 12,000g for 5min at 4°C.

2. Homogenise tissue in sterile PBS. Centrifuge at 14,000g for 10 min at 4°C. Discard supernatent and resuspend the cell pellet in 180ul of DNA extraction buffer (0.1M EDTA, 0.2M NaCl, 0.05M Tris-HCl, pH 8.0, 0.5% SDS, 50µg/mL DNAse free RNAse).

2. Add proteinase K to a final concentration of 100µg/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 100μl of ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

4. Centrifuge at 12,000g for 3 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 300μL isopropanol into a clean 1.5mL 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 12,000g 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 300μL 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 12000g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

8. Add 50μL 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 260nm and 280nm 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 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50ug/Ml / nanodrop. The DNA sample is aliquoted into cooled cryostorage tubes and stored at -80^{o}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 100kbp and preferably exceeds 200kbp.

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			
Authoriser			

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

Procedure
1. Homogenisation: The tissue should be homogenised in 1mL TRI reagent per 50-100mg tissue.

2. Resuspend the cells in 20mL 1X TRI reagent. Store the homogenate for 5 min at RT (18-22°C).

3. RNA extraction: Add 0.1mL bromochloropropane or 0.2mL of chloroform to the mixture and mix vigorously. Store sample for 2-15 min at RT (18-22°C). Centrifuge at 12,000g for 15 min at 4°C.

4. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5mL of isopropanol and mix, then store for 5-10 min at RT. Centrifuge at 12,000g for 8 min at 4-25°C.

5. RNA wash: Mix RNA pellet with 1mL of 75% ethanol. Centrifuge at 7,500g for 5 min at 4-25°C.

6. Solubilization: Air dry the RNA pellet for 5-10 min. Dissolve by pipetting in 50-200µL of FORMAzol, 0.5%SDS, or DEPC treated RNAase free water and incubate at 55-60°C for 10min.

7. The absorbance of the RNA at 260nm and 280nm 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 1cm light path is equivalent to a RNA concentration of 40µg/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 10min 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			
Authoriser			

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 12,000g
- A vortex
- -80^{o}C Freezer
- A spectrophotometer capable of reading 260 and 280nm

Procedure

1. Homogenisation: The tissue should be homogenised in 1mL Cell Lysis Buffer (CelLytic^{TM}-M lysis/extraction reagent, Sigma Aldrich).

2. A commercial protease inhibitor solution (for example P3840 Sigma-Aldrich) may be added to the CelLytic^{TM}-M reagent to reach a final concentration of 1X in the buffer.

3. Centrifuge the lysed cells for 15 min at 12,000-20,000g at 4°C to pellet the cellular debris.

4. Remove the protein-containing supernatant to a chilled test tube 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			
Authoriser			

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 2,000g

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 5mL of 1X PBS.

3. Remove the 1X PBS and then add 1-2mL 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 1,200g at 4°C for 5min, 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			
Authoriser			

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^{o}C
- A vortex mixer
- A refrigerated centrifuge capable of 12,000g
- A spectrophotometer capable of reading absorbance at 260 and 280nm/Nanodrop

Procedure

1. Resuspend 10^6-10^7 cells in 125µL Cell Lysis Buffer (CelLytic^{TM}-M lysis/extraction reagent, Sigma Aldrich).

2. Add proteinase K to a final concentration of 100µg/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 100µL ammonium acetate protein precipitation solution and vortex vigorously for 20 sec at high speed.

4. Centrifuge at 12,000g 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 300μL isopropanol into a clean 1.5mL 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 12,000g 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 300μL 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 12,000g for 3 min and drain to remove ethanol, ensure that the DNA pellet remains in the tube.

9. Add 50μL 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.

10. The absorbance of the DNA at 260nm and 280nm 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 260nm with an A_{260} of 1.0 in a 1-cm light path being equivalent to a DNA concentration of 50ug/Ml / nanodrop. The DNA sample is aliquoted into cryostorage tubes and stored at -80^{o}C

11. 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 100kbp and preferably exceeds 200kbp.

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			
Authoriser			

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 12,000g
- A vortex mixer
- -80°C Freezer
- A spectrophotometer capable of reading 260 and 280nm/Nanodrop
- BioRad Experion or Agilent Bioanalyser 2100

Procedure
1. RNA extraction shall be performed by resuspending 5-10 x10^6 cells per 1mL TRI-reagent. Store the homogenate for 5 min at RT (18-22°C).

2. RNA extraction: Add 0.1mL bromochloropropane or 0.2mL of chloroform to the mixture and mix vigorously. Store sample for 2-15 min at RT (18-22°C). Centrifuge at 12,000g for 15min at 4°C.

3. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5mL of isopropanol and mix, then store for 5-10 min at RT. Centrifuge at 12,000g for 8min at 4-25°C.

4. RNA wash: Mix RNA pellet with 1mL of 75% ethanol. Centrifuge at 7,500g for 5 min at 4-25°C.

5. Solubilisation: Air dry the RNA pellet for 5-10min. Dissolve by pipetting in 50-200µLof FORMAzol, 0.5%SDS, or DEPC treated RNAse free water and incubate at 55-60°C for 10min.

6. The absorbance of the RNA at 260nm and 280nm 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 40µg/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 10min and loaded into the wells and electrophoresed for 40min at 60V. 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			
Authoriser			

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 20,000g
- A vortex mixer
- -80^{o}C Freezer

Procedure

1. Resuspend the cells in Cell Lysis Buffer. The volume of CelLytic^{TM}-M lysis/extraction reagent (Sigma Aldrich) to be added to the cells varies according to cell size and protein concentration required. In general: 125ul CelLytic^{TM}-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^{TM}-M reagent to reach a final concentration of 1X in the buffer.

3. Centrifuge the lysed cells for 15 min at 12,000-20,000g 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.

SOP 3.13 Processing of Bronchoalveolar Lavage

SOP Number: 3.13
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for the processing of a bronchoalveolar lavage sample.

Responsibility

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

Procedure:

1. During processing and transport keep BAL sample in a container on ice at 4^{o}C.

2. Measure the volume of BAL fluid on receipt at the processing laboratory. Record the volume of BAL fluid in the study specific documentation and/or data management system.

3. Filter BAL fluid through sterile gauze to remove aggregated debris

4. Centrifuge the fluid for 10 min at 300g at RT as soon as possible after collection.

5. Pour off and store the supernatant on ice at 4^{o}C. If enzymatic activity, is to be measured in the supernatant it should be done immediately. Supernatant protein or lipid concentration can also be measured.

5. Re-suspend the cell pellet in 1mL of 1X PBS.

6. Perform a total cell count on an aliquot of the sample as outlined below.

Cell count

- A haemocytometer slide is used to perform cell counts using trypan blue exclusion dye stain to count viable cells.
- Add 20µL trypan blue to 100µL 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.
- A 20µL sample of the mixture is applied to the haemocytometer counting chamber and the cells are visualised by light microscopy.
- Viable cells exclude the dye and remain clear while dead cells stain blue. Cells in the four outer quadrants are counted and an average obtained.
- The number of cells is determined as follows: total count /4 (average number of viable cells) x 1.2 (dilution factor) x 1 x 10^4 (area under coverslip) = viable cells/mL.

7. Similarly, perform a differential cell count using for example Wright's stain. Express each cell type as a % of total cells. At least 200 cells should be counted when doing a differential, preferably from different areas on the slide.

8. Aliquot fluid into labeled storage tubes and store at -80^{o}C. To avoid excess freeze/thaw cycles it is recommended that 50% of collected fluid be stored in a single cryostorage tube and the remainder in smaller aliquots.

9 The cell pellet may be used immediately in cell culture experiments or can be stored at -80^{o}C for future RNA analysis. The cell pellet should be re-suspended in 1mL of TRIZOL or equivalent solution for RNA analysis and follow the procedure outlined in SOP 3.5.

10. Consideration should be given to the analysis to be performed on the BAL sample. If protease sensitive proteins are to be measured, the sample should be incubated with protease inhibitor prior to freezing. Samples to be used for nucleic acid extraction should be incubated with a specific agent to facilitate extraction (such as RNAlater) prior to freezing.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.

Appendix A REFERENCES

1. Collins, F.S., Green E. D, Guttmacher A.E., Guyer M.S. A vision for the future of genomics research. *Nature* 2003; 422: 835-847.
2. Kaiser J. Biobanks. Population databases boom, from Iceland to the U.S. *Science* 2002; 298: 1158-1161.
3. Bouchie A. Coming soon: a global grid for cancer research. *Nat. Biotechnol*. 2004; 22: 1071-1073.
4. Hagen H.E., Carlstedt-Duke J. Building global networks for human diseases: genes and populations. *Nat. Med*. 2004; 10: 665-667.
5. Manolio T.A., Bailey-Wilson J.E., Collins F.S. Genes, environment and the value of prospective cohort studies. *Nat. Rev. Genet*. 2006; 7 :812-820
6. Makarow M. and Hojgaard L. Population Surveys and Biobanking. May 2008. *European Science Foundation*. www.esf.org
7. http://www.ntnu.no/research/research_excellence/hunt
8. http://www.ukbiobank.ac.uk/
9. http://www.geenivaramu.ee/index.php?lang=eng
10. http://www.walescancerbank.com/
11. Smith B.H., Campbell H., Blackwood D., Connell J., Connor M., Deary I.J., Dominiczak A.F., Fitzpatrick B., Ford I., Jackson C., Haddow G., Kerr S., Lindsay R., McGilchrist M., Morton R., Murray G., Palmer C.N., Pell J.P., Ralston S.H., St Clair D., Sullivan F., Watt G., Wolf R., Wright A., Porteous D., Morris A.D. Generation Scotland: the Scottish Family Health Study; a new resource for researching genes and heritability. *BMC Med Genet.* 2006;7: 74.
12. Yuille M, Dixon K, Platt A, Pullum S, Lewis D, Hall A, Ollier W. The UK DNA Banking Network: a “fair access” biobank. *Cell Tissue Bank*. 2009; Aug 12. DOI 10.1007/s10561-009-9150-3.
13. http://www.p3gobservatory.org/studylist.htm
14. https://www.pcrc.tchpc.tcd.ie
15. GeneLibrary Ireland – An all-island Biomedical Research Infrastructure. Molecular Medicine Ireland 2009. http://www.molecularmedicineireland.ie/uploads/files/GeneLibraryIrelandReport_Final.pdf
16. Cambon-Thomsen A. The scoial and ethical issues of post-genomic human biobanks. *Nat. Rev. Genet*. 2004; 5 :866-873.
17. www.bbmri.eu

18. Yuille M, van Ommen GJ, Bréchot C, Cambon-Thomsen A, Dagher G, Landegren U, Litton JE, Pasterk M, Peltonen L, Taussig M, Wichmann HE, Zatloukal K. Biobanking for Europe. *Brief Bioinform.* 2008; 9(1): 14-24.
19. Guidelines for Human Biobanks and Genetic Research Databases (OECD) 2009.
20. Common Minimum Technical Standards and Protocols for Biological Resource Centres Dedicated to Cancer Research. IARC/WHO International Agency for Research on Cancer 2007.
21. Best Practice Guidelines for Biological Resource Centres Organisation for Economic Co-operation and Development (OECD) 2007.
22. Best Practices for Repositories Collection, Storage, Retrieval and Distribution of Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER) (2008).
23. National Cancer Institute Best Practices for Biospecimen Resources http://biospecimens.cancer.gov/bestpractices/to/.
24. Elliot P., Peakman T.C. on behalf of the UK Biobank. The UK Biobank sampling handling and storage protocol for the collection, processing and archiving of human blood and urine. International *J. Epidemiol.* 2008; 37: 234-244.
25. Dunn W.B., Broadhurst D., Ellis D.I., Brown M., Halsall A., O'Hagan S., Spasic I., Tseng A., Kell D.B. A GC-TOF-MS study of the stability of serum and urine metabolomes during the UK Biobank sample collection and preparation protocols. International *J. Epidemiol.* 2008; 37: i23–i30.
26. WHO Guidance on regulations for the Transport of Infectious Substances 2009–2010.
27. The United Nations Model Regulations guiding the transport of infectious substances (15th edition).
28. The Canadian Tumour Repository Network Standardised Procedures. http://www.ctrnet.ca/uploads/media/english_29_english_file_73.pdf.
29. Boyanton BL Jr. and Blick KE. Stability studies of twenty-four analytes in human plasma and serum. *Clin. Chem.* 2002; 48: 2242-2247.
30. Ciulla TA, Sklar RM, Hauser SL. A simple method for DNA purification from peripheral blood. *Anal. Biochem*. 1988; 174: 485-488.

Appendix B Reagent and Equipment Preparation and Maintenance

Reagent Preparation and Stock Solution for Sample Processing

A. Calculation of Molar, % and "X" Solutions.

A molar solution is one in which 1 liter of solution contains the number of grams equal to its molecular weight. Eg. To make up 100 mL of a 5M NaCl solution = 58.456 (mw of NaCl) g x 5 moles x 0.1 liter = 29.29 g in 100mL.

2. Percent solutions.

Percentage (w/v) = weight (g) in 100 mL of solution

Percentage (v/v) = volume (mL) in 100 mL of solution.

For example: To make a 0.7% solution of agarose in TE buffer, weight 0.7 of agarose and bring up volume to 100 mL with TE buffer.

3. "X" Solutions.

Many enzyme buffers are prepared as concentrated solutions, e.g. 5X or 10X (five or ten times the concentration of the working solution) and are then diluted such that the final concentration of the buffer in the reaction is 1X.

For example: To set up a restriction digestion in 25µL, add 2.5µL of a 10X buffer, the other reaction components, and sterile water to a final volume of 25µL.

B. Preparation of Working Solutions from Concentrated Stock Solutions.

Many buffers require the same components but often in varying concentrations. It is useful to prepare several concentrated stock solutions and dilute as needed.

For example: To make 100mL of TE buffer (10 mM Tris, 1 mM EDTA), combine 1mL of a 1M Tris solution and 0.2mL of 0.5 M EDTA and 98.8mL sterile water. The following is useful for calculating amounts of stock solution needed:

$C_i \times V_i = C_f \times V_f$, where C_i = initial concentration (conc), or conc of stock solution;

V_i = initial volume (vol) or amount of stock solution needed

C_f = final concentration, or conc of desired solution;

V_f = final vol, or volume of desired solution

C. Steps in Solution Preparation:

1. Refer to the laboratory manual and manufacturer's instructions for use for specific instructions on preparation of the particular solution and the bottle label and MDSS for any specific precautions in handling the chemical.
2. Weigh out the desired amount of chemical(s). Use an analytical balance if the amount is less than 0.1g.

3. Place chemical(s) into appropriate size beaker with a stir bar.
4. Add less than the required amount of water. Prepare all solutions with double distilled water.
5. When the chemical is dissolved, transfer to a graduated cylinder and add the required amount of distilled water to achieve the final volume. An exception is in preparing solutions containing agar or agarose. Weigh the agar or agarose directly into the final vessel.
6. If the solution needs to be at a specific pH, calibrate the pH meter with fresh buffer solutions and follow the manufacturer's instructions for using a pH meter.
7. Autoclave, if possible, at 121^{o}C for 20 min. Some solutions cannot be autoclaved, for example, SDS. These should be filter sterilized through a 0.22μm filter.
8. Solid media for bacterial plates can be prepared in advance, autoclaved, and stored in a bottle. When needed, the agar can be melted in a microwave, any additional components, e.g. antibiotics, can be added and the plates can then be poured.
9. Concentrated solutions, e.g. 1M Tris-HCl pH=8.0, 5M NaCl, can be used to make working stocks by adding autoclaved double-distilled water in a sterile vessel to the appropriate amount of the concentrated solution.

D. Glassware and Plastic Ware

1. Glass and plastic ware used for molecular biology must be scrupulously clean. Unclean test tubes, bacterial contamination and traces of detergent can inhibit reactions or degrade nucleic acid.
2. Glassware should be rinsed with distilled water and autoclaved or baked at 150^{o}C for 1 hour. For experiments with RNA, glassware and solutions are treated with diethyl-pyrocarbonate to inhibit RNases which can be resistant to autoclaving.
3. Plastic ware such as pipettes and culture tubes are often supplied sterile. Tubes made of polypropylene are turbid and are resistant to many chemicals, like phenol and chloroform; polycarbonate or polystyrene tubes are clear and not resistant to many chemicals. Make sure that the tubes used are resistant to the chemicals used in any experiment. Micropipette tips and microcentrifuge tubes should be autoclaved before use.

Preparation of Master Mixes

1. The reagent preparations are critical to any research laboratory in which nucleic acid amplification assays are performed. Each reagent to be used in amplification assays must be carefully quality controlled to ensure maximum assay performance. However, a reagent preparation laboratory should always prepare stock solutions in an area separate from specimen processing and never prepare pre-amplification materials in a post-amplification area.
2. Master mixes for each amplification-based assay should be prepared by combining as many of the individual reagent components (excluding target) as is feasible while maintaining performance and stability requirements.
   a. Preparation of master mixes minimizes the number of pipetting and mixing steps required in later processes (sample preparation amplification analysis), thereby decreasing the potential for volume errors, introduction of contaminants, performance variability, and mistakes due to the omission of a necessary reagent component.

   b. If master mix solutions are prepared in bulk (i.e., quantities sufficient for testing over a several weeks), they should be carefully dispensed in small-volume, single-use aliquots to minimise freeze-thaw cycles, and the number of times a particular tube is opened
   c. Mixes should be used in a timely manner, and not be stored for extended periods of time (i.e. the longest a master mix should be stored is based on the earliest expiration date of one of the components).

3. Some reagents such as buffers need not be dispensed in single-use aliquot, but appropriate controls must be included in the amplification assay to detect contamination of these reagents.

4. All master mixes and buffers prepared by the laboratory for use in amplification testing should be tested for low-level product contamination by multiple amplification reactions (without addition of any target nucleic acid sequence) for each aliquot. Repetitions depend on the size of the batch; a rule of thumb is at least 10%.

5. Stocks of positive-control samples should never be prepared or stored in the reagent preparation laboratory. Positive-control stocks and aliquot of low-copy number working dilutions should be prepared in an area separate from space used for pre- or post-

amplification tasks. Small volume aliquot of diluted controls can be stored in the specimen preparation laboratory.

Instrument installation and maintenance

1. All instruments in the research laboratory should be used for its intended purpose and maintained in proper working conditions in accordance with the manufacturer's instructions for use/manual.

2. All instruments in the research laboratory must have the manufacturer's manual which should contain a general description of the instrument, procedures for use, service and maintenance and troubleshooting. Special attention should be paid to any safety precautions (for example ethidium bromide and other chemicals, UV light, etc.). The manufacturer's manual should be arranged and stored in a defined space where all operators can access and read the manuals prior to instrument use.

3. Each laboratory shall provide notebooks and routine maintenance procedures to document all maintenance procedures in order to meet the minimum requirements as defined by the manufacturer. Information to be documented includes date, maintenance done, and the initials of the individual performing the maintenance procedure.

4. All instruments or pieces of equipment used in the performance of diagnostic tests and for reagent preparation and handling in the research laboratory must function properly. They must perform in accordance with the manufacturer's specification and be validated for use based on established standards. This will help to ensure the reliability of diagnostic test results.

Temperature-Dependent Equipment:

1. Place thermometers (NBS calibrated) in any equipment in which the temperature is critical for proper performance of a parameter involved in diagnostic testing or reagent handling. Label each thermometer.

2. If certain temperature dependent equipment is not used daily, record the temperature each time a test is performed in the temperature monitoring notebook to be kept beside that piece of equipment.

3. Do not allow thermometers to touch the walls or sides of an instrument during temperature measurement.

4. Typical tolerance limits for equipment are highlighted below:
   - Incubators: $\pm$ 2°C unless a specific temperature is required
   - Water baths and heating blocks: $\pm$ 1°C
   - Refrigerators: 4 to 8°C
   - Standard laboratory freezers: -20 $\pm$ 5°C
   - Ultra low freezers: $\pm$ 10°C as long as below -60°C
   - Do not use "frost-free" freezers. Temperature fluctuations lead to reagent degradation

5. Electrical Precautions. Have all electrical equipment checked for proper grounding and electrical safety by the biomedical engineering departments before installation and at least annually.

Disclaimer:
MMI has prepared these guidelines as a service to the research community. All SOPs should be adopted only with reference to local ethical and regulatory requirements. MMI does not accept any liability for loss or damage relating to the implementation of these guidelines.