PROCEDURE

Metro South Health Research Biorepositories – Collection, Processing, Handling and Retrieval

PR2017/104
Version No. 3.0

PURPOSE

Metro South Health (MSH) is committed to promoting and informing research biorepositories to ensure adherence to high ethical standards and practices in the collection, processing, handling and retrieval of biospecimens and accompanying information for research purposes.

The purpose of this procedure is to outline general principles that can be used to ensure that biospecimens and data are collection, processed, handled and retrieved in a manner sensitive to the rights of the patient/participant. This procedure applies to all types of biospecimens, including, but not limited to, wet tissue, frozen tissue, paraffin-embedded tissue, glass slides, blood, serum and urine.

OUTCOME

Whilst research biorepositories must be operated in accordance with the MSH Research Biorepository Governance Framework, principles may be adapted so that they are appropriate to the mission and goals of each research biorepository.

This procedure applies to all MSH or Queensland Health (QH) employees whose usual reporting line is through a MSH facility or service (including visiting medical officers, visiting health professionals, students and researchers) who operate or access, or who propose to establish or access, a research biorepository that includes biospecimens collected, processed or stored within MSH facilities.

Failure to comply with this procedure may amount to research misconduct on the part of the responsible individual. This procedure must be read in conjunction with other MSH Research Management and Research Biorepository procedures.

KEY PRINCIPLES

The following key principles guide MSH research biorepositories in operational and practical considerations that arise in the process of collection, processing, handling and retrieval tissue, derivative products and samples. The way in which individual MSH research biorepositories put these principles into operation may be scaled in relation to the research biorepository's size of operations.

- Throughout the existence of the research biorepository, Custodians and/or directors must ensure that the collection and use of patients/participants' biospecimens and data are scientifically, legally and ethically appropriate.
- The research biorepository must define standards for all preparations used in the growth and/or maintenance of the living biospecimens held; these should be documented with the appropriate mechanisms in place to allow changes to processes. Materials used should be of high quality and documented.

- The aim of every research biorepository should be to collect, maintain, and disseminate high quality biospecimens, based on the intended research use and future research projects. High-quality biospecimens are defined as those whose biology most closely resembles the biology of the biospecimen prior to its removal from the human research patient/participant.
- Biospecimen availability and the intended analytical objectives for their utility must be considered prior to initiating a research biorepository in MSH. Biospecimen collection, processing, handling and retrieval practices have many elements in common, although biospecimen processing will vary according to the specific type of research activity.
- The Custodian must ascertain the sensitivities of different types of biospecimens to collection, processing, handling and retrieval processes. These may vary, particularly between viable and non-viable samples; therefore, research biorepository collection Standard Operating Procedures (SOPs) must incorporate any special requirements necessary for the preservation of viability, functionality, structural integrity and stability of cells, tissues, organs, cell free fractions and macromolecules and/or analytes. In addition to biospecimen type, other factors include: disease and/or other relevant status of the patient/participant or population providing the biospecimen, collection method, sample vessel and security of containment.
- Specific personnel responsibilities, training, risk management and skills are required for biospecimen collection. Planning of collection logistics must take into account distance from the collection point to the processing lab, interim transport containment and security of the storage facility (if this is a different location). SOPs for stabilisation and/or preservation of samples during transit will be necessary for the research biorepository.
- Due to the importance of pre-analytical processes (defined as those procedures undertaken between the times of biospecimen collection to the moment of their analysis) it is important to apply strategies that maintain the stability and functionality of biospecimens and macromolecules of interest.
- Stringent SOPs are similarly required for biospecimen labelling and tracking as initiated from the point of collection to receipt in the research biorepository and further to the end-user.
- Based on the availability of biospecimens, a research biorepository may collect and process many different biospecimens (eg solid tissue, blood, saliva and urine) from the same case. Furthermore, a research biorepository may process each biospecimen into a variety of formats (eg formalin-fixed paraffin embedded, OCT blocks and/or snap frozen). Such redundancy of sample supply allows for optimised future usage; however, limitations of the research biorepository's storage capacity must be considered when collecting multiple biospecimen types and processing them in a variety of formats and amounts.
- Scientific enquiry requires reproducibility in that experiments performed in one laboratory by one set of investigators must be replicable in another laboratory; therefore, consistency in collection, processing, handling and retrieval amongst MSH research biorepositories is vital.
- All biospecimens and data within the research biorepository must be subject to proper quality control measures at every stage of processing to ensure high standards of quality.
- To foster the interoperability of systems and facilitate the scientific exchange of data and biospecimens, Custodians should strive to collect, process, handle and store biospecimens and data in a manner consistent with internationally accepted technological standards and norms.

LEGISLATION OR OTHER AUTHORITY

Legislation

- *Hospital and Health Boards Act 2011 (Qld)*
- *Information Privacy Act 2009 (Qld)*
- *Human Rights Act 2019 (Qld)*
- *Public Health Act 2005 (Qld)*
- *Therapeutic Goods Act 1989 (Cth)*
- *Transplantation and Anatomy Act 1979 (Qld)*

To the extent an act or decision under this document may engage human rights under the *Human Rights Act 2019*, regard will be had to that Act in undertaking the act or making the decision. For further information on the *Human Rights Act 2019* see: https://www.qhrc.qld.gov.au/

Regulation

- Transplantation and Anatomy Regulation 2004 (Qld)

Statements, papers and guidelines

- Canadian Tissue Repository Network: Policies and Standard Operating Procedures
- International Society for Biological and Environmental Repositories (ISBER): Best Practices: Recommendations for Repositories Fourth Edition
- National Cancer Institute: Best Practices for Biospecimen Resources
- Organisation for Economic Co-operation and Development (OECD)
    - Best Practice Guidelines for Biological Resource Centres
    - Guidelines on Human Biobanks and Genetic Research Databases
- The Royal College of Pathologists of Australasia: Biobanking Guideline 2014
- World Health Organisation (WHO): Common Minimum Technical Standards and Protocols for Biological Resource Centres Dedicated to Cancer Research

MSH policies, procedures, manuals and frameworks

- Metro South Health Research Management Policy (PL2017/55)

RESPONSIBILITIES

Executive Management

Ensure collaborative, harmonised, clear and detailed publicly available policies, procedures and SOPs are in place for the establishment and operation of all MSH research biorepositories.

Metro South Research

Support Custodians in the establishment and operation of a research biorepository through the provision of guidance and support when interpreting principles and provisions contained within the MSH Research Biorepository Governance Framework.

MSH Research Biorepository Strategic Oversight Committee

Provide clarification and assistance in collection, processing, handling and retrieval of biospecimens for each research biorepository. The Committee also ensures that the goals of the MSH Governance Framework are met and that the quality and value of MSH research biorepositories are maintained.

Custodian/Principal Investigator – responsible officer

Ensure the research biorepository's collection, processing, handling and retrieval processes follow the MSH Research Biorepository Governance Framework and appropriate for the type of biospecimens collected.

Research biorepository manager

Implement appropriate collection, processing, handling and retrieval methodologies on behalf of the research biorepository and report any matters which require action to the Custodian.

Laboratory technician/technologist assistant/clinical personnel

Research biorepository personnel must possess sufficient educational background, experience and training to assure that assigned tasks pertaining to the collection, processing, handling and retrieval of biospecimens from MSH patients/participants are performed in accordance with the MSH Research Biorepository Governance Framework and applicable SOPs.

SUPPORTING DOCUMENTS

Attachment 1 - Application

Attachment 2 - Recommended Quality Control Processes

Attachment 3 - Recommended Preservation Methods

Attachment 4 - Collection, Processing, Handling and Retrieval Checklist

DEFINITIONS

See the MSH Research Biorepositories Glossary

PROCEDURE - Collection, Processing and Handling

STEP 1: Standard Operating Procedures (SOPs)

The research biorepository manager must implement SOPs which outline processes and protocols for the collection, processing, handling and retrieval of biospecimens. Standardised SOPs must be applied consistently in storing biospecimens to ensure quality and to avoid introducing variables into research studies. Personnel should record storage conditions along with any deviations from SOPs, including information about temperature, thaw/refreeze episodes, and equipment failures.

Validation of storage equipment, for example, identifying "hot spots" in a freezer and ensuring that back-up equipment and temperature monitoring systems are functional, is essential. Please see Facility, Equipment, Storage and Security Procedure (PR2017/103) for more information.

STEP 2: Collection of biospecimens

Biospecimens for the research biorepository must be obtained only after all patient/participant diagnostic needs have been met and should be accompanied by documented, informed consent or a waiver of consent from the MSH Human Research Ethics Committee (HREC). The Participant Information and Consent Form (PICF) consent must specify the biospecimens to be removed for storage and scientific purposes. Please see MSH Research Management - Biospecimen Ethics and Participant Information and Consent Form Procedure (PR2017/115) for more information.

The removal of tissue from a patient's/participant's body is authorised if it is removed by a registered health practitioner and/or appropriately authorised/qualified scientific employee for the for the purpose of research as approved by a Human Research Ethics Committee and consent is given to the removal in accordance with the requirements stated in the National Statement.

A unique collection number must be allocated to the biospecimen, which is never reassigned if the sample is later discarded. It is extremely important to note that the clinical requirements of MSH patients/participants are always considered a priority over the collection of biospecimens for biobanking purposes. Please see Access and Applications for Samples Procedure (PR2017/106) for more information.

STEP 3: Processing

To ensure suitability for research, the processing of the biospecimen must be done in a manner to protect tissue architecture and the integrity of molecular products. Biospecimens must be handled as being potentially biohazardous and laboratory staff must take appropriate precautions when handling biospecimens. Please see Facility, Equipment, Storage and Security Procedure (PR2017/103) for more information.

STEP 4: Handling

To meet the needs of the users, the biospecimen should be handled in a manner that optimally maintains the architecture of the tissue and the molecular integrity of the biospecimen.

In certain circumstances, a risk assessment may be carried out on the biospecimen and the methods recorded to determine, as far as possible, the potential harm to personnel, the public and the environment. SOPs must be developed to mitigate risks to particular biospecimens. Please see Facility, Equipment, Storage and Security Procedure (PR2017/103) for more information.

STEP 5: Storage methods

Biospecimens should be stored in a stabilised state. Unnecessary thawing and refreezing of frozen biospecimens or their derivatives should be avoided. Appropriate size for aliquots and samples should be determined in advance. Biospecimens must be stored in a manner optimal for their category and intended use and this should be documented. Storage conditions must ensure that loss of material is prevented:

- Storage temperatures must be monitored continuously, and incident and alarms must be documented and traceable.
- The freezer container must be equipped with an alarm system that ensures an immediate intervention.
- Processes for transfer in the event of a breakdown must be defined, including the obligations of personnel.

Please see Facility, Equipment, Storage and Security Procedure (PR2017/103) for more information. Multiple empty functioning freezers must be available in the case of single freezer failure. Please see Emergency Preparedness and Work Health and Safety Procedure (PR2017/108) for more information.

When thawing/refreezing is necessary, a research biorepository must follow consistent and validated processes/SOPs to ensure continued stability of the analytes of interest. Methods such as inventory tracking should be established to minimise disruption of the stable environment during sample retrieval.

STEP 6: Storage temperature

In selecting biospecimen storage temperature, consideration should be given to the type, the anticipated length of storage, the biomolecules of interest, and whether study goals include preserving viable cells. Paraffin-blocks should be stored at temperatures below 27°C in an area with pest and humidity control. Factors such as blocks stored in areas above these 'room temperatures,' duration of fixation, and most importantly humidity fluctuations have been shown to seriously compromise the expression of certain antigens when evaluated by immunohistochemistry.

In the case of liquid biospecimens, such as blood, consideration should be given to fractionation to produce components such as plasma or serum, which should be separated before storage to preserve each constituent under its optimal condition. Whole blood (rather than fractionated blood) cryopreservation may be an efficient and cost-effective option for processing viable cells in large-scale studies. If the intent is to maximise possible future uses, tissues should be stored in the vapour phase of liquid nitrogen (LN_2) freezers or frozen at -80°C to ensure long-term viability. Lower storage temperatures and cryoprotectant (such as dimethyl sulfoxide) may be used to maintain viable cells for long periods of time. The difference in temperature between the bottom and top of a LN_2 freezer should be measured and taken into consideration in planned analyses. Regular temperature mapping of the interior of freezers is recommended to insure uniform temperature through the storage unit.

STEP 7: Retrieval

Retrieval of biospecimens for shipment of analysis requires strict adherence to SOPs for proper biospecimen inventory and tracking.

STEP 8: Validation methods

In some circumstances the validation of methods and processes used for preservation must be carried out to ensure the reproducibility and reliability by using one of the following approaches; performing blind tests, calibration, comparing results of the same method performed at different times, with different methods and performed by different persons. Please see Facility, Equipment, Storage and Security Procedure (PR2017/103) for more information.

STEP 9: Self-audit, review and compliance

Utilise Attachment 4 - Collection, Processing, Handling and Retrieval Checklist to aid in self-auditing, review and compliance.

PROCEDURE DETAILS

Procedure Number
PR2017/104

Procedure Name
MSH Research Biorepositories – Collection, Processing, Handling and Retrieval Procedure

Policy Reference
PL2017/53
MSH Research Biorepositories Policy

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Procedure Author
Erica Wright, Manager, Research Development, Metro South Research, Metro South Health

Portfolio Executive Director
Professor John Upham, Chair, Metro South Research, Metro South Health

Approving Officer
Professor John Upham, Chair, Metro South Research, Metro South Health

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05 July 2024 (within the next 3 years)

ATTACHMENT 1 - Application

1.0 General considerations

Translational research, using advances in molecular biology, archived tissue samples and annotated data, is pursued to aid in the elucidation of the disease process and discovery of new diagnostic and treatment modalities. A collection of appropriately stored and well-annotated biospecimens and derivatives is a valuable resource, important to the research process. The public and patients/participants must have confidence that research biorepositories and researchers will use and handle such material with sensitivity, responsibility and concern for maintaining the value of the collection. Biospecimen storage is fundamental to contemporary practice in diagnostic pathology. Pathologists are required to store biospecimens (principally as formalin-fixed paraffin embedded tissue blocks, although also as fresh tissue, genetic material, serum, cultures etc). Pathologists and pathology practices are central to patient multidisciplinary care and to research.

The requirements of clinical care and the interests of researchers often overlap and the governance arrangements for the annotation, collection, processing, handling and retrieval of biospecimen is critical to the management of this limited resource. Individual types of biospecimens should be handled according to SOPs specific to the type and the biomolecules to be analysed (eg ribonucleic acid (RNA), deoxyribonucleic acid (DNA), protein and lipid) when possible, recognising that collection in the context of a clinical trial might be constrained by trial-specific protocols. Although most of the practices in this section assume freezing or formalin fixation of samples, dry or ambient temperature storage procedures may be appropriate for many samples. The quality of the samples and the extent of the accompanying data is a determinant of value. The goal of MSH is to harmonise procedures for handling biospecimens and annotated data thus ensuring that the quality and integrity of the collection is consistently maintained at a high level.

1.1 Timing of biospecimen collection

The relative importance of the period of time between receipt and processing and/or collection and freezing of a biospecimen varies with the research application. Cells, tissues and organs lose functionality and molecules degrade at different rates dependent upon type and status of participant and collection circumstances. It is important to identify critical factors which predispose different biospecimens to deterioration and contamination. These factors are generally well understood within the medical/research community. Records of; collection time/date, receipt time/date and processing time/date, freezing time/date are critical to record and provide when required.

The speed at which the degradation occurs will depend upon many complex factors including: patient/participant and/or organ health status, collection procedures and the temperature and hydration at which the biospecimen is maintained and the stability of molecules of interest. Every effort must be made to maintain tissue biospecimens at cold temperatures as soon as they become ischemic or are removed from the body. For certain biospecimens, it is also important to prevent dehydration and desiccation of tissues during transportation by covering it with sterile gauze moistened in biopreservation media. In general, biospecimens should be processed as rapidly as possible. The collection, processing, handling and retrieval times, and procedures must be documented in the research biorepository's SOPs since pre-analytical variables may affect research and clinical outcomes. End-users should be provided with the pre-analytical variables so that they can make informed, evidence-based assumptions and conclusions about their experimental data.

1.2 Temperature

Because cold preservation is a critical stabilising factor for many biospecimen types, the temperatures at which biospecimens are collected, processed and stored must be carefully considered. These range from chilling/hypothermic (2 - 8°C) to low subzero (-4°C to 0°C), freezing (-20°C to -150°C) in mechanical/electrical freezers and storage at the ultra-low temperatures of liquid and vapour-phase liquid nitrogen (LN_2) (to a minimum -196°C).

Choice of collection and storage temperature depends upon biospecimen resilience to chilling, freezing and cold-induced dehydration, duration of exposure and tolerance to cryoprotective treatments, biospecimen type and use in intended analyses. The general rule is that a warm storage environment, even for a short period of time, can lead to physiological stress and macromolecular degradation. For this reason, it is necessary to maintain appropriate temperature(s) from the point of collection through processing and storage. Hypothermic temperatures (2 - 8°C) should be considered as the default condition for biospecimen transport/storage when not frozen.

The type and duration of low temperature treatments are also dictated by biospecimen use. Blood samples collected to yield serum will need to be maintained at room temperature for a minimum of thirty (30) minutes to allow clotting. The collection and processing time must be documented and reported to the end-user. This information is critical for quality control measures (eg will help explain the presence of fibrin, a common occurrence when insufficient time is allowed for clotting to occur).

Biospecimens can also be collected, shipped and stored at ambient temperatures using new technologies that have been developed specifically for such purposes. Ambient preservation is available for purified analytes (RNA and DNA) as well as in complex systems (eg saliva, blood, cells and tissue). Preservation can be for a few days and in some cases, for longer than twenty-five (25) years. Technology for preserving dried blood spots on cellulose-based cards/filter papers at ambient temperature for longer than fifteen (15) years has been well described in literature.

1.3 Biospecimens received externally to MSH

Conditions of deposit must be determined and agreed upon, if pertinent, in a Material Transfer Agreement. Please see Material Transfer Agreements, Packaging and Shipping Procedure (PR2017/107) for more information. Where deposits are outside the remit of the research biorepository, suitable research biorepositories should be recommended. The depositor must provide proof that prior informed consent to collect and deposit the biospecimen in a MSH research biorepository has been obtained or reasonable efforts have been taken to obtain such consent (with proof of Human Research Ethics Committee review). On the deposit of biospecimen/s in a research biorepository the research biorepository must record Custodianship and terms and conditions for further distribution.

In any situation in which a research biorepository has in its possession information that could identify a patient/participant, such information must be dissociated from the biospecimen concerns and any other associated data. Specific care must be taken to ensure that data in the possession of the research biorepository is not misused in such a way as to cause harm to individuals or groups of individuals. Please see Facility, Equipment, Storage and Security Procedure (PR2017/103) for more information.

2.0 Collection

Tissue for the research biorepository must be obtained only after all patient/participant diagnostic needs have been met and should be accompanied by documented, informed consent or a waiver of consent from the MSH HREC.

Broader molecular profiles can be obtained from samples that have been collected using rigorous and standardised procedures. Collection procedures should be geared to allow use of the samples in genomic and proteomic research. To ensure suitability for genomic and proteomic research the time elapsed between surgical resection of the tumour and freezing ideally should be rapid to ensure preservation. Adequate documentation, relevant to the research biorepository, must capture the timeframe for quality assurance purposes (eg date of surgery and date of processing etc).

Depending on the needs of the investigator for whom the samples are collected or the protocol of the research project for which the material is collected, tissues can be collected from several sources (eg surgery or autopsy).

If possible, biospecimens should be collected in sufficient quantity and diversity to be of value in a variety of research project designs. A variety of protocols exist for the collection of different biospecimen types. The protocol chosen should be suited to the particular needs of the research project.

Once the biospecimen is collected (and sometimes prior to its removal) the biospecimen may begin to take on new characteristics based on changes to the material's environment (eg changes in exposure to certain nutritional, chemical, or other environmental factors that may occur during a surgical or collection process). Such changes may result in inaccurate determinations of the molecular and physical characteristics of the biospecimen during subsequent analysis. Every attempt should be made to minimise the effects of material handling on biospecimen integrity.

For optimal preservation, formalin-fixed, paraffin-embedded tissue should be stored as a block and not sectioned until analysis is imminent because degradation will occur under even the best storage conditions. However, when slide-mounted cut sections must be stored prior to analysis several steps may be taken to minimise degradation, including thorough dehydration and processing prior to storage, and storing the slides frozen and protected from exposure to moisture. Optimal storage conditions might vary according to the final use to which stored sections will be put, and empirically determining the optimal storage conditions is recommended.

2.1 Solid tissues

Solid tissues are collected by biopsy or during surgical procedures. Collection should be carefully planned with surgeons, clinical staff and pathologists. Tissues may be collected prospectively, as a part of a population-based research project, or for general purposes for future research activities. All materials and instruments should be prepared in advance.

2.1.1 Diagnostic integrity

The collection of samples for research should never compromise the diagnostic integrity of a biospecimen. Only tissue which is excess to diagnostic purposes can be collected for the resource.

This is especially important when human solid tissue biospecimens are collected during surgery and where pathology is involved in the subsequent diagnostic process. This is because attributes of the sample (eg the margin of the tumour or the number of tumour positive lymph nodes) may have a direct impact on the care that a patient/participant subsequently receives (eg chemotherapeutic treatment vs radiation therapy vs no treatment).

2.2.2 Pathology

A pathologist may supervise the procurement of the tissue. The appropriate handling of tissues procured for research purposes is facilitated if a practicing pathologist supervises the actual procurement of the tissue; this is especially important to prevent the compromise of diagnostic biospecimens. The intact surgical biospecimen or biopsy should be sent to pathology as soon as possible.

Ideally, information from the pathologist on the characteristics of the biopsy or surgical material (eg % normal, % tumour, % necrosis and/or % fibrosis) may be recorded for future use so that the end user will be able to determine the usefulness of the tissue. Where possible, multiple sections or samples should be created to allow for greater use of the biospecimens.

The pathologist will examine the sample, and, allowing adequate tissue for diagnosis, will remove a portion of the tumour and adjacent normal tissue. If applicable, involved lymph nodes and metastasis will also be collected. Tissues must be sliced with sterile forceps and scalpel blades, and the staff must use sterile gloves.

A pathologist may review all potentially diagnostic tissue biospecimens to determine what material can be made available for research. Blood, and other body fluids, as well as some other solid tissues not required for diagnosis or prognosis can be collected in accordance with approved protocols and might not require pathologic review.

Research biorepository personnel must be present to freeze or fix the tissue as quickly as possible. Tissues must be snap frozen either directly or enclosed in a container immersed in the freezing medium (eg precooled isopentane). Liquid nitrogen (LN_2) is not recommended as a suitable freezing medium for direct snap freezing due to the potential formation of cryo-artefacts. When dry ice/LN_2 is not readily available, tissue collections into RNAlater may be a good alternative provided that this tissue is not required for diagnostic purpose, and permission is given by the pathologist.

2.2.3 Surgical samples

Remnant samples may be collected from diagnostic surgical procedures. With proper Human Research Ethics Committee approval and appropriate informed consent, biospecimens may be resected specifically for research. Generally, it is recommended that surgical biospecimens or biopsy be preserved within one (1) hour of excision; however, tissue subject to a delay up to two (2) hours should still be collected. Detailed record of the timing of events from excision to fixation or freezing must be kept by the MSH research biorepository.

Tissues placed in any type of “preservative” should be dissected into appropriately sized pieces so as to allow adequate permeabilisation of the tissue by the preservative.

When pathological diagnosis is required, a pathologist should first examine the biospecimen to identify tissue that can be made available for research without compromising diagnostic integrity. Generally, biospecimens should remain fresh, not fixed, and be placed in a clean or sterile container on wet ice (2 - 8°C) for transport from surgery to pathology or to the MSH research biorepository. The optimal process would be to handle all biospecimens in a sterile manner; however, that is not always practical, as few surgical pathology gross rooms have a sterile hood.

Many research protocols do not require biospecimens be procured or processed following sterile procedure. A “clean” area must be set up for biospecimen procurement/processing. During biospecimen procurement, contact between different biospecimens must be avoided (biospecimen contamination) and equipment used for procurement should be cleaned or disposable equipment replaced after each procurement. Fresh blades and instruments should be used with each new biospecimen as well as in different areas of the same biospecimen. The surgeon/pathologist/scientist must be provided with gloves and clean instruments for resection of the tissue. Biospecimens must not be resected on a dry towel, or other absorbent material, as this procedure rapidly desiccates the biospecimen and may compromise its usefulness.

Unless a researcher specifies otherwise, tissue provided to the research biorepository may be placed directly in appropriately labelled clean containers of cold biopreservation media (2-8°C) for transport to the research biorepository for processing. If the tissue is to be frozen immediately, it is not necessary to place it in preservation media, which may cause ice crystals to form on the outside of the biospecimen when freezing. It is important to educate/train all personnel who will be handling the biospecimen on the specific handling requirements unique to each protocol. Please see Operational Arrangements Procedure (PR2017/101) for more information.

Samples requiring snap-freezing or flash freezing (cooling at sufficiently high rates to limit damage to cell structure from intracellular ice formation or prevent compositional changes in labile molecules) can be frozen in a Dewar of liquid nitrogen or on dry ice at the time of collection. Generally, where biospecimen morphology needs to be conserved, snap-freezing should be done in pre-cooled isopentane (at a maximum of -80 °C) or sub-cooled in liquid nitrogen. Data should be maintained and tracked on the time that elapses between relevant time points (eg collection, processing, preservation and storage). A date and time stamp can be utilised for maintaining these records efficiently. This information can then be transferred into a database.

If a frozen section is cut for diagnostic reasons, then the research biorepository personnel must make every effort to obtain an extra slide for Quality Control (QC). If sufficient amounts of tissue are available following a diagnosis, the research biorepository personnel should save some of the tissue as snap-frozen and a representative section for making a paraffin block that will become the property of the research biorepository. If possible, a Hematoxylin and Eosin (H & E) slide may then be cut from each paraffin block which may serve as the QC for that biospecimen. If insufficient amounts of tissue are provided by the pathologist to allow for making a paraffin block, then the research biorepository personnel may be able to request an H & E slide to be made from the pathology department's diagnostic paraffin block to serve as the QC material for the research biorepository.

All samples must be labelled appropriately and all relevant accompanying data should be documented. Please see Quality Management System (Assurance and Control) Procedure (PR2017/110) for more information. Ideally and where practicable, research biorepository personnel must pre-label biospecimen collection containers with patient/participant ID/barcode before surgery to ensure accurate labelling and biospecimen tracking. In some circumstances a generic collection kit may be provided in a central location (ie theatre fridge) which enables biospecimens to be placed in the kit with a patient/participant ID. Multiple tube labelling can then occur outside of theatre in research biorepository premises. All personnel who will be handling the biospecimen (eg surgeons, nurses, pathologists and research biorepository personnel) must be trained on the specific handling requirements of each protocol.

2.2 Blood or blood-derived products

2.2.1 Blood

Blood samples may be collected in different manners depending upon the amount of blood needed for a research project and the distance between the sample source, processing location, and the research biorepository. For example, blood samples may be collected at a location that is far away from the research site, in either small quantities as dried blood spots on treated or untreated matrices or collected in large quantities in tubes that provide stability at room temperature for several days. Consideration should be given that cell viability and functionality from blood samples may be compromised during extended ambient storage/transport; however new technologies have been developed to significantly extend ambient storage/transport of blood samples for up to one year. Hypothermic temperatures (2 - 8°C) may allow for extended stability of blood-derived cell products.

Detailed instructions and protocols for collection of blood or blood derivatives are given in the research biorepository's SOPs. The following general guidelines should be considered:

1. Blood samples should be processed and stored within a research project dependant time frame, depending on the analytical endpoints.
2. All biospecimens should be treated as potentially infectious.
3. Blood should not be collected after prolonged venous occlusion.
4. Tubes into which the blood is collected should be clearly labelled.
5. For the preparation of plasma, blood may be collected into EDTA (Ethylene Diamine Triacetic Acid), ACD (Acid Citrate Dextrose), lithium heparin, or into a clotting tube containing separating gel.
6. Ideally, blood should be processed within one (1) hour of collection. After that time, cell viability decreases rapidly, resulting in poor cell structure and degradation of proteins and nucleic acids.
7. If a long time elapses between collection and processing (2–3 hours) it is recommended to use ACD tubes.
8. Lithium heparin is generally used if cytology studies have to be performed, but it is not recommended for proteomics work.
9. Heparinised blood may interfere with amplification of DNA templates and should be considered when performing PCR.
10. Either EDTA or ACD tubes can be used if DNA is to be extracted or if lymphoblastoïd cell lines to be derived. Lithium heparin is not recommended for proteomics application and lymphoblastoïd cell lines establishment.
11. EDTA tubes are recommended if protein studies will be performed. The use of EDTA tubes results in less proteolytic cleavage than heparin and citrate-anticoagulated plasma.
12. For the preparation of plasma, the blood should be centrifuged as soon as possible. For the preparation of serum, the blood should be processed within one (1) hour after collection.
13. The amount of blood collected should be justified when applying for ethical clearance.
14. Reduced volume of blood in a tube containing additives should be recorded to avoid confounding results.
15. The time and date of blood collection and time of freezing should be recorded, as well as any deviations to the standard processing protocol.
16. Blood should be transported at room temperature or on melting ice depending on the particular applications. Samples to be used for proteomics assays should be processed immediately at room temperature. Cool temperature can activate platelets and release peptides into the samples *ex-vivo*.
17. Blood spot collection should be considered an alternative to whole blood when conditions necessitate easier collection and cheap room temperature storage. Different types of collection cards are available (Guthrie cards, FTA cards, Isocode) (see a research biorepository's SOPs for further information).

"Guthrie cards" (903 filter paper, Schleicher and Schuell) are used to collect heelstick blood from newborns for metabolic disease screening. The 903 paper is manufactured from 100% pure cotton linters with no wet-strength additives. The critical parameters for newborn screening sample collection are blood absorbency, serum uptake and circle size for a specified volume of blood. Blood spots archived as long as seventeen (17) years have also provided valuable sources of amplifiable DNA.

Modified cards (Isocode® or FTA cards®) have been developed consisting of filter paper impregnated with a proprietary mix of chemicals that serves to lyse cells, to denature proteins, to prevent growth of bacteria and other microorganisms, and to protect nucleic acids from nucleases, oxidation and UV damage.

Room temperature transport in folders or envelopes (by hand or mail) has been common for years. The papers protect DNA within the samples for some years at ambient conditions. The main variable is expected to be the quality of the storage atmosphere, particularly the content of acid gases and free-radical-generating pollutants, although FTA® paper can protect against such conditions (Smith and Burgoyne, 2004). Genomic DNA stored on FTA® Cards at room temperature for over fourteen (14) years has been successfully amplified by PCR. In contrast, genomic DNA stored at room temperature on non-FTA Cards for over six months did not amplify. Sample integrity is optimised when FTA cards are stored in a multi-barrier pouch with a desiccant packet.

One of the primary decisions in storing blood samples is whether to collect anticoagulated (plasma/buffy coat/RBC) whole blood or coagulated (serum/clot) blood. When serum is collected without anticoagulant, the blood clot obtained after processing can be used as a source of DNA for genotyping and other DNA related studies. In similar fashion, blood collected with anticoagulant can yield a packed cell volume (containing both the buffy coat and RBC) to be used as a source of DNA. When multiple blood collection devices/containers are involved there is a prescribed priority order of draw. The order of draw for clinical testing may be different from an investigator's requested order of draw. It is also important to determine which anticoagulants are acceptable for a particular downstream procedure.

2.2.2 Buffy coat cells

For DNA testing, if DNA cannot be extracted from blood within three (3) days of collection, the buffy coat may be isolated and stored at -70°C or lower prior to DNA isolation. Buffy coat biospecimens that are being used for immortalisation by Epstein-Barr virus should be transported frozen on dry-ice. RNA should be isolated from buffy coat within one to four hours of biospecimen collection; alternatively RNA stabilisation solution (eg RNAlater) should be used.

2.3 Other biospecimens

2.3.1 Urine

Urine is easy to collect and is a suitable source of proteins, DNA and other metabolites. Urine should be routinely stored at -80°C. Ambient temperature storage before freezing should be kept to a minimum.

Urine samples should be maintained on ice or refrigerated after collection. Collection containers should be sterile and dry, and have a 50 mL to 3 L capacity, a wide mouth, and a leak-proof cap. Depending upon the analyte to be measured, a preservative may be needed.

The type of preservative may differ according to test methodologies, time delay and transport conditions. EDTA and sodium metabisulfite are examples of preservatives commonly used in urine collections. Urine containers used for environmental toxicology assays should be high density polypropylene to minimise phthalate contamination. Because urine may contain cellular and other elements, a urine sample is typically centrifuged to remove cells and debris. The acellular urine and separate cell pellet can then be analysed and/or frozen as aliquots.

There are various methods of collection (eg first morning urine, random, fractional and timed) depending on the type of analysis intended. The collection method must be documented in the sample record.

2.5.1.a First morning urine samples

Before going to sleep, the patient/participant voids a urine biospecimen. Immediately on rising, the patient/participant collects the "first morning" urine biospecimen.

First morning biospecimens are best for detecting substances in a more concentrated solution (eg white and red blood cells or urinary hormones).

2.5.1.b Random urine samples

A random urine sample is good for routine screening and cytology studies.

2.5.1.c Fractional urine samples

Fractional urine samples are used to compare the concentration of an analyte in urine with its concentration in blood. First morning urine, which contains solutes and metabolites from the evening meal, is discarded and a second urine sample following a period of fasting is collected.

2.5.1.d Timed urine sample

Timed urine collections allow for comparisons of patterns of excretion of certain biomolecules. Typical collection times are 12 and 24-hour. For the 24-hour collection on day one, the patient/participant empties his/her bladder and for the next (twenty-four) 24 hours all subsequent urine is collected.

2.3.2 Buccal cells

The collection of buccal cells is not difficult and does not require highly trained staff. Buccal cell collection is considered when non-invasive, self-administered, or mailed collection protocols are required for DNA analysis. However, buccal cell collection will yield only limited amounts of DNA in comparison to blood. Different methods of self-collection are available depending on the endpoints and the analyses to be performed. Buccal cell biospecimens may be useful as a source of DNA. A variety of collection techniques and containers have been developed specially for these collections.

2.3.3 Cytobrush

This method is to collect cells on a sterile cytobrush by twirling it on the inner cheek for fifteen (15) seconds. The operation is repeated three times, on the two (2) cheeks. The swabs are separated from the stick with scissors and transferred to a cryotube. The duration of the collection can influence the DNA yield. Cytobrushes produce DNA with good quality; however mouthwash is superior for reactions requiring long fragments.

2.3.4 Mouthwash

Buccal cells are collected by rinsing the mouth for then (10) seconds with 10ml of sterile water and expectorating the rinse into a 50ml centrifuge tube. This operation is repeated three (3) times. The amounts of extracted DNA can vary according the time of brushing. The effect of lag time of storage at room temperature is visible for mouthwashes, while the cytobrushes are less sensitive to the lag time at room temperature.

2.3.5 Treated cards

These cards are treated to inhibit the growth of bacteria and kill viruses thereby minimising nucleic acid degradation. The patients/participants expectorate saliva into a sterile cup. The tip of the treated card triangle is placed into the saliva, which is wicked onto the matrix. The treated card is air-dried and placed in a bag with dessicant. Treated cards correspond to the lowest efficiency for DNA yield because of the small quantity of collected saliva. Moreover, some proteins are let in the solution of extracted DNA. Therefore the DNA cannot be kept for long-term conservation. An advantage for this method of buccal cells collection is its low cost due to the absence of an extraction step. Finally, cytobrushes and mouthwashes are generally considered unsuitable for children because cytobrushes are abrasive. Mouthwashes require patients/participants to expectorate and may be aspirated or swallowed.

2.3.6 Saliva

Saliva is used as a biological fluid for the detection of different biomarkers such as proteins, drugs and antibodies. Saliva meets the demand for non-invasive, accessible, and highly efficient diagnostic medium.

The sample collection is non-invasive (and thus not painful) and can easily be done without various devices. Whole saliva is collected by expectoration into a provided tube, while for the collection of submandibular saliva and sublingual saliva different ducts need to be blocked by cotton gauze. For the collection of paratoid saliva, a paratoid cup should be used. Saliva samples are used for drug testing, HIV detection or monitoring of hormone levels and as a source of DNA. Collection devices for these biospecimens include non-covered cotton roll, polypropylene-covered polyether roll and paraffin wax chewing stimulation. Some researchers may request participants to provide saliva samples directly into a container with an opening large enough to facilitate this collection.

Saliva can be stored either as non-centrifuged aliquots or centrifuged, which results in supernatant and pellet aliquots which can then be analysed and/or stored separately.

2.3.7 Bronchoalveolar lavage (BAL)

The airways, and particularly the alveoli, are covered with a thin layer of epithelial lining fluid (ELF), which is a rich source of many different cells and soluble components of the lung that play important functions by protecting the lung from undue aggressions and preserving its gas-exchange capacity. Bronchoalveolar lavage performed during fiber-optic bronchoscopy is the most common way to get samples of ELF. The cellular and protein composition of the ELF reflects the effects of the external factors that contact the lung and is of primary importance in the early diagnosis, assessment and characterisation of lung disorders as well as in the search for disease markers.

Bronchoalveolar lavage is classically performed by instillation of buffered saline solution divided into 3–4 aliquots (typically a total volume of 100–150ml) through a flexible fiberoptic bronchoscope, after local anesthesia. The first 10ml should be processed separately and is denoted as bronchial lavage (BL).

The rest of the lavage, denoted as bronchoalveolar lavage (BAL), should be pooled into a sterile siliconised bottle and transported on ice immediately to the laboratory. At the laboratory, the total volume of the lavage is measured, and cells and proteins are separated by centrifugation. The lavage fluid should be frozen and stored at -80°C until use.

2.3.8 Bone Marrow Aspirate (BMA) and Fine Needle Aspirate (FNA)

Bone marrow is the soft tissue found in the hollow interior of bones. In adults, marrow in large bones produces new blood cells. There are two (2) types of bone marrow: red marrow (also known as myeloid tissue) and yellow marrow. In cancer research red bone marrow from the crest of the ilium is typically examined. Bone marrow should be collected by a registered health practitioner who is well trained in this procedure. Bone marrow should be aspirated by sterile percutaneous aspiration into a syringe containing an EDTA anticoagulant, and the biospecimens should be chilled immediately. Heparin is not recommended as an anticoagulant for molecular testing. If a biospecimen contains erythrocytes, it should be processed to remove the erythrocytes before freezing. The bone marrow samples should be freshly frozen and stored at -80°C.

2.3.9 Cerebrospinal fluid (CSF)

Cerebrospinal fluid (CSF) originates from the blood. The choroid plexes in the 1st, 2nd and 3rd ventricles of the brain are the sites of CSF production. CSF is formed from plasma by the filtering and secretory activities of the choroid plexus and lateral ventricles. CSF circulates around the brain and the spinal cord. CSF nourishes the tissues of the central nervous system and helps to protect the brain and the spinal cord from injury. It primarily acts as a water shock absorber as it surrounds the brain and the

spinal cord and thus absorbs any blow to the brain. CSF also acts as a carrier of nutrients and waste products between the blood and the central nervous system (CNS). CSF is the most precious biospecimen. Often, only small volumes of CSF are available for analysis due to difficulty in collection. Hence handle this with care. Only a registered health practitioner or a specially trained nurse must collect the biospecimen. After sampling the biospecimen should be transferred into a clean penicillin vial containing about 8mg of a mixture of EDTA and sodium fluoride in the ratio of 1:2. Centrifuging of CSF is recommended before freezing if the sample contains red blood cells or particulate matter. The biospecimen should be frozen and stored at -80°C or in liquid nitrogen. Do not delay freezing the CSF because cells are rapidly lysed once the CSF is removed from the body.

2.3.10 Semen

Seminal fluid is the liquid component of sperm, providing a safe surrounding for spermatozoa. At pH 7.35-7.50, it has buffering properties, protecting spermatozoa from the acidic environment of the vagina. It contains a high concentration of fructose, which is a major nutriment for spermatozoa during their journey in the female reproductive tract. The complex content of seminal plasma is designed to ensure the successful fertilisation of the oocyte by one of the spermatozoa present in the ejaculum.

Seminal plasma is a mixture of secretions from several male accessory glands, including prostate, seminal vesicles, epididymis and Cowper's gland. After collecting ejaculate, the fresh ejaculate should immediately be spun down at 4°C to separate seminal fluid from spermatozoa. Protease inhibitors should then be added to the sample to avoid digestion by powerful proteases present in seminal fluid. To ensure complete separation of cell debris or occasional spermatozoa from seminal plasma, the sample can be centrifuged a second time. The sample should be stored at -80°C.

2.3.11 Cervical and urethral swabs

The quality of collected cervical and urethral biospecimens depends on appropriate collection methods. Swabs, brushes or other collection devices should be placed in a transport medium, or transported dry in a sealed tube and resuspended in the transport medium upon arrival. The transport fluid may either be stored at -70°C or lower or immediately centrifuged, and the pellet processed for DNA or RNA extraction. Cervical vaginal lavages (CVL) can be obtained and used for HPV studies.

2.3.12 Nail and hair

Currently, hair analysis is used for purposes of assessing environmental exposures. Hair analysis is also used to test for illegal drug use and to conduct criminal investigations. Nail clippings may contain analytes of interest that were deposited during the growth of the nail. Nail biospecimens can be collected for drugs, nutritional, poisons and toxicity testing. Nail and hair clippings can be used for trace metal analysis to provide a longer-term measure of exposure. These samples are simple to collect, store and ship. They can also be used as a source of DNA.

2.3.13 Breast milk samples

Breast milk collection can be initiated when breast-feeding starts. Breast milk can be collected by manual expression or vacuum pump and should be collected in autoclaved or specially cleaned bottles and are typically stored frozen. If certain analytes such as phthalates are of interest, the sample can be collected in a glass bottle with Teflon™ cap and stored in the participant's household freezer.

2.3.14 Stool samples

Samples are self-collected by patient/participant into a container that can be lined with plastic wrap or placed inside another container to provide an impervious storage container. After collection the sample should be frozen. Some procedures will allow for lyophilising the sample for long-term storage which provides a more inert (less odoriferous), smaller sample for analysis.

2.4 Post mortem collection (autopsy/necropsy)

Remnant samples may be collected from autopsy/necropsy procedures consistent with relevant regulations, as appropriate. Requests should specify a maximum time interval post mortem prior to processing. Autopsy/necropsy procedures may yield “normal” tissues or large quantities of a biospecimen that would not otherwise be available from surgical procedures (eg heart or brain). Biospecimens that are not removed as part of the routine autopsy procedure (ie leg, arm, hand, foot or face tissue) are not usually available as their procurement may result in disfigurement of the body.

Tissue biospecimens collected at autopsy should be appropriately labelled as to the organ site, tissue type, and time of resection, and then placed immediately into a container of cold biopreservation media (2 – 8°C) on wet ice for transport to the tissue repository for processing. These organs could be dissected into smaller sections for processing and storage. Detailed information should be obtained about the decedent such as disease condition, age, sex, race, cause of death, time and date of death, and time of organ procurement. Information about the procured organ should include the condition (normal or diseased). Please see MSH Research Management - Biospecimen Ethics and Participant Information and Consent Form Procedure (PR2017/115) for more information on ethical requirements.

2.5 Transplant

Occasionally, organs that are inappropriate for transplant may be offered or made available to a research biorepository for research purposes. It is not unusual for the organ to have been out of the body for many hours beyond the normal time frame identified for procurement of samples. However, because transplant tissue is usually placed in a biopreservation media at 2 - 8°C to keep it viable for transplant, most researchers will still accept transplant tissue as it is likely to be of superior quality to either surgical or autopsy biospecimens.

Transplant organs may also be dissected into smaller sections for processing and storage. Information about the donor from whom the organ was procured should be obtained from the transplant centre. All organs/tissues intended for research should be maintained in appropriate biopreservation media at 2 - 8°C until processed. Isotonic saline or culture media may not be considered optimal for hypothermic biopreservation of viable cells/tissues/organs.

In general, it is important to remove as much blood and other native fluids from the resected tissue/organ as soon as possible prior to processing. The reason for doing this is because for larger or highly vascularised tissues in preservation solution, clot formation within the vasculature obstructs the penetration of the preservation solution into the tissue. This situation results in biospecimens that are not homogeneously preserved and there could be localised tissue damage due to ischemia. Also, tissue damage could occur because ischemia and the clotting cascade impact the molecular profile of the tissue. These activities produce changes in the molecular pathways and in the protein expression thus resulting in the tissue section being less representative of its original resected state.

It is also important to have pathology on the organ consented to use in research to ensure there is no other incidental disease which may affect research results (eg occult cancer).

3.0 Processing and handling

3.1 Tissue processing

To ensure suitability for genomic and proteomic research, the processing of the tissue sample or blood must be done in a manner to protect tissue architecture and the integrity of molecular products. Biospecimens must be handled as being potentially biohazardous and laboratory staff should take

appropriate precautions when handling tumour tissue or whole blood and blood products.

Desiccation and degradation of biospecimens should be avoided. The method of transport of the tissue sample from the operating room to the pathology or processing laboratory should be documented. Please see Material Transfer Agreements, Packaging and Shipping Procedure (PR2017/107) for more information.

All precautions to avoid cross-contamination of biospecimens during processing, product isolation or aliquoting must be employed. This should include using fresh containers, pipette tips and blades between biospecimens and between different areas of the same biospecimen (eg between malignant and associated uninvolved tissue). Snap freezing or freezing in a cryoprotectant must be done by suitable means.

3.2 Biospecimen handling – general considerations

MSH aims to provide users of its research biorepositories with standardised, high quality biological samples that are readily accessible for their research needs. To meet the needs of the users, the biospecimen should be handled in a manner that optimally maintains the architecture of the tissue and the molecular integrity of the DNA, RNA, and proteins in the biospecimen. All steps should be performed by staff that are suitably qualified or have adequate training to perform the tasks.

To ensure a minimum number of transfer or generation from the original biospecimen, where this is appropriate, the research biorepository could use a master (or seed) and distribution stocks. If undertaking this process for cells lines, nucleic acid and derivatives, the research biorepository should produce a master stock from the original biospecimen. This master stock should be used to generate distribution stock. The research biorepository should use the distribution stock to supply biospecimen. The research biorepository should adapt the size of these matters and distribution stocks to the anticipated distribution rate and potential uses.

3.3 Safeguards

The security safeguards must protect biospecimens against loss or theft as well as unauthorised access, disclosure, copying, use or modification. MSH research biorepositories must protect biospecimens regardless of the format in which it is stored. Methods of ensuring security of biospecimens should include the following methods:

- Physical measures such as locking research biorepository freezers, fridges and restricting access to offices and laboratories.
- Organisational measures, such as limiting access on a ‘need-to-know’ basis.

Overall MSH research biorepository security must be implemented by limiting access of unauthorised personnel to the workplace. Ideally, Custodians must also give consideration to maintaining a duplicate collection, preferably on another site as a ‘disaster’ protection measure and to avoid accidental loss. Please see Facility, Equipment, Storage and Security Procedure (PR2017/103) for more information.

3.4 Biospecimen labelling

Biospecimens contained within the research biorepository must receive a unique identifier that is linked, preferably electronically, to the uniform diagnostic data set.

Each biospecimen should be labelled in such a manner that the labelling will survive all potential storage conditions, in particular dry ice and liquid nitrogen. Ink used on the label should be resistant to all common laboratory solvents.

It is recommended to print labels with a barcode (linear or 2D), thus providing a direct link to database software. However, it is also essential to include human-readable indications of contents. The barcode template should be documented. The software used for labelling should allow data import and export in standard formats.

All biospecimens should ideally be labelled with at least two human-readable forms of identification without revealing the identity of the patient/participant. Information on the label should include research biorepository's unique identifier number and/or the number of the place within the storage system, with the same information repeated in the barcode if available.

Annotation data (eg person, lifestyle, diagnosis, laboratory, clinical and research generated) should be accurate, quality-controlled and standardised as far as possible. See Databases, Tracking, Records and Documentation Procedure (PR2017/109) for more information.

3.3 Transport

The transport and transfer of biospecimens must be carried out as soon as possible in order to minimise the effect of hypoxia upon gene expression, degradation of RNA, proteins and other tissue components. For transport from surgery to pathology, or to the MSH research biorepository, fresh biospecimens must be placed in a closed, sterile container on wet ice. All tissue must be treated as potentially infectious; the collection process should be carried out in the most aseptic conditions possible.

The use of biospecimen tracking systems for transportation, location within the laboratory, archival storage and inventory management is strongly supported. Biospecimen transportation and storage environmental conditions should be continuously monitored and protected by alarm systems and back up and emergency procedures. Each biospecimen receptacle must be clearly labelled. Please see Material Transfer Agreements, Packaging and Shipping Procedure (PR2017/107) for more information.

4.0 Storage

The biospecimen should be stored under environmental parameters that assure the stability of its properties. Details of the inventory control, lead times and re-stocking practices must be documented. The research biorepository should select preservation and maintenance methods according to recommendations from the depositor and/or previous experience. The research biorepository must document these preservation processes to ensure they are reproducible and that key parameters of the process are recorded and monitored. The storage method of the tissue sample, or derived product, affects the suitability of the sample for use in specific genomic or proteomic studies. Storage SOPs must be geared to protecting the integrity of the collection and should allow for efficient and accurate retrieval of samples. Samples must be stored in a manner optimal for their intended category and use and this should be documented. Please see Facility, Equipment, Storage and Security Procedure (PR2017/103) for more information.

In some circumstances, the biospecimen should be preserved by at least two (2) methods (where two (2) distinct methods are not applicable to the biospecimen, cryopreserved stocks should be maintained in separate locations) and as a master cell banks and as stocks for distribution. The details of the preservation techniques are laid down in the domain specific criteria.

The labels should include at least the batch date of number and the research biorepository accession number. Where possible an indication of expiry date should be provided to the user of the biospecimen. Biospecimen with specific hazards must be clearly differentiated.

Frozen samples should be stored in screw-capped, plastic containers or cryovials that can be sealed. Vials should permit appropriate labelling, prevention of contamination or samples desiccation and should withstand freezing in liquid nitrogen.

If mechanical or liquid nitrogen (LN_2) systems are used for storage of frozen samples, adequate back-up capacity should be in place to ensure that operating temperatures are maintained at all times. Events such as equipment failure or power-outage emergency should be planned for and processes should be in place to deal with possible emergencies. For mechanical freezers, manual defrost feature is optimal as freeze-thaw cycles of automatic units can degrade biologic samples. Alarm systems must be used to monitor temperatures in the storage freezers and SOPs be in place to permit corrective action before the temperatures falls out of range. See Emergency Preparedness and Work Health and Safety Procedure (PR2017/108) for more information.

4.1 Cryopreservation

Cryopreservation is the recommended standard for preservation of biospecimens for a wide range of research applications. Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures, typically -80°C (freezer) or -196°C (nitrogen liquid phase). Cryopreservation involves the process of cooling cells or whole tissues to low sub-zero temperatures at which any biological activity, including the biochemical reactions that would lead to cell death, is effectively stopped. There are generally two (2) distinct methods of cryopreservation:

(1) Preservation in the frozen state which can involve either ultra-rapid freezing (direct introduction into LN_2) or controlled-rate (slow programmable) cooling.

(2) Vitrification, which is preservation in the glassy, non-crystalline state (not commonly utilised in research biorepositories).

Both methods usually require the addition of cryoprotectants which have different protective properties (eg colligative or osmotic) although generally they will lower the freezing temperature. Cryoprotectants are applied in different regimes, combinations and concentrations dependent upon the mode of cryopreservation (eg frozen or vitrified).

Controlled-rate cooling minimises the potential for lethal, intracellular ice forming during the freezing process; this is achieved by controlling extracellular ice nucleation (also termed ‘seeding’) and applying optimally slow cooling rates that allow sufficient water to leave the cell during the progressive freezing of the extracellular fluid. Controlled-rate cooling requires the careful control of cooling rate (eg around 1°C/minute is appropriate for many mammalian cells), ice nucleation temperature and terminal freezing temperature and hold time (ie before biospecimens are transferred to LN_2); the optimisation of which will vary between cells of differing size and water permeability.

To circumvent cryoinjury caused by toxic cell volume changes and the excessive concentration of solutes the application of colligative cryoprotectants, such as dimethyl sulfoxide (DMSO), is required during controlled rate cooling.

In contrast, vitrification is a process that avoids the potential damage to cells caused by intracellular and extracellular ice formation. This is usually achieved by the addition of cryoprotectants at higher concentrations which increase the viscosity of the sample and prevent ice crystals from forming.

Common cryoprotectants, such as DMSO are often toxic in high concentrations and care must be taken to limit the damage produced by the cryoprotectant itself. When possible, researchers should test available preservation solutions to determine what works best for their specific research activities and, as required, optimise the cryoprotectant strategy for their preserved samples.

Using an appropriate cryopreservation media and cryoprotectant(s) will reduce the rate of degradation at hypothermic temperatures and offset the risks of inadvertent devitrification at ultra-low temperatures. When possible, researchers should test available preservation solutions to determine what works best for their specific research activities and, as required, optimise the cryoprotectant strategy of cryopreserved samples. Using an appropriate biopreservation media will reduce the rate of degradation at hypothermic temperatures and risks of inadvertent devitrification at ultra-low temperatures.

The temperature at which frozen and vitrified preparations are stored affects the length of time after which cells can be recovered in the viable state (generally, the lower the storage temperature, the longer the viable storage period). As temperature is reduced, metabolic and degradation processes in cells are slowed; however, they are not effectively slowed to allow for long-term storage (years to decades) until the temperature falls below the glass transition temperature of pure water (effectively < -132 °C for most mixtures of cells and aqueous cryopreservation media).

The Tg of some vitrification solutions can be higher and it may be prudent to determine the actual critical Tg using thermal analyses. In addition to storage temperature, handling during removal from storage will affect the viability of cells and may result in degradation of cellular components. Every time a sample is warmed above the glass transition temperature, it experiences a micro-thaw event. Repeated thermal cycling episodes lead to increased cell death via apoptosis and necrosis.

The temporal nature of delayed onset cell death resulting from preservation stress may affect the quality of data obtained from these samples depending on the timing of experiments post-preservation and the ability of the cells to recover from cryoinjury in the long-term.

At these low temperatures, any biological activity, including the biochemical reactions that would lead to cell death, is effectively stopped. However, due to the particular physical properties of water, cryopreservation may damage cells and tissue by thermal stress, dehydration and increase in salt concentration, and formation of water crystals.

The below table lists the most commonly accepted cryopreservation standards for human tissue and body fluids. Specific applications (eg proteomics or storage of primary cell cultures) may require more complex cryopreservation procedures.

Temperature in °C	Properties of water/ liquid nitrogen	Cryopreservation method	Biological relevance
0 to +4	Ice melting	Refrigerator	Processing of fresh material
-0.5 to -27	Ice fusion area	Freezer	
-27 to -40	Ice	Freezer	Limit of protein mobility/ DNA stability
-40 to -80	Limit of water molecules mobility	Freezer	RNA stability
-80 to -130	Ice transition	Freezer/Liquid Nitrogen	Recommended storage for blood and urine
-130 to -150	Liquid nitrogen (vapour phase)	Liquid nitrogen	Recommended storage of tissues
-150 to -196	Liquid nitrogen (liquid phase)	Liquid nitrogen	Possible micro-fractures. Recommended storage of living cells

Table 1: Basic standards of cryopreservation and applications to biological biospecimens

Biospecimen freezing is generally performed by placing the biospecimen in a sealed (but not airtight) container and immersing the container in the freezing medium. The ideal medium for rapid freezing is isopentane that has been cooled to its freezing point (-160°C). To achieve this, the vessel containing the isopentane should be placed in a container of liquid nitrogen. The freezing point approximately corresponds to the moment when opaque drops begin to appear in the isopentane. Direct contact of the biospecimen with liquid nitrogen should be avoided, as this can damage tissue structure.

4.2 Biopreservation

Biopreservation is a general term used to describe the preservation of all types (eg viable and non-viable cells, blood, cell fractions and DNA) of biospecimens using a range of low temperature and some ambient storage methods. Whereas, cryopreservation is a more specific form of preservation that involves the storage of cryoprotected living cells, tissues, organs and organisms at ultra-low temperatures, usually in LN_2 or its vapour to a minimum temperature of -196°C.

4.3 Other fixation and preservation methods

Formalin or alcohol fixation and paraffin embedding may be used as an alternative method to preserve tissues at relatively low cost when adequate freezing procedures and storage facilities are not available. Fixed paraffin blocks may be stored in light and humidity-controlled facilities at 22°C.

Tissues fixed according to strict protocols may be used for DNA extraction. The DNA is usually fragmented but remains suitable for PCR-based analysis of short DNA fragments (up to 1–2 kbp). However, fixed tissues are of limited usefulness for RNA extraction.

RNAlater is a commercial aqueous, non-toxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA. RNAlater eliminates the need to immediately process tissue samples or to freeze samples in liquid nitrogen for later processing.

Tissue pieces can be harvested and submerged in RNAlater for storage for specific periods without jeopardizing the quality or quantity of RNA obtained after subsequent RNA isolation. However, biospecimens once placed in RNAlater cannot be further used for histomorphopathological analyses. Please see Recommended Preservation Methods contained within Attachment 3 for more information.

4.4 Liquid Nitrogen (LN_2) tanks

The critical temperature for sensitive tissues, organisms and cells is generally considered to be -132°C, the glass transition temperature (Tg). Vapour-phase storage is preferred over liquid-phase storage, but the design of the tank/ freezer is critical to maintain a sufficient amount of LN_2 in the vapour phase. Use of vapour phase avoids some of the safety hazards inherent in liquid-phase storage, including the risk of transmission of infectious agents. In contrast, liquid-phase storage necessitates less frequent resupply of LN_2 and thus affords better security in case of a crisis in LN_2 supply.

4.5 Freeze/thaw and cooling/re-warming cycles

Research biorepositories must avoid unnecessary thawing and refreezing of frozen biospecimens. Freeze/thaw cycles for biospecimens cryopreserved in the frozen state and cooling/re-warming cycles for biospecimens in the vitrified state can be damaging to the macromolecules and cells intended for analysis.

Damage can also occur via osmotic and dehydration injury during exposure and removal of cryoprotective additives and vitrification treatments. Therefore, it is important to select aliquot sizes that are appropriate for the intended uses for the biospecimens in order to minimize the number of times a sample is thawed and frozen or vitrified before it is used.

Samples are often maintained at liquid/vapour nitrogen temperatures in order to achieve biopreservation of the sample below the glass transition temperature (below which the cell biochemical activity is virtually stopped).

Thermal cycling intervals resulting in sample temperature increase above the glass transition temperature allows for repeated freeze/thaw cycles even within the sub-zero frozen state. For these reasons it is essential to limit the potential of freeze/thaw and vitrification/devitrification cycles occurring when samples are introduced or removed from storage.

Checks must be in place to assure and validate storage stability.

4.6 Validation of methods and processes

Validation of the methods and processes used for preservation must be carried out to ensure their reproducibility and reliability, and general compliance during the quality control of biospecimen. The validation of quality check, characterisation and preservation methods should be carried out by using at least one of the following approaches:

- Performing blind tests.
- Comparing the results of the same method performed at different times (reproducibility).
- Comparing results obtained with different methods (reliability).
- Comparing the results obtained for the same method performed by different persons.

The results of quality checks and the process used should be recorded.

5.0 Biospecimen stability

In addition to the issues of temperature discussed above, biospecimen stability may also be affected by other parameters such as the use of anticoagulants and stabilising agents like EDTA and ascorbate. It is important to know in advance, biospecimen collection requirements by manufacturers of customised assay kits. For some applications, rapid dehydration is an effective method to stabilise molecules. Dehydration methods may be more practical in field settings where access to refrigerants or chemical fixatives is dangerous or cumbersome. Where cold temperatures are applied it is essential to maintain the continuity of the cold chain from the point of collection to deposition in the research biorepository. Selected methods for collecting and preserving biospecimens should be followed when possible to ensure that any preservatives, dehydration or other protective treatments used do not have a deleterious effect on future analyses.

5.1 Collection and storage containers

Collection and storage containers vary according to biospecimen types being collected and the analytical goals of the research project. During selection of container type, consideration should be given to the long-term use, standardisation and applicability to new platforms and automation. Also, the same containers used for biospecimen collection may not be suitable for biospecimen storage. In some cases contaminants associated with the container (eg persistent organic pollutants or heavy and trace metals) may interfere with subsequent analysis. This issue is especially true for biospecimens stored for environmental analysis. Container labels should be permanent and able to endure excursions in and out of cold conditions and exposure to high humidity and ambient temperatures especially when samples are taken from extreme environments. Light sensitive material should be stored in containers that do not allow penetration of light such as amber vials or amber coated bags.

5.2 Sterility

Risk assessments and mitigation exercises must be undertaken in the context of a biospecimens requirement for asepsis. While complete sterile conditions may not be required for many biospecimen collections and processing, adequate consideration must be given to the cleanliness of instruments, surfaces and equipment used in biospecimen processing and handling.

RNA is particularly sensitive to RNAses which may be present on tools and surfaces that have not been properly cleaned/sterilised. Where disposable instruments are used, every biospecimen should be handled with fresh new instruments and when non- disposable instruments are used, they should be appropriately cleaned after each biospecimen processing. Sterility of preservatives, cryoprotectants and liquid nitrogen supplies should also be considered.

5.3 Pilot research projects and proof of performance research projects

MSH research biorepositories should implement small-scale pilot research projects to validate new protocols, equipment, laboratory tests or testing services when possible. Pilot or feasibility studies can identify problems or critical points and instigate preventative actions at an early stage of collection, handling and processing before a larger study is undertaken. Pilot studies can also help determine new processes and identify training required before implementing a new protocol.

5.4 Methods research

All research endeavours should be based on well-characterised and validated assays, where possible. These methods can often initially be guided by experienced researchers that will be involved in end use of these research biorepositories. Even assays that are developmental in nature should be tested to ensure that they are reproducible over time. ‘Proof of Performance’ tests allow for testing replicate samples over time to allow for measurement of standard deviations in the assays performed.

Where possible, research should be performed to ensure that the storage and handling procedures implemented are ones that will be conducive to stabilisation of the molecular components and particular targets of interest within the biospecimen.

6.0 Retrieval

Samples should be retrieved from storage according to research biorepository SOPs that safeguard sample quality. Retrieval of biospecimens for shipment or analysis requires strict adherence to protocols for proper biospecimen inventory and tracking, as well as adherence to established safety standards in working with freezers and other storage equipment. Please see Access and Applications for Samples Procedure (PR2017/106) for more information.

6.1 Locating biospecimens in storage

The location of biospecimens to be retrieved must first be verified in the appropriate biospecimen inventory system. A biospecimen requisition is generated according to procedures applicable to the research biorepository’s biospecimen requesting, tracking and inventory protocols. The requisition is checked for accuracy before transmission to the research biorepository, according to established SOPs and QC standards. Please see Databases, Tracking, Records and Documentation Procedure (PR2017/109) for more information.

6.2 Biospecimen retrieval

Biospecimens should be located and pulled from storage as documented on biospecimen requisition forms. If biospecimens are frozen or vitrified, speed is necessary during the retrieval process. Such speed may require that at least two (2) individuals carry out the retrieval process.

If possible, biospecimens being retrieved should be maintained at the storage temperature throughout the process (eg biospecimens stored at -80°C should be kept on dry ice during the retrieval process). Forceps may be used when withdrawing biospecimens stored at LN_2 temperatures to prevent warming of the biospecimens from contact with hands. Once retrieved, research biorepository personnel must confirm that all requisitioned biospecimens have been accounted for. Recommended Quality Control (QC) checks/processes must be performed to confirm that all biospecimens listed on the requisition were retrieved (Attachment 2).

If biospecimens appear to be missing, SOPs must be followed in order to locate the missing biospecimens. Inventory systems should be updated to indicate that samples are in fact missing or that they were improperly located when placed in the inventory. A second, independent quality control check should be performed to ensure that the correct biospecimens have been retrieved. Mechanisms should be established to track before the last aliquot of a critical material is about to be used.

6.3 Thawing, re-warming and aliquoting biospecimens

6.3.1 Liquid and solid tissue

Frozen tissue biospecimens should be thawed at room temperature for a brief period of time or at refrigerated temperature (4 - 8°C) overnight. Biospecimens in resin straws can be thawed directly at room temperature or in a 37ºC water bath. Care should be taken that surface moisture from water baths does not enter the sample containers. In the case of vitrified samples, optimisation of re-warming is critical and it may be necessary to apply a two-phase and/or rapid re-warming process to ensure that the samples do not form ice crystals as they pass through the Tg.

Large-volume liquid samples (eg sera, plasma and urine) may need to be divided into smaller aliquots for distribution to multiple end-users. The proper pipette and tip to use is determined by the required volumes and eventual analysis. If analysing for persistent organic pollutants, using a plastic pipette and tip may contaminate the sample further. A different pipette tip should be used for each biospecimen.

A new alternative to thawing a biospecimen for aliquoting is a drilling system that includes a motor that produces a sonic, linear oscillatory motion that removes a frozen biological sample from a stored frozen biospecimen without thawing the remainder of the biospecimen. Biospecimen containers must be opened and the biospecimens aliquoted in a biological safety hood. Sterile vials and pipettes should be used to avoid contaminating samples.

6.3.2 Viable cells

The rate and method of freezing/thawing and cooling/rewarming biospecimens can have serious effects on the viability of cells. Exact freezing/thawing, vitrification and cooling/re-warming protocols should be developed, including the validation of appropriate biopreservation media (cryopreservation solution), devices and cooling rate to ensure that the method used supports the known or anticipated use for the biospecimens.

Although slow cooling is generally best to ensure cell viability in frozen samples, the opposite process is required when thawing from the frozen state. Agitation of the vial/ampoule/straw in a 37°C water bath for a brief period of time is preferable, but this process may be detrimental to certain cell types if the time required is too lengthy. Samples should be rapidly thawed just enough to thaw visible crystalline ice, and so that sample temperature is still hypothermic (2 – 8°C). Cells should be further quickly diluted in appropriate media to minimise toxicity from the cryopreservation media; however, dilution protocols (which may include several washing steps to gradually dilute and wash out the cryoprotectant) should be optimised to circumvent damaging osmotic effects. In the case of vitrified biospecimens, optimising cooling and re-warming regimes is critical for ensuring the formation and maintenance of a stable glassy state and preventing de-vitrification and ice nucleation during re-warming. Therefore, in contrast to controlled rate freezing protocols, vitrification can involve rapid cooling and re-warming regimes.

6.3.3 Assessment of cell viability

The number of recovered cells can be determined by several methods, including: dye exclusion (ie Trypan Blue) or vital staining using tetrazolium salts, and fluorescent dyes such as fluorescien diacetate. Accurate measurement of the number of viable cells in a non-motile population can be difficult and it is essential to standardise viability assays and where possible back them up with unequivocal tests of functionality and clear evidence of cell division and growth. As indicated previously, the phenomena of delayed onset cell death results in a temporal disconnection between evaluations of viability immediately post-thaw and actual long term cell survival and functionality.

As such, it would seem logical that post-thaw assessment of cell viability would need to be expanded beyond a singular assessment via Trypan Blue (or similar assays) immediately post-thaw or after re-warming in the case of vitrified samples. An outline for an expanded assessment of post-thaw recovery (this may be referred to as True Yield/Viability) would include multiple methods of assessment including Live/Dead assays, cell death mechanism assays such as Annexin/PI, metabolic assays such as alamarBlue or MTT, cell function assays (dependent on cell type), and assessment at multiple time points post-thaw/re-warming, especially within the timeline of delayed onset cell death.