ATTACHMENT 3 - Quality Control Considerations for Specific Types of Biospecimens

Generic Quality Control (QC) processes

Many of the Quality Control (QC) processes are generic across all types of research biorepositories and they concern the three "pillars" or responsibilities of all collections which are:

- **Authenticity:** correctly assigned identity.
- **Purity:** freedom from contamination (when applicable).
- **Stability:** capability of a sample material to retain the initial value of a measured quantity for a defined period of time within specific limits when stored under defined conditions. For example, the stability of serum ("sample") as pertaining to the Protein S activity ("measured quantity") when stored at -80 °C ("under defined conditions") is the initial Protein S activity value ("the initial value") plus or minus 20% ("within specific limits") for five years ("for a defined period of time").

Depending upon the molecular analyses that will be performed by the end-user, it may be advisable to extract and analyse matching molecular entities (eg DNA, RNA or proteins) as a part of the biospecimen QC testing.

Discard processes

QC testing for potential discard (prior to selection and dispatch to a researcher) must be looked at cautiously by research biorepositories. Whilst a research biorepository may have had ensured complete sample control (ie from collection to delivery) to processing to freezing, thus ensuring standardised process, other collections may have had samples arrive from a host of institutions, with no ability to standardise each step (apart from a request to each site to do so). Given this scenario, QC testing for potential discard might be considered reasonable by a research biorepository however it must be considered on a case by case basis.

QC testing and selection

QC testing may be performed immediately prior to dispatch (to researcher) as this still gives time to find and replace an alternate sample to the initial selection. QC selections may need to be research biorepository specific (ie if a research biorepository is 'cherry picking' samples for QC testing) personnel might decide to choose a specific 'sample collection date' (eg 01-01-2008). Custodians should however give consideration to identifying if sample selected was collected on that date BUT didn't arrive for processing until two days later for example. This may result in the integrity of that sample differing from a similar sample not selected for testing (ie collected on the 01-01-2008 but was processed same day).

There are a myriad of factors that would influence the integrity of 'same type' samples (dates, collection type, sample type, temperature, time, transport, processing, freezing, diagnosis) to name but a few.

Whilst QC selection based on one 'type' could work extremely well for a research biorepository who have no sample deviation across their holdings that may not be the case for the majority. It is important to also note that QC measures for specific types of biospecimens can also be dictated by national/federal or international rules and regulations (eg work health and safety and bioethics).

QC considerations for solid tissue biospecimens

QC examination of tissues designated for research should be appropriate for the research protocol. QC of tissue ranges from microscopic examination of an aliquot representative of a specific tissue by a pathologist or cell biologist, or an equivalently trained individual, to molecular quality control in which nucleic acids and proteins are characterised. The highest quality control measures ("platinum" level) involve enriching the diseased population of tissue through macro- or micro- dissection of frozen sections and potentially performing molecular analyses as well. Platinum-based approaches are, however, cost prohibitive and potentially exhaust biospecimen availability. A cost effective approach for tissue resources requires simple methods of QC that can be expanded per investigator request.

Prospective research projects may require specific QC measures and this can be performed for defined research projects per an investigators request. The additional QC required may then also be part of the cost recovery process as part of the research project budget. However, for general research biorepositories a standardised (simple) QC program should be implemented to keep labour and testing costs to a minimum, whilst still being able to assure a quality product for researchers.

For pathology research, if tissue is prospectively removed from a patient/participant with a particular diagnosis, verification of disease state criteria meeting the research request should be confirmed. The percent of biospecimen that is diseased should be documented along with the percent necrosis/fibrosis and percent of mucin formation present in the tissue. If tumour is present, tumour cellularity should be assessed.

For each biospecimen collected, an aliquot, representative of that biospecimen, should be microscopically examined by a trained pathologist or other trained professional experienced with the organism from which the tissue originated. This aliquot can be the diagnostic biospecimen from whence the research tissue was obtained, as long as the aliquot reviewed is as close as possible to the area where the tissue supplied for research was procured. Please note that this will require a pathologist to support research biorepository QC and examination will be required ASAP on the day of collection as solid tumour is also 'fresh frozen' by some research biorepositories ie not just kept as FFPE.

QC considerations for digital/virtual microscopy

Virtual Microscopy (research biorepository specific and dependent on sample type/instrumentation)

Virtual Microscopy (VM) is the method of producing a digital image of a tissue section or cytological preparation mounted on a glass microscope slide that is suitable for visual examination, annotation of regions of interest and interpretation.

This method uses scanning equipment at a range of magnifications to produce digital images suitable for remote web-based viewing and archiving. These digitised images can approximate the process of viewing slides microscopically, including the capacity to adjust viewing magnification and focus on specific regions of the image. When optimised, image quality may be sufficient for diagnosis or QC to confirm the composition of research biorepository research biospecimens. The use of this technology in certain situations may provide advantages compared with microscopic examination of slides, including: elimination of shipping glass slides, facilitating rapid review, reducing costs of tracking and replacing lost or broken glass slides, and allowing accessibility anytime via the Internet as well as allowing concurrent review of the same slide image by multiple viewers.

Depending upon the availability and type of imaging systems, at some locations it may be more cost effective to provide a tissue section on a glass slide to investigators. Also, high quality images require optimal scanning and significant data storage capabilities; thus the storage capacity required for a large number of such images must be taken into consideration and only the most "diagnostically difficult" cases may necessitate digital storage.

Digital pathology

Digital pathology, built around the examination of digital virtual microscope images, is a workspace environment which integrates with other electronic applications such as laboratory information systems, electronic medical records, medical imaging, molecular testing systems and biospecimen tracking and receiving systems.

Digital pathology also allows complex image analysis of both morphology and tissue based assays (ie immunohistochemistry, immunofluorescence) and can allow simultaneous viewing of multiple different images concurrently. Image analysis of biospecimens could ultimately be used to automate quality control in tissue research biorepositories by augmenting or replacing the traditional morphologic review of actual tissue sections. It could aid in assessing tissue quality by detecting and measuring features such as % tumour, % stroma, % necrosis, % cellularity and other morphologic features.

In a hospital setting research biorepositories may utilise 'excess samples' to clinical requirements. In this circumstance the research biorepository may require pathology laboratories and utilise this type of equipment for clinical samples. It may also be possible to have a dedicated pathologist whom is willing to undertake QC processes for research biorepository biospecimens.

If a research biorepository is reliant upon clinical biospecimen pathology reports, it is presumed that research biorepository biospecimens are exactly the same as the clinical samples taken diagnosis. This is assumed for bone marrow and blood samples however this may not be correct for tumour biospecimens. The biospecimens destined for the research biorepository would also need to undergo the same dissection and evaluation process. The clinical biospecimen cannot be used to provide % for the research biorepository biospecimen.

QC considerations for fluid biospecimens

The different collection, processing and storage procedures may adversely affect the structure and/or function of molecular components in fluid biospecimens.

In some situations, fluid biospecimens (eg serum, plasma, urine, saliva and cerebrospinal fluid) may require assessment as to their integrity in view of the detection or measurement of specific analytes.

It is imperative of all QC that 'timing of testing' is determined by the research biorepository. Questions such as how often is testing going to be performed and on 'what' research biorepository samples should be asked.

It is also important for the research biorepository to note if it is expending samples for QC purposes thus rendering each unusable for future research projects (eg QC would be counterproductive if done on rare entities at any time other than just prior to researcher dispatch).

Molecular markers to assess specific pre-analytical variables can be used, such as the haemoglobin content to assess haemolysis or the sCD40L content to assess exposure to room temperature. In the absence of a sufficient number of such quality control tools, this is an ongoing field of biospecimen research. In many instances quality control can only be performed in reference samples and in a targeted manner once the end-use analysis is known. For example, if it is known that the biospecimens are going to be used for the measurement of a specific cytokine, the level of this cytokine in a previously collected panel of control samples can be compared to the reference interval in a panel of freshly acquired specimens of the same type.

This would involve utilising a pathology laboratory reference range (for clinical testing). In a research biorepository setting Custodians would need to 'test' the research biorepository sample for all potential analytes PRIOR to freezing. Whilst research biorepositories are not set up as pathology testing labs, it is potentially possible to request testing of the samples (via pathology). Provided the sample is within reference range at the time of freezing, the result of each analyte tested needs to be recorded against each of the patient's/participant's samples. A minimum 'analyte' data set would need to be established for each sample type.

Frozen samples are not thawed before being dispatched to a researcher. In order to perform QC testing prior, another of the patient's/participant's s samples would have to be tested to see if time or any process undertaken in the research biorepository has impacted on analyte integrity. A second sample and a second test would be required for the analyte in question.

Additionally, the cost of performing the first set of analysis followed by QC testing on an identical biospecimen and the resourcing needed would in all likelihood make the overall costs of research biorepositories unsustainable and if costs were passed on to researcher the samples would become cost prohibitive.

QC control considerations for Cell specimens - research biorepository specific, dependent on sample type

Contamination control methods for eubacteria, fungi, mycoplasma and viruses can be applied to primary cell cultures or cell lines. Cell viability and/or purity of the cell suspensions can be assessed after thawing of a representative frozen aliquot. DNA fingerprinting methods can be applied for identification of established cell lines.

QC considerations for microorganisms - research biorepository specific, dependent on sample type

Phenotypic characterisation includes both macroscopic and microscopic morphology assessment. Genotyping (eg DNA sequencing, PCR-based profiling, microarrays), ribotyping, classical biochemical tests and/or serotyping methods can be applied for taxonomical identification purposes. Functional assays include viability assays, or assays for cytopathic effects.

QC for purity can be performed; however, certain cultures need to be maintained in a non-axenic state (eg obligate plant pathogens and assemblages of microorganisms, symbiotic and beneficial associates found in microalgae and cyanobacterial collections).

QC considerations for plant biospecimens - research biorepository specific, dependent on sample type

The overarching QC process of plant biorepositories (ie gene research biorepository, culture collections, germplasm repositories, seed and field research biorepositories) ideally involves germplasm characterisation before and after storage and at the point of dissemination as well as plant health (phytosanitary) checks, safety duplication and passport documentation with assignment of an accession number.

In the case of seed materials, the International Seed Testing Organisation (ISTA) has the mission to develop and publish standard procedures in the field of seed testing and encourage and establish uniformity in seed testing world-wide.

For clonally propagated plants (and other non-seed genetic resources such as pollen and dormant buds) quality testing includes assessment of viability, phytosanitary status and disease management (eg comprising quarantine, disease indexing and eradication).

Phenotype and genotype authentication can be a regulatory requirement for some crops and commercial forestry species and can include formal confirmation of certification status (trueness-to-type) by field-testing plants that are evaluated using specific phenotypic descriptors and as appropriate confirmation using molecular markers.

The detection, expression and stability of genetically modified materials may be necessary. The risk assessment and management of transgene contamination is a requirement for certain types of collections.

Post-storage QC measures include assessments of viability, morphogenetic competence, totipotency, regeneration, biochemical stability (eg, for secondary product producing cell lines) phenotypic and genotypic stability (eg, characterisation of somaclonal variation) and trueness-to-type assessment under field or glasshouse conditions using descriptors.

QC considerations for nucleic acid biospecimens

DNA and RNA can be assessed for integrity and fragmentation (eg molecular weight; RNA Integrity Number), quantity/concentration and purity. DNA can be assessed for the absence of cross-linking, the absence of PCR inhibitors, the bisulfate conversion rate and the methylation status. RNA can be assessed for amenability to reverse transcription and the maximum length of the quantitative real time polymerase chain reaction (qRT-PCR) products.

Generally, most research biorepositories will not carry out all QC considerations given here on their research biorepository genomic samples. They may one or a combination eg RNA = RIN plus spectrophotometry/nanodrop A260/280 or DNA = nanodrop or picoGreen® dsDNA quantitation. It will largely depend on the research biorepositories accessibility or ownership of QC instrumentation.

Potentially a 'minimal QC testing requirement' per genomic sample should be stipulated for sample types similar between Metro South Health research biorepositories.

QC considerations for macroscopic cut-up of histopathology biospecimens

Procedures for Macroscopic cut-up of histopathology biospecimens (see RCPA Macroscopic Cut-up Manual), standardised units and terminology in pathology requesting and reporting (see RCPA PUTS and PITUS projects) and Structured reporting protocols (see RCPA Structured reporting protocols) should be used wherever possible. The pathologist is responsible for biospecimen sampling for patient care and is in the best position to prioritise patient care and retention of biospecimens for future diagnostic or therapeutic purposes over research interests.

RCPA is an accreditation body for diagnostic laboratories. The uniform use of RCPA Structured Reporting protocols facilitates the collection of diagnostic information critical to the value of a research biorepositories' biospecimens. The capture of critical diagnostic elements in discrete data fields for electronic transmission between diagnostic pathology laboratories and cancer registries as well as researchers reduces errors and saves time currently spent re-entering diagnostic information. The QUPP funded PITUS project is aimed at developing data transmission standards to support this functionality. Diagnostic pathologists may be required for this process.

If a future goal is to have Metro South Health research biorepositories accredited, it would be best to align all research biorepository quality and technical aspects with diagnostic laboratory standards.