Attachment 4 - Collection, Processing, Handling and Retrieval Checklist

Handling

The collection, processing, embedding, and quality check for all biospecimens is critical to the overall quality and diversity of the sample inventory.

Self-audit/review assistance

- Review SOPs for sample handling, including sample types, samples with potentially infectious materials, aliquoting, relabelling, de-identifying or anonymising and biospecimen retrieval.
- Ensure there is an SOP for the type of samples suitable for submission to the research biorepository and rejection criteria policy and records of rejection.
- Undertake a sample of records for the assessment of the quality of stored biospecimens.
- Review Participant Information and Consent Forms (PICFs).
- Observe biospecimen processing area for clean environment, aliquot sizes of biospecimens, biospecimen identifiers, biospecimen storage conditions during sample receipt and processing and tracking of samples as they move from one station to another.
- Ensure temperature excursion information is maintained and tracked.
- Review quality assessment process for stored biospecimens and risks of biospecimen misidentification monitored and the process improved.
- Review biospecimen coding system for sample identification and process for confirming patient/participant consent prior to processing and banking.
- Review SOP in relation to sample size and process if the sample size is too small relative to the requirements or if it does not meet researchers' needs.
- Follow a tissue sample released for research from the pathologist to storage, verifying biospecimen identification throughout the process.
- Select several biospecimens and follow their tracking throughout the life of the biospecimen, including from parent to child etc.

1. **Biospecimen types submission criteria** Y/N/NA

   There is a clearly defined SOP defining types of biospecimens submitted to the research biorepository that is based on:

   1. Purpose - intended use of the biospecimen
   2. Required biospecimen data
   3. Safety - laboratories are suitable for the type of biospecimen/pathogen

requiring processing (biosafety/risk level)

4. Duration of storage (may be indefinite)

The SOP may be an overarching statement contained within the Research Protocol that defines the criteria required for all collections held in the research biorepository. This may include the receipt or transfer of an entire collection.

2. **Collection/processing oversight** Y/N/NA

A pathologist or the Custodian must ensure that the documented collection processes and SOPs reflect published best practices.

Blood and other body fluids not required for the diagnosis or prognosis must be collected with approved protocols and may not require pathologist review. To determine remnant tissue at the site of the collection, the appropriate medical/legal designee must be involved in the decision. This does not apply to downstream processing.

3. **Quality Control (QC)/Quality Assurance (A) for stored biospecimens** Y/N/NA

A mechanism for periodic assessment of the quality of stored biospecimens is in place for each class of biospecimens in the research biorepository.

The frequency of the checks may be determined by the; type of biospecimens being stored, preservation method and turnover of the material. This may take a variety of forms including direct observation of materials, sampling, integrity of records etc. The form and frequency that this takes is to be defined by the research biorepository.

Quality assurance may be assessed at the time of disbursement.

Evidence of compliance:

✓ Documentation of inventory sampling OR

✓ Documentation of unsuitable specimens by collection, as applicable OR

✓ Documentation of inventory QA/QC processes OR

✓ Assessment from researchers using the biospecimens

4. **Aliquot size** Y/N/NA

Aliquot sizes are appropriate for the intended use of the biospecimen.

Freeze/thaw cycles may be deleterious to the macromolecules intended for analysis; therefore, it is important to provide some aliquots that have a suitable volume for single-use. Storage and cost logistics may require that some larger volume aliquots are maintained.

Evidence of compliance:

✓ Documentation of sample size stated in SOPs

5. **Temperature excursions** Y/N/NA

Temperature excursions beyond recommended storage requirements are tracked during routine processing and distribution.

The research biorepository has all known relevant annotations on a given biospecimen that may be made available to the researcher.

6. **Clean environment** Y/N/NA

Biospecimens are processed in a clean environment, when required.

RNA is particularly sensitive to RNases that may be present on tools and surfaces that have not been sterilised.

7. **Biological safety cabinet** Y/N/NA

Aliquots are made using sterile pipettes within a biological safety cabinet, when required.

8. **SOP for handling biospecimens for infectious diseases** Y/N/NA

There is a SOP for receipt and management of potentially infectious material that includes application of universal precautions.

Elements of the SOP must include proper handling of biospecimens for biohazard protection. The SOP may include information about prior testing for infectious hazards.

9. **SOP surgical pathology biospecimens release for research** Y/N/NA

A sample of a surgical pathology gross biospecimen may be submitted for research only if all of the following criteria are met.

1. The pathologist determines that the sample(s) is not necessary for diagnostic purposes.
2. Formal written authorisation is obtained in accordance with the requirements of the HREC if identifiable patient information is released.
3. The research biorepository meets other relevant requirements, including but not limited to, the requirements of the institution, the directives of the Metro South Health Research Biorepository Project Steering Committee or similar entity, and state and local laws and regulations.
4. De-identified/anonymised sample of a surgical pathology gross biospecimen may be submitted for research if a waiver of consent has been obtained.

10. **Histological characteristic review** Y/N/NA

A pathologist reviews all solid tissue biospecimens to determine the histological characteristics of the biospecimens that are submitted to the research biorepository.

Characteristics may vary depending on the tissue type and the nature of any pathological changes (when present). For example, solid tissue biospecimens from the colon of a patient/participant with ulcerative colitis and colonic adenocarcinoma may include a section of normal colon, a section of colon involved by the chronic active inflammatory process, and a section from the colonic adenocarcinoma. Solid tissue samples can be banked and/or processed according to the previously established protocol for handling normal, disease involved, and neoplastic colonic tissue. Pathology review may occur prior to banking or distribution.

11. **Biospecimen identity and unique identifier** Y/N/NA

The identity of every biospecimen is maintained through each step of processing and slide preparation. Each biospecimen received into the biorepository receives a unique identifier.

An unambiguous system of unique biospecimen identification coupled with a legible, sequential container labelling system that withstands exposure to anticipated reagents and temperature extremes are essential to fulfil this requirement. Containers can be various shapes and sizes and constructed from multiple materials (plastic, glass, cardboard). It is important to ensure that the container is suitable for the type of biospecimen and how it will be used/stored.

12. **Misidentification risk** Y/N/NA

The research biorepository has a documented SOP to ensure that the risk of misidentification is monitored and subjected to continual process improvement.

The research biorepository must actively monitor the key elements of all sample types throughout the entire process. The program may include, but is not limited to:

1. maintaining identification of nucleic acids and protein derivatives from a biospecimen
2. Quality Control and application of a barcode or other identifier, and
3. record of the number of sample derivatives prepared.

13. **Biospecimen tracking mechanism** Y/N/NA

The identity of every biospecimen is maintained and tracked throughout the life of the biospecimen and its derivatives (eg parent to children to grandchildren etc).

An effective tracking system must be in place to ensure that biospecimens can be tracked accurately from the collection site through biospecimen arrival and subsequent shipment from the research biorepository.

14. **Biospecimen rejection criteria** Y/N/NA

There are documented criteria for the condition exceptions that should be recorded and communicated to researchers regarding items that could impact research results.

This requirement is not intended to imply that all "unacceptable" biospecimens be discarded or not analysed. For example, if an unacceptable biospecimen is received, there must be a mechanism to notify the requesting researcher, and to note the condition of the sample on the report. For example, many semen samples are sub-optimal; all samples should be evaluated and unusual properties noted. The research biorepository may wish to record that a dialogue was held with the requesting researcher.

15. **Relabelling SOP** Y/N/NA

There is a SOP in place for relabelling of a biospecimen and/or aliquots.

Circumstances under which relabelling may occur may include, but are not limited to: a) inadvertent duplication of ID from internal or external sources; b) for full de-identification; c) replacement of a label (eg original label has fallen off).

Evidence of compliance:

✓ Documentation of reason for relabelling

16. **De-identification for research and coding** Y/N/NA

For biospecimens that are released for research, there is a SOP for de-identifying/blinding or anonymising biospecimens without compromise to research-related demographic information, when required. There is a defined coding system for sample identification.

17. **Participant Information and Consent Form (PICF)** Y/N/NA

For biospecimens that are released to a research biorepository, appropriate patient/participant/donor informed consent is secured. This is not applicable when biospecimens are obtained under waiver of consent.

18. **Biospecimen collection/handling SOP** Y/N/NA

Collection, processing, and storage times are documented as required by the research biorepository Research Protocol in place at the time of biospecimen procurement. Time is kept to a minimum between when a biospecimen is removed from its site of origin and when it is preserved (e. fixed, cooled or frozen).

19. **Retrieval SOPs** Y/N/NA

All biospecimen retrieval procedures ensure biospecimen integrity.

The integrity of the biospecimen must be maintained throughout the retrieval process.

Evidence of compliance:

✓ Procedure defining the process

20. **Paraffin embedding and/or fixation QC** Y/N/NA

The research biorepository has a SOP for paraffin embedding and/or fixation and quality checks to include the frequency requirements for quality checks (eg 24 hours/48 hours).

This requirement applies only to research biorepositories that perform their own fixation and embedding and are not a part of an accredited laboratory.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources* *Biorepository Checklist.*

Preservation

Self-audit/review assistance

- Undertake a sampling of biospecimen Quality Assurance (QA) reports for key elements of processing and preservation of solid and fluid biospecimens.
- If collection occurs on-site, observe the processing/preservation procedure.
- Ascertain how the research biorepository captures variables that could impact biospecimen usage.
- Identify when the research biorepository would communicate pre-analytic variables to researchers.
- Review process to ensure accuracy of pre-analytic data capture.

1. **Pre-analytic variables** Y/N/NA

   There is a mechanism to capture pre-analytical variables that could impact potential uses of the biospecimens.

   While intended use of biospecimens is not always known, the biospecimens are typically stored for anticipated types of analysis (ie serology, molecular, proteomic) and should be fit for purpose for the anticipated applications. Preservation procedures are optimised for the greatest number of molecular analytes/analysis platforms.

2. **Processing/preservation - solid biospecimens** Y/N/NA

   The key elements related to the processing and preservation of solid biospecimens are documented in the biospecimen Quality Assurance (QA) report when available.

   These elements may include, but are not limited to:

   1. Chilling/heating/drying of tissue during handling
   2. Size and number of tissue pieces
   3. Percentage of tumour/necrosis/stroma in the tissue
   4. Liquid collection media
   5. Use of gauze wrapping, additives, and embedding compounds
   6. Variation in fixation (eg temperature, buffer, pH of formalin, start/end time in fixative)
   7. Freezing protocols
   8. Time in fixative
   9. Time to preserve.

The research biorepository has all known relevant annotations on a given biospecimen that may be made available to the researcher. Information regarding some of these elements may not be available to the research biorepository for all biospecimen collections, especially those that were procured before recent best practices for research biorepositories were published.

3. **Processing/preservation - fluid biospecimens** Y/N/NA

The key elements related to the processing and preservation of fluid biospecimens are documented.

Key elements may include, but are not limited to:

1. Collection preservative

2. Original volume received

3. Temperature and duration of biospecimen prior to processing

4. Temperature and speed of first centrifugation step

5. Temperature and speed of subsequent separation steps

6. Method used for separation

7. Derivative(s) preserved and their volume

8. Quality control results for derivatives (ie cell viability, purity, hemolysis status, human versus non-human content)

9. Tumour content (%), if applicable.

The research biorepository has all known relevant annotations on a given biospecimen that may be made available to the researcher. Under some circumstances some of this information may be "unknown" depending on the site and age of specimen. It is recommended that the research biorepository encourage their source sites to gather/provide as much information as possible.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources Biorepository Checklist.*

Processing

Self-audit/review assistance

- Undertake sampling of DNA/RNA extraction and amplification SOPs.
- Review records of; DNA quantity measurement, nucleic acid integrity and purity assessment and internal controls.
- Ensure nucleic acid amplification procedures for proper physical containment are in place and procedural controls to prevent carryover.
- Review quantitation and quality control assessments.
- Assess the adequacy of nucleic acid isolation and preparation evaluation (ie how often).
- Follow a sample from extraction through storage.

1. **Biospecimen identification** Y/N/NA

   There is a system to positively identify all patient/participant biospecimens, biospecimen types, and aliquots through all phases of the analysis, including biospecimen receipt, nucleic acid extraction, nucleic acid quantification, hybridisation, detection, documentation and storage.

2. **Extraction/purification methods** Y/N/NA

   Nucleic acids are extracted and purified by methods reported in the literature, by an established commercially available kit or instrument, or by a validation of a method developed in-house.

   The method should be assessed for its suitability for each source type that requires extraction. Any modification to established SOPs must be documented, as well as variations to procedures depending on anatomic site and biospecimen preservation format (eg fresh frozen vs OCT-embedded).

   Evidence of compliance:

   ✓ Written SOP for each extraction process

3. **Nucleic acid quantity and integrity/purity assessment** Y/N/NA

   The quantity of nucleic acid is measured. The quantity of nucleic acid must be measured prior to use by a SOP that allows for the accurate determination of the concentration/quantity of the nucleic acid. The integrity and purity of nucleic acid is assessed, when appropriate for downstream use.

   Evidence of compliance:

   ✓ Records detailing the concentration and yield of nucleic acid per biospecimen, per extraction

4. **Human/non-human DNA** Y/N/NA

When the downstream application requires an estimation of the ratio of human versus non-human genomic DNA in the biospecimen, the human/non-human DNA quantity is measured.

5. **Neoplastic cell content assessment** Y/N/NA

There is documentation of histological assessment of neoplastic cell content for tumour biospecimens from which DNA or RNA is extracted for analysis.

In addition to confirming the presence or absence of neoplastic cells by a pathologist, it may be necessary for some assays to assess neoplastic cellularity for some downstream assay to ensure that the percentage of neoplastic cells exceeds the limit of detection for the assay.

A corresponding H&E section from the same tissue block used for DNA or RNA extraction may be used to assess sample adequacy. In the case of a frozen tissue block, a validation formalin-fixed paraffin-embedded mirrored to the frozen tissue biospecimen may be used for histological examination of sample adequacy.

Alternatively, a stain such as toluidine blue may be used to stain the slide that is being used for DNA extraction. When assessment of sample adequacy is performed outside of the testing facility, documentation of such assessment should accompany the sample.

6. **Carryover** Y/N/NA

Nucleic acid amplification procedures (eg PCR) are designed to minimise carryover (false positive results) using appropriate physical containment and procedural controls.

This item is primarily directed at ensuring adequate physical separation of pre- and post-amplification samples to avoid amplicon contamination. The extreme sensitivity of amplification systems requires that special precautions are taken. For example, pre- and post-amplification samples should be manipulated in physically separate areas; gloves must be worn and frequently changed during processing; dedicated pipettes (positive displacement type or with aerosol barrier tips) must be used; and manipulations must minimise aerosolisation.

In a given run, biospecimens should be ordered in the following sequence: patient/participant samples, positive controls, negative controls (including "no template" controls in which target DNA is omitted and therefore no product is expected). Enzymatic destruction of amplification products is often helpful, as is real-time measurement of products to avoid manual manipulation of amplification products.

7. **Internal controls nucleic acid amplification** Y/N/NA

In all nucleic acid amplification procedures, internal controls are run to detect a false negative reaction secondary to extraction failure or the presence of an inhibitor, when appropriate.

The research biorepository should be able to distinguish a true negative result from a false negative due to failure of extraction or amplification. Demonstration that another sequence can be successfully amplified in the same biospecimen should be sufficient to resolve this issue. For quantitative amplification assays, the effect of partial inhibition must also be addressed.

The internal control should not be smaller than the target amplicon. There are some rare exceptions to this rule due to sequence length and design. In this situation the internal control should not be more than 10% smaller than the target amplicon and the use of a smaller internal control should be justified.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources* *Biorepository Checklist.*

Digital image capture

Self-audit/review assistance

- Undertake a sampling of qualification data.
- If significant differences in slide/staining characteristics are expected identify how the qualification taken this into account
- If clear digital images cannot be obtained, identify the process for determining the cause and correcting any potential problems with the scanning system.
- Review process on what occurs if tumour content is insufficient.

1. **System qualification** Y/N/NA

   If digital whole slide imaging is used as an integral part of the research biorepository operation, there is documentation that the system has been qualified for the intended use.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources* *Biorepository Checklist.*

Tissue Microarray (TMA)

TMA technology helps expedite discovery of the novel targets important in disease treatment by providing a tool for high-throughput screening of multiple tissues using immunohistochemical, in situ hybridisation, and fluorescent in situ hybridisation (FISH) analyses.

Self-audit/review assistance

- Review a sampling of tissue microarray SOPs.
- Ensure records of methods selected for region of interest of tissue and communication with the microarray technologist.
- Review the system to positively identify biospecimens, biospecimen types and aliquots throughout the process.
- Identify who is responsible for selecting tissues and performing analysis for tissue microarray.
- Understand the selection and number of cores determined.
- Follow a tissue biospecimen for TMA from processing to final analysis. Observe biospecimen identification, core selection and analysis.

1. **Specimen identification** Y/N/NA

   There is a system to positively identify all patient/participant biospecimens, biospecimen types, and aliquots through all phases of the analysis.

   The phases include, but are not limited to:

   1. Biospecimen receipt
   2. Biospecimen ID key
   3. Tissue core selection from parent paraffin block
   4. Location and identification within the new tissue microarray recipient tissue block
   5. Documentation
   6. Utilisation (number of times sectioned)
   7. Storage.

2. **Preparation procedures** Y/N/NA

   There is documentation describing the tissue types and purpose for the TMA, including the size and placement of the tissue cores as well as control tissue cores.

   Criteria for selection and documentation of the tissue cases are required. The usefulness and analysis of tissue microarray cores can be affected by the location (edges versus centre) and loss of tissue cores as the tissue microarray block is thin sectioned.

Consideration is of size, frequency, and location of cores therefore, should be considered and documented to match the intended use of the tissue microarray. Examples of the intended purpose of the TMA include, but are not limited to, disease-specific TMA, disease-progression TMA, tissue staining control TMA, cell line TMA etc.

3. **Original paraffin tissue block** Y/N/NA

SOPs are in place to determine to what extent the original paraffin tissue block lesion can be removed.

4. **Tissue core selection** Y/N/NA

Tissues selected (paraffin block and tissue region of interest) to make a TMA must be selected by a qualified anatomic pathologist.

5. **Method of core selection** Y/N/NA

Methods of the selection of the regions of interest of tissue and clear documentation to transfer the correct information to a tissue microarray technologist must be documented.

6. **Number of cores** Y/N/NA

Methods for determining the relevant number of cores to accurately represent the parent tissue block must be documented. A SOP is in place to determine the optimum number of cores required per TMA as dictated by each research project's Research Protocol.

7. **TMA procedure** Y/N/NA

There is a SOP to ensure that the correct tissue is placed in the correct location of the TMA, for example, a TMA map (tissue type, key ID, and location in the TMA).

This would include the placement and location of tissue controls and orientation markers. There is software available to manage the map of a TMA. This resource is very useful in helping the pathologist evaluate and read results from the TMA after it has been stained.

8. **TMA evaluation** Y/N/NA

Analysis of TMAs are performed by an anatomic pathologist and documented.

The analysis may include software-assisted analysis or manual reading by a pathologist.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources Biorepository Checklist.*

Laser Capture Microdissection (LCM)

LCM "captured" cells can be used in a wide range of downstream assays such as loss of heterozygosity (LOH) studies, gene expression analysis at the mRNA level or in a wide range of proteomic assays such as 2D gel analysis, Western blotting, reverse phase protein array, and surface-enhanced laser desorption ionisation (SELDI) protein profiling. Commercial kits for the isolation of RNA and DNA are available and adaptable to the micro samples obtained by LCM.

Self-audit/review assistance

- Undertake a sample review of LCM SOPs.
- Review records of LCM laser focus and alignment and system to positively identify biospecimens, biospecimen types and aliquots throughout the process.
- Identify how the quality of LCM tissue material is ensured.

1. **Biospecimen identification** Y/N/NA

   There is a system to positively identify all patient/participant biospecimens, biospecimen types, and aliquots through all phases of the microdissection and processing procedures to the point of storage or use.

2. **LCM procedures** Y/N/NA

   There is a procedure in place to monitor and document the LCM process.

   LCM tissues are derivative of a parent block and condition of tissue management is important for the quality outcome of tissue components. This is especially important if the collection is from frozen tissue.

3. **LCM equipment** Y/N/NA

   The LCM Laser focus and alignment is maintained and documented to ensure optimal performance.

   Documentation related to the critical components of the LCM as noted by the manufacturer is required.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources Biorepository Checklist.*

Cell fractionation

The purpose of cell fractionation is to obtain a pure sample of part of the original whole, such as mitochondria, plasma membranes, DNA, RNA, soluble proteins or even specific macromolecules. There are many procedures defined for each target material, such as tissue, plant cells, animal cells, cell membranes and molecular components. Fractionation can simply be the separation of components of a biospecimen, such as blood into white blood cells, serum, and red blood cells.

Self-audit/review assistance

- Undertake a sampling of cell fractionation SOPs.
- Review the system to maintain the identification of the derivatives to the parent biospecimen.
- Review cell fractionation process follows the steps in the SOP.
- Identify how the quality of the cell fractionation process is ensured.

1. **Biospecimen identification** Y/N/NA

   Derivatives from fractionation of biospecimens maintain the identification associated with the parent biospecimen during the fractionation process.

   Documentation of biospecimen type, handling conditions, and, if applicable, storage information are elements of the identification that are maintained until the process is complete. If anonymity from the parent biospecimen is required, this can be accomplished after the fractionation is complete.

2. **SOPs** Y/N/NA

   There are written SOPs for all steps in the fractionation process.

   Deviations from the manufacturer instructions must be validated and documented.

3. **Quality Control (QC)/Quality Assurance (QA)** Y/N/NA

   Research biorepositories providing cell fractionation procedures must document all quality control and quality assurance measures.

   These measures would include the establishment of validation sets performed by the laboratory to establish consistent success in quality fractionation and where possible, enrolment in proficiency testing or performance of alternative assessment to demonstrate expertise and quality fractionation.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources Biorepository Checklist.*

Cell and tissue culture

Self-audit/review assistance

- Undertake a sampling of cell and tissue culture SOPs.
- Review records of microbial contamination and other cell line testing.
- Identify how the research biorepository ensures that the quality of cell lines is maintained.

1. **Culturing environment** Y/N/NA

   Culturing is performed under aseptic conditions in a biological safety cabinet.

2. **Cell line loss** Y/N/NA

   There is a system in place to prevent loss of the cell line in case of culture failure, contamination or other problems. Potential systems may include the use of duplicate or independently established cultures, harvesting in duplicate or at different times, or other control processes.

3. **Monitoring of passage numbers** Y/N/NA

   The research biorepository's SOPs must define the maximum number of passages for each cell line by either reference or laboratory method.

   When passages have reached the maximum passage number, the cell line should be re-established using working stock with a lower passage number.

   Evidence of compliance:

   ✓ Documentation of tracking of cell line passages OR

   ✓ Documentation of growth curves

4. **Testing for microbial contamination** Y/N/NA

   Cell lines must be tested for microbial contamination at intervals defined by the research biorepository Custodian.

   Evidence of compliance:

   ✓ Records detailing the type(s) of tests and test outcomes

5. **Testing for functionality and/or unique characteristics** Y/N/NA

   Cell lines are tested for functionality or unique characteristics.

   Such testing may be performed by analysing aspects of the phenotype (eg expression patterns), genotype or morphology. The research biorepository should have a SOP that addresses the need for identity testing.

Evidence of compliance:

✓ Records of cell line evaluation AND

✓ Records of (short tandem repeats) STR profiling or another method for cell lines to accomplish this goal

6. **Recording of failures** Y/N/NA

Culture failures are recorded. Records must indicate corrective actions.

Evidence of compliance:

✓ Documentation indicating the results of testing and indication when a cell line has failed to pass the criteria established for successful passage of the quality tests

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources Biorepository Checklist.*

Histology section – general Quality Control (QC)

Self-audit/review assistance

- Undertake a sampling of biospecimen preparation records, histology Quality Control (QC) SOPs, QC records (histochemical), tissue blocks (identification), slides (labelling, quality) and reagents (expiration date).
- Identify how the histology section ensures biospecimen identity throughout processing.
- If problems are identified during the review of histology procedures, further evaluate the responses, corrective actions and resolutions.
- Select a representative biospecimen and follow from receipt in the department through accessioning, grossing, processing, time reported and availability database.

1. **Biospecimen preparation records** Y/N/NA

   The histology section maintains records of the number of blocks, slides, and stains prepared and appropriately denotes the block from which the slide was prepared.

2. **Reagent expiration date** Y/N/NA

   All reagents are used within their indicated expiration dates.

   The research biorepository must assign an expiration date to any reagents that do not have a manufacturer-provided expiration date. The assigned expiration date should be based on known stability, frequency of use, storage conditions, and risk of contamination.

   This checklist requirement applies to all reagents used in the research biorepository (histochemical, immunohistochemical, and immunofluorescent reagents, and reagents used for molecular tests).

   The acceptable performance of histochemical stains is determined by technical assessment on actual case material, use of suitable control sections, and as part of the biospecimen evaluations as determined by the protocol.

   Exception to the above is that some histochemical reagents used in histology are not subject to outdating, so that assignment of expiration dates may have no meaning. The acceptable performance of such reagents should be confirmed at least annually by technical assessment, as described above. (If the manufacturer assigns an expiration date, it must be observed.)

   Expired reagents may be used only under the following circumstances, as long as they will not have a negative impact on downstream studies: 1. The reagents are unique, rare or difficult to obtain; or 2. Delivery of new shipments of reagents is delayed through causes not under control of the research biorepository.

The research biorepository must document verification of the performance of expired reagents in accordance with written SOPs.

If expired reagents are stored in histology, there must be a SOP to describe the intended use. The reagents must be stored separately and clearly labelled for the intended purpose (eg for training purposes, not for diagnostic use).

Evidence of compliance:

✓ Written SOP for evaluating reagents lacking manufacturer's expiration date

3. **Special stain quality** Y/N/NA

All histochemical stains are of adequate quality, and daily controls are demonstrated on each day of use for the tissue components or organisms for which they were designed.

Positive tissue controls assess the performance of the special stain. Special stains are performed on sections of control tissue known to contain components specific to each special stain. Verification of tissue used as a positive control must be performed and documented before being used with clinical biospecimens.

Evidence of compliance:

✓ Written SOP for special stains AND

✓ Records of special stain QC AND

✓ Documented results of verified special stain control tissue block

4. **Special stains/studies** Y/N/NA

For special stains and studies using immunologic and FISH/ISH methods, results of controls are documented to be acceptable before reporting results, when applicable.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources Biorepository Checklist.*

Immunohistochemistry (IHC)

Self-audit/review assistance

- Undertake a sampling of IHC SOPs, new antibody validation records, new reagent/shipment confirmation of acceptability records, antibody QC records, buffer pH records, batch control records and of slides (quality).
- Identify how the research biorepository validates new antibodies, confirms the acceptability of new reagent lots and distinguishes non-specific false-positive staining from endogenous biotin.

1. **Biospecimen modification** Y/N/NA

   If the research biorepository performs immunohistochemically staining on biospecimens other than formalin-fixed, paraffin-embedded tissue, the written SOP describes appropriate modifications for biospecimen types.

   Such biospecimens include frozen sections, air-dried imprints, cytocentrifuge or other liquid-based preparations, decalcified tissue, and tissues fixed in alcohol blends or other fixatives.

2. **Buffer pH** Y/N/NA

   The pH of the buffers used in immunohistochemistry is routinely monitored. pH must be tested when a new batch is prepared or received.

   Evidence of compliance:

   ✓ Written SOP defining pH range for each buffer in use AND

   ✓ Records of initial and subsequent QC on each buffer

3. **Quality Control (QC) – antibodies (positive tissue controls)** Y/N/NA

   Positive tissue controls are used for each antibody.

   Positive controls assess the performance of the primary antibody. They are performed on sections of tissue known to contain the target antigen, using the same epitope retrieval and immunostaining protocols as the donor tissue. Results of controls must be documented, either in internal research biorepository records, or in the donor report. A statement in the report such as, “All controls show appropriate reactivity” is sufficient.

   Ideally, the positive control tissue would be the same biospecimen type as the patient/participant test biospecimen (eg small biopsy, large tissue section, cell block), and would be processed and fixed in the same manner (eg formalin-fixed, alcohol-fixed, decalcified) as the patient/participant biospecimen.

However, for most research biorepositories, it is not practical to maintain separate positive control samples to cover every possible combination of fixation, processing and biospecimen type. Thus, it is reasonable for a research biorepository to maintain a bank of formalin-fixed tissue samples as its positive controls; these controls can be used for patient/participant biospecimens that are of different type, or fixed/processed differently, providing that the research biorepository can show that these biospecimens exhibit equivalent immunoreactivity. This can be accomplished by parallel testing a small panel of common markers to show that biospecimens of different type, or processed in a different way (eg alcohol-fixed cytology biospecimens, decalcified tissue) have equivalent immunoreactivity to routinely processed, formalin-fixed tissue.

A separate tissue section may be used as a positive control, but test sections often contain normal elements that express the antigen of interest (internal controls). Internal positive controls are acceptable for these antigens, but the research biorepository manual must clearly state the manner in which internal positive controls are used. A positive control section included on the same slide as the tissue is optimal practice because it helps identify failure to apply primary antibody or other critical reagent to the test slide; however, one separate positive control per staining run for each antibody in the run (batch control) may be sufficient provided that the control slide is closely scrutinised by a qualified reviewer.

Ideally, positive control tissues possess low levels of antigen expression, as is often seen in neoplasms. Exclusive use of normal tissues that have high levels of antigen expression may result in antibody titers of insufficient sensitivity, leading to false-negative results.

Evidence of compliance:

✓ Written SOP for the selection and use of positive tissue controls for each antibody AND

✓ Patient/participant reports or worksheet with control results

4. **Quality Control (QC) – antibodies (negative tissue controls)** Y/N/NA

Appropriate negative controls are used.

Negative controls must assess the presence of nonspecific staining in patient/participant tissue as well as the specificity of each antibody with the exception listed below. Results of controls must be documented, either in internal research biorepository records, or in the patient/participant report. A statement in the report such as, "All controls show appropriate reactivity" is sufficient.

For research biorepositories using older biotin-based detection systems, it is important to use a negative reagent control to assess non-specific or aberrant staining in donor tissue related to the antigen retrieval conditions and/or detection system used.

A separate section of donor tissue is processed using the same reagent and epitope retrieval protocol as the donor test slide, except that the primary antibody is omitted, and replaced by any one of the following:

- An unrelated antibody of the same isotype as the primary antibody (for monoclonal primary antibodies)
- An unrelated antibody from the same animal species as the primary antibody (for polyclonal primary antibodies)
- The negative control reagent included in the staining kit
- The diluent/buffer solution in which the primary antibody is diluted

In general, a separate negative reagent control should be run for each block of donor tissue being immunostained; however, for cases in which there is simultaneous staining of multiple blocks from the same biospecimen with the same antibody (eg cytokeratin staining of multiple axillary sentinel lymph nodes), performing a single negative control on one of the blocks may be sufficient provided that all such blocks are fixed and processed identically. This exception does not apply to stains on different types of tissues or those using different antigen retrieval protocols or antibody detection systems. The research biorepository Custodian must determine which cases will have only one negative reagent control, and this must be specified in the SOP manual.

The negative reagent control would ideally control for each reagent protocol and antibody retrieval condition; however, large antibody panels often employ multiple antigen retrieval procedures. In such cases, a reasonable minimum control would be to perform the negative reagent control using the most aggressive retrieval procedure in the particular antibody panel. Aggressiveness of antigen retrieval (in decreasing order) is as follows: pressure cooker; enzyme digestion; boiling; microwave; steamer; water bath. High pH retrieval should be considered more aggressive than comparable retrieval in citrate buffer at pH 6.0.

Immunohistochemical tests using polymer-based detection systems (biotin-free) are sufficiently free of background reactivity to obviate the need for a negative reagent control and such controls may be omitted at the discretion of the research biorepository Custodian, following appropriate validation.

It is also important to assess the specificity of each antibody by a negative tissue control, which must show no staining of tissues known to lack the antigen. The negative tissue control is processed using the same fixation, epitope retrieval and immunostaining protocols as the donor tissue. Unexpected positive staining of such tissues indicates that the test has lost specificity, perhaps because of improper antibody concentration or excessive antigen retrieval. Intrinsic properties of the test tissue may also be the cause of "non-specific" staining. For example, tissues with high endogenous biotin activity such as liver or renal tubules may simulate positive staining when using a detection method based on biotin labelling.

A negative tissue control must be processed for each antibody in a given run. Any of the following can serve as a negative tissue control:

1. Multi-tissue blocks. These can provide simultaneous positive and negative tissue controls, and are considered “best practice” (see below).

2. The positive control slide or donor test slides, if these slides contain tissue elements that should not react with the antibody.

3. A separate negative tissue control slide.

The type of negative tissue control used (ie separate sections, internal controls or multi-tissue blocks) must be specified in the research biorepository SOP manual.

Multi-tissue blocks may be considered best practice and can have a major role in maintaining quality. When used as a combined positive and negative tissue control as mentioned above, they can serve as a permanent record documenting the sensitivity and specificity of every stain, particularly when mounted on the same slide as the donor tissue. When the components are chosen appropriately, multi-tissue blocks may be used for many different primary antibodies, decreasing the number of different control blocks needed by the research biorepository. Multi-tissue blocks are also ideal for determining optimal titers of primary antibodies since they allow simultaneous evaluation of many different pieces of tissue. Finally, they are a useful and efficient means to screen new antibodies for sensitivity and specificity or new lots of antibody for consistency, which should be done before putting any antibody into diagnostic use.

Evidence of compliance:

✓ Written procedure for the selection and use of negative reagent (as appropriate) and tissue controls for IHC AND

✓ Donor reports or worksheet with control results

5. **Endogenous biotin** Y/N/NA

If the research biorepository uses an avidin-biotin complex (ABC) detection system (or a related system such as streptavidin-biotin or neutravidin-biotin), there is a SOP that addresses nonspecific false-positive staining from endogenous biotin.

Biotin is a coenzyme present in mitochondria, and cells that have abundant mitochondria such as hepatocytes, kidney tubules and many tumours (particularly carcinomas) are rich in endogenous biotin.

Biotin-rich intranuclear inclusions are also seen in gestational endometrium and in some tutors that form morules. If steps are not included in the immunostaining method to block endogenous biotin before applying the ABC detection complex, nonspecific false-positive staining may occur, particularly when using heat-induced epitope retrieval (which markedly increases the detectability of endogenous biotin).

This artefact is often exquisitely localised to tumour cells and may be easily misinterpreted as true immunoreactivity. Blocking endogenous biotin involves incubating the slides with a solution of free avidin (which binds to endogenous biotin), followed by incubation with a biotin solution (which saturates any empty biotin-binding sites remaining on the avidin).

Biotin-blocking steps should be performed immediately after epitope retrieval and before incubation with primary antibody.

6. **Control slide review** Y/N/NA

The research biorepository Custodian or designee reviews all control slides each day biospecimens are stained.

Records of this review must be maintained and should clearly document that positive and negative controls for all antibodies stain appropriately. Control records must be retained for one inspection cycle (every 3 years).

The control slides must be readily available upon request. The location of the slides should be stated in the SOP manual.

7. **Antibody validation** Y/N/NA

The research biorepository has documented validation of new antibodies, prior to sample characterisation, including appropriate positive and negative controls.

The performance characteristics of each assay in the immunohistochemistry research biorepository must be appropriately validated before being made available as characterisation data for the specimen type. The initial goal is to establish the optimal antibody titration, incubation time, temperature, detection system, and antigen retrieval protocol. Once optimised, a panel of tissues must be tested to determine the assay's sensitivity and specificity. The scope of the validation is at the discretion of the research biorepository Custodian and will vary with the antibody. For a well-characterised antibody with a limited spectrum of antigenic targets, like chromogranin or prostate specific antigen, the validation can be limited. A panel of 10 positive and 10 negative cases would be sufficient in this setting. For an antibody that is not well characterised and/or has a wide range of reported reactivity, a more extensive validation is necessary. The number of tissues tested should, in this circumstance, be large enough to determine whether the staining profile matches that previously described.

For most antibodies, normal controls are available for use in validation. In the exceptional case where only limited control tissue is available (fewer than 10 cases), the research biorepository Custodian should alert the investigator of this limitation.

Evidence of compliance:

✓ Written SOP for the evaluation/validation of new antibodies

8. **New reagent lot confirmation of acceptability** Y/N/NA

The performance of new lots of antibody and detection system reagents is compared with old lots before or concurrently with being placed into service.

Parallel staining is important to control for variables such as disparity in the lots of detection reagents or instrument function. New lots of primary antibody and detection system reagents must be compared to the previous lot using an appropriate panel of control tissues. This comparison must be made on slides cut from the same control block.

Evidence of compliance:

✓ Written SOP for the confirmation of acceptability of new reagent lots prior to use AND

✓ Records of confirmation of new reagent lots

9. **Slide quality** Y/N/NA

The immunohistochemical stains produced are of acceptable technical quality.

The research biorepository Custodian or designee reviews slides and determines if they are of acceptable technical quality. The inspector must examine examples of the immunohistochemical preparations offered by the biorepository. A reasonable sample might include 5-10 diagnostic antibody panels.

Completed by:_____

Date: _____

Adapted from National Cancer Institute *NCI Best Practices for Biospecimen Resources* *Biorepository Checklist.*