NIH NATIONAL CANCER INSTITUTE

NCI Best Practices for Biospecimen Resources: Appendices

Biorepositories and Biospecimen Research Branch

National Cancer Institute

National Institutes of Health

U.S. Department of Health and Human Services

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Appendix 1. Minimal Clinical Data Set for Biobanking

The Minimal Clinical Data Set for Biobanking in this appendix represents the recommended elements for the annotation of disease state or risk of cancer in biospecimen resources, usually including demography information, medical history and diagnosis, treatments and medications, key test results and relevant procedures. The items in this recommended data set are not meant to be inclusive and are only suggested examples. Different biospecimen resources and different studies may require more or less detailed annotations that focus on the primary use of the clinical biospecimens. Good practice suggests that the data set for clinical annotation be tailored to the needs of the users of the biospecimen resource. This Minimal Clinical Data Set is not to be confused with other data sets, such as that used by the Centers for Medicare and Medicaid Services (CMS) to evaluate nursing home patients, aka Minimum Data Set (MDS).

Item		Notes
Age		or ≥ 90, at collection
Exposures (where age > 18)	Smoking	
	Drinking	
	Occupation	
Gender		
Race		
Ethnicity		
Disease diagnosis/Normal		
Source/Method of diagnosis		
Treatment type/None		
Height		
Weight		
Family history of cancer		
For tissue biospecimens only,	Histologic type	Also record for blood biospecimens in bloodborne cancers
	Grade	
	Size	
	Nodal status (pos/neg, # pos/total nodes, etc.)	
	Pathologic TNM status	
	Pathologic TNM stage	
	Procedure	Procedure by which biospecimen was obtained
Biomarkers		Biomarkers used in routine care; e.g. Estrogen and Progesterone receptor sensitivity
Outcome—or will it be possible to get these data when outcome is known	Death	Year only
	Date of last cancer follow-up	Year only

	Recurrence (local, distant, unknown)	
Collection method		
Comorbidity		
Social determinants of health		

Appendix 2. Additional Resources Related to Ethical, Legal, and Policy Issues in Biospecimen Research

The resources listed below are not intended to be exhaustive but rather to provide useful examples and references for biospecimen resources. All Web links were last accessed on January 9, 2025.

I. General Resources Related to Ethical, Legal, and Policy Issues in Biospecimen Research

The reports and resources listed below provide an overview of ethical, legal, and policy challenges in biospecimen research. Topics include State and international regulations related to biospecimens and tools for IRBs and biospecimen resource managers.

A. *NCI Documents*

NCI Brochure: Donating Your Blood, Tissue and Other Samples

- English
- Spanish

NCI ELSI Workshop Summaries:

(1) Cancer Moonshot Biobank Ethical, Legal and Social Implications (ELSI) Mini-Symposium

(2) NCI Think Tank on Identifiability of Biospecimens and 'Omic Data (PMID 23579437)

(3) Workshop on Release of Research Results to Participants in Biospecimen Studies

(4) Workshop on Ethical Use of Pediatric Biospecimens in Research

(5) Workshop on Custodianship and Ownership in Biospecimen Research

(6) International Symposium to Harmonize Biorepository Practices

B. *Documents from Other Sources*

The President's Commission for the Study of Bioethical Issues published two key reports impacting research involving biospecimens and biospecimen resources.

- The first (October 2012) examines the use of biospecimens to conduct whole genome sequencing, including considerations or informed consent, privacy and data sharing.

- The second (December 2013) explores the ethical issues surrounding the return of incidental findings in research involving biospecimens.

The Secretary's Advisory Committee on Human Research Protection (SACHRP) has developed a number of documents relevant to practical and regulatory issues about the collection, storage, distribution, and future research use of biospecimens and associated data, including recommendations and frequently asked questions and informed consent.
Public Responsibility in Medicine & Research (PRIM&R) hosts a series of podcasts that address ethical issues in research: More than meets the IRB and Research Ethics Reimagined.

Research Involving Human Biological Materials: Ethical Issues and Policy Guidance—Volume I: Report and Recommendations of the National Bioethics Advisory Commission

This 1999 report from the National Bioethics Advisory Commission (NBAC) addresses the question of whether the Common Rule is effective in protecting human subjects from harm in research involving biospecimens. The NBAC report also provides recommendations related to biospecimen research, including interpretations of several key terms and concepts in the Common Rule.

International Compilation of Human Research Protections
This compilation was developed by the Office for Human Research Protections (OHRP) for IRBs/ethics committees, researchers, sponsors, and others who are involved in international research. The report includes a table for each country that lists the key organizations, legislation, regulations, and guidelines related to human biological materials.

II. Sample Informed Consent Documents

The following list of sample informed consent documents is provided to guide and inform biospecimen resources about possible approaches to the informed consent process. These documents may be adapted depending on the nature of the resource and its mission.

A. *NCI Documents*

The Cancer Genome Atlas (TCGA)
The NCI and the National Human Genome Research Institute (NHGRI) have developed informed consent documents that are consistent with the goals and activities of TCGA, a comprehensive and coordinated effort to accelerate the understanding of the molecular basis of cancer through the application of genome analysis technologies. Both documents, one for retrospective biospecimen collections and another for prospective collections, specifically address genetic research, broad sharing of biospecimens and clinical data, the possibility of future research use, the deposition of genomics data into electronic database with partial public access, and the risk of loss of privacy.

The Genotype-Tissue Expression (GTEx) Project
The NCI and NHGRI have developed an informed consent document for the Genotype-Tissue Expression (GTEx) project. GTEx was a NIH Common Fund project that collected biospecimens and clinical data from 900 non-diseased postmortem donors. This document provides a sample for obtaining research authorization from next-of-kin or a family decision maker to obtain and use these tissues for genomic and other research endeavors.

B. *Documents from Other Sources*

National Human Genome Research Institute (NHGRI)
Informed consent resource

All of Us Research Program
Informed Consent Process

III. Patient Information Documents

The following list of sample patient information documents is provided to guide and inform biospecimen resources about additional resources that may be useful during the informed consent process. These documents are intended to explain the informed consent process and/or the importance of biospecimens in research to a general audience and may be adapted depending on the nature and mission of the resource.

A. *NCI Documents*

Guide to Understanding Informed Consent

This guide explains what a human research participant should expect during the informed consent process, explains the importance of the informed consent process to clinical human research participants, and describes how informed consent fits into a larger system that protects the welfare of people who take part in clinical trials.

Providing Your Tissue for Research
This six-page booklet is meant to complement the face-to-face education that occurs between clinicians and potential clinical trial participants. It provides a balanced discussion of questions and answers on how biospecimens are collected and used in research.

- English
- Spanish

B. *Documents from Other Sources*

Research Advocacy Network
The Research Advocacy Network (RAN) is a nonprofit organization working to bring together all participants in the medical research process. The RAN has developed booklets about the importance of biospecimens in research directed toward human research participants and IRB members. Documents are available in English or Spanish.

IV. Resources for Simplifying Informed Consent Documents

Several groups have been established to provide recommendations on simplifying and improving the readability of informed consent documents. The following resources are not specific to biospecimen resources but instead provide general information on how to improve the informed consent process to meet the needs of human research participants.

A. *U.S. Government Documents*

NCI
NCI Consent Template for Adult Cancer Trials (May 12, 2013):
The NCI, in conjunction with a working group of multidisciplinary experts, created a consent template for use in adult cancer trials (NCI Consent Template for Adult Cancer Trials).

FDA
FDA informed consent information sheet from 2014:
This guideline on informed consent provides guidance for IRBs, clinical investigators, and sponsors.

B. *Documents from Other Sources*

Association of American Medical Colleges
The summary from a May 2007 strategic planning meeting titled "Universal Use of Short and Readable Informed Consent Documents: How Do We Get There?" includes a review of informed consent literature, potential approaches for improving informed consent, and success stories from the field.

The AAMC provides letters containing feedback on FDA guidance on informed consent.

Group Health Center for Health Studies
The Project to Review and Improve Study Materials (PRISM) is a Group Health Center for Health Studies initiative to improve the readability of print materials used in communication with study participants. The PRISM Readability Toolkit is a comprehensive resource that includes sample

informed consent language, editing checklists, a reference guide for improving readability, and examples of how to improve readability.

https://kpwashingtonresearch.org/index.php/about-us/capabilities/research-communications/prism

Appendix 3. Governance Plan

This governance plan is provided as an example to biospecimen resources to help with planning the resource and defining the authorities, processes, and procedures that are needed to guide key operational decisions. The governance plan should become part of the resource's documents and be available if requested. (Please see Section B. Governance of the *NCI Best Practices for Biospecimen Resources (2025)* for more information and additional recommendations related to custodianship. All sections referenced below refer to the *NCI Best Practices for Biospecimen Resources (2025).*

Principal Investigator:
Grant Number:
Project Title:
Project Period:
Name of the Biospecimen Resource (if different than the project):

A. Name of the Custodian:

B. Summary of the Project:

C. Governance Structure of the Project (See Section B.2. Roles, Responsibilities and Ownership):

1. Outline the resource's management structure and discuss the roles and responsibilities of each management or oversight body.
2. Outline the resource's protocols and procedures that guide its operations and discuss whether the protocols are documented and approved by the IRB and/or a project oversight committee.

D. Integrity of Biospecimens and Data (See Sections B.1. Overview and B.2. Roles, Responsibilities and Ownership):

1. Describe the resource's protocols to ensure the physical integrity of collected biospecimens.
2. Describe the resource's protocols to ensure the integrity of the human research participants' data that accompany the biospecimens.

E. Access to Biospecimens and Data (See Section B.8. Access to Biospecimens and Data):

1. Outline the resource's protocols and procedures for the distribution of samples to investigators. Describe how the scientific merit, prioritization of access requests, and proposed research use are assessed and by what review group.
2. Describe whether samples will be accompanied by data and the type of data. Outline the safeguards that are in place to ensure that confidentiality of the data is not compromised.

F. Release of Research Results (See Section B.6. Return of Results):

1. Outline the protocols that are in place for publication and dissemination of research results from biospecimen research. Describe the process for handling results that are potentially stigmatizing to groups.
2. Outline any process to provide educational materials to the public such as brochures, literature, meetings, or public websites.

G. Legacy and Contingency Plans (See Section B.12. Legacy and Contingency Plans):

1. Outline the resource's plans for the handling and disposition of biospecimens and associated data when reaching any of the following points: (a) End of the budget period of the grant, (b) loss of management or termination of funding, (c) accomplishment of the specific research objectives of the study, (d) depletion of biospecimens, or (e) achievement of critical data end points.

H. Retention of Biospecimens, Data, and Records (See Section B.5.7 Issues Pertaining to Discontinuation in a Study):

1. Outline the resource's protocols for the handling and disposition of biospecimens and associated data sets following the discontinuation of participation by a human research participant.
2. Outline the resource's protocols for the retention of biospecimens, data, and records pertaining to informed consent and the identity of human research participants.

I. Sharing of Resources (See Section B.8. Access to Biospecimens and Data):

1. Outline the resource's protocols and procedures for the sharing of research data and tools generated from biospecimen research consistent with the NIH Scientific Data Sharing Policy and the NIH Research Tools Policy.
2. Outline the resource's protocols for communicating information to human research participants regarding the general type of research performed on biospecimens and the sharing of biospecimens with other researchers, when practicable.

J. Conflicts of Interests (COIs) (also see Section B.10 Conflict of Interest):

1. Describe the protocols for managing and limiting any potential COIs for the resource's staff consistent with 42 CFR Part 50 Subpart F, as well as applicable NIH COI policies.

Appendix 4. Sample Material Transfer Agreements

The following MTAs are intended to serve as sample agreements for use between biospecimen resources and approved end-users receiving biospecimens and/or data [1]. These sample MTAs may need to be modified depending on the material and data that are being transferred and the specific requirements of the research project. Please note, these MTAs are intended for the transfer of deidentified biospecimens and data. (Please see Section B.9.1. Material Transfer Agreements of the *NCI Best Practices* for more information and additional recommendations related to MTAs).

Sample Material Transfer Agreement
For Transfers from Biospecimen Resources to Approved Third-Party End Users

This Material Transfer Agreement (the "Agreement") is by and between <*insert name of biospecimen resource*> ("Provider") and <*insert name of third-party institution*> ("Recipient") regarding the transfer of human specimens, with or without associated data, from the <*insert name of biospecimen resource*> to approved third-party end users for research purposes as further defined below. Throughout this Agreement, Provider and Recipient are collectively referred to as the "Parties." This Agreement will become effective upon the date of the last signature affixed below.

The Provider and Recipient agree as follows:

1. DEFINITIONS. Within this Agreement, the following terms will have the same meaning and effect as those used in the Standards for Privacy of Individually Identifiable Health Information set forth in 45 CFR Parts 160 and 164 ("HIPAA Privacy Rule"). These terms are repeated here for convenience:

(a) "De-identified" information is information that formerly contained individually identifiable health information but which has had all unique identifying information, numbers, characteristics, and codes removed such that the information a record contains cannot be used alone or in combination with other information to identify the individual who is the subject of the information (45 CFR 164.514). Identifying information includes, but is not limited to, the 18 categories of identifiers described in 45 CFR 164.514(b)(2).

(b) "Protected Health Information" or "PHI" means any information, whether oral or recorded in any form or medium: (i) that relates to the past, present, or future physical or mental condition of an individual; the provision of health care to an individual; or the past, present, or future payment for the provision of health care to an individual, and (ii) that identifies the individual or with respect to which there is a reasonable basis to believe the information can be used to identify the individual (45 CFR 164.103).

2. DESCRIPTION OF MATERIAL AND DATA. The Provider will transfer to the Recipient the following biospecimens and/or derivatives ("MATERIAL"): <*insert description of specific samples to be transferred*> with the following data ("DATA"): <*insert description of specific data to be transferred, if applicable*>.

3. COLLECTION OF MATERIAL AND DATA. The MATERIAL and DATA were collected and/or processed from human biospecimens as part of <*insert name of biospecimen resource*> in accordance with appropriate Federal and local laws, Assurances, and Institutional Review Board approvals related to human subjects research, as appropriate.

4. TRANSFER OF MATERIAL AND DATA. The MATERIAL and DATA provided by Provider will be de-identified and all Protected Health Information (PHI), as defined by the Federal Health Insurance Portability and Accountability Act (HIPAA, 45 C.F.R. 164) will have been removed.

5. RESPONSIBILITIES AND AUTHORIZATIONS OF RECIPIENT

(a) Recipient agrees to use the MATERIAL and DATA for the approved research project only (see Appendix 1 "Research Project") and will not use the MATERIAL and DATA for any unapproved commercial purposes, including selling or transferring to a third party for commercial purposes.

(b) Recipient is responsible for obtaining any necessary Human Subjects research approvals or exemptions required to use the MATERIAL and DATA at the respective institution. The MATERIAL and DATA will be used by the Recipient in compliance with all applicable Federal, state, and local statutes and regulations.

(c) Recipient will allow the use of MATERIAL and DATA only by <*insert name of third party P.I.*> ("Recipient Investigator") and Recipient Investigator's research team that are under the direct supervision of Recipient Investigator, and only after they have been informed of and agreed to the provisions and restrictions stated herein. Any transfer of MATERIAL and DATA to other than Recipient Investigator's research team requires the advanced written approval of the Provider.

(d) It is acknowledged that the Recipient may already have in its possession or will obtain from another source, PHI related to the MATERIAL and DATA, and to which the Recipient may be subject to additional restrictions or obligations under separate agreements. Recipient shall notify Provider in writing within five (5) working days of its discovery of any unauthorized use or disclosure of PHI related to the MATERIAL and DATA of which Recipient, its officers, employees, or agents become aware. Recipient shall take (i) prompt corrective action to cure any deficiencies or (ii) any action pertaining to such unauthorized disclosure required by applicable federal law.

(e) Recipient agrees to not identify or contact any donor, or living relative of a donor, who may have provided the MATERIAL or any DATA received by Recipient under this Agreement from Provider.

(f) Recipient agrees to report data, inventions, and publications resulting from the use of the MATERIAL and/or DATA to Provider.

6. THE MATERIAL AND DATA ARE NOT FOR USE IN HUMAN SUBJECTS OR FOR THE TREATMENT OR DIAGNOSIS OF HUMAN SUBJECTS.

7. DISCLAIMER. Any MATERIAL delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties. THE PROVIDER MAKES NO REPRESENTATIONS AND EXTENDS NO WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE HUMAN MATERIAL WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHTS. To the extent allowed by law, Recipient assumes liability for claims for damages against it by third parties which may arise from its use, storage, processing, distribution, or disposal of the MATERIAL except that, to the extent permitted by law, Provider shall be liable to Recipient when the damage is caused by the gross negligence or willful misconduct of Provider.

8. TERMINATION AND DISPOSAL. Either Party may terminate this Agreement with sixty (60) days written notice to the other Party. When the Research Project is completed or this Agreement is terminated, whichever comes first, any unused MATERIAL and DATA will either be destroyed in compliance with all applicable statutes and regulations or will be returned to the Provider as requested by the Provider.

9. ACKNOWLEDGEMENT. In all oral presentations or written publications resulting from the use of the MATERIAL and DATA, the Recipient will acknowledge the *<insert name of biospecimen resource>* as the source of the MATERIAL and DATA, unless requested otherwise by Provider, as follows:

“Biospecimens (and/or Derivatives) and associated data were provided by the*<insert name of biospecimen resource>*, an initiative developed through funding from the *<insert funding source, if applicable>*.”

10. COST AND SHIPPING. The MATERIAL and DATA are provided at no cost to Recipient. Provider will notify Recipient when the MATERIAL and DATA are ready for shipment. Recipient will be responsible for the pick-up and shipment, including shipping costs, of the MATERIAL and DATA.

The Parties have executed this Agreement by their respective duly authorized officers on the day and year hereinafter written. Any communication or notice to be given shall be forwarded in writing to the respective addresses listed below.

SIGNATURES APPEAR ON THE FOLLOWING PAGE

Signatures for Provider

Provider Scientist:
Provider Organization:
Address:

Name of Authorized Official:
Title of Authorized Official:

_____
Signature of Authorized Official Date

Certification of Provider Authorized Official: This Agreement __has / __has not been modified. If modified, the modifications are attached.

Signatures for Recipient

Recipient Scientist:
Recipient Organization:
Address:

Name of Authorized Official:
Title of Authorized Official:

_____
Signature of Authorized Official Date

Certification of Recipient Scientist: I have read and understood the conditions outlined in this Agreement and I agree to abide by them in the receipt and use of the MATERIAL and DATA.

_____
Scientist Receiving Material Date

MATERIAL TRANSFER AGREEMENT

FOR THE TRANSFER OF HUMAN MATERIALS

FOR RESEARCH PURPOSES

This Human Material Transfer Agreement ("Agreement") is between _____ [IC] ("Provider"), part of the National Institutes of Health (NIH), a component of the United States Department of Health and Human Services (HHS) and _____ ("Recipient"), for the transfer of material isolated from individuals who have participated in clinical research (each a "Human Subject"), with or without accompanying data, to be used for research purposes as further defined below. Provider and Recipient may each be referred to as a Party or collectively as Parties. This Agreement will become effective on the date of the last authorized signature below ("Effective Date").

Recipient and Provider agree as follows:

1. Provider will transfer to Recipient the following materials: _____ and/or the following data: _____ (collectively "Human Material").

2. Recipient will only use the Human Material for the following internal research project: _____ ("Research Project").

3. Recipient agrees <u>not</u> to do any of the following:

   (a) **Use the Human Material in humans or for any diagnostic, prognostic, or treatment purposes;**

   (b) Use the Human Material for any commercial purposes, including selling, commercial screening, or transferring Human Material to a third party for commercial purposes;

   (c) Transfer the Human Material to anyone who is not under the Recipient Investigator's (as listed on the signature page of this Agreement) direct supervision unless advanced, written approval of Provider is obtained before any transfer.

4. If Recipient receives:

   (A) Information from Provider, or information ascertained through Recipient's use of the Human Material, that can be used to determine a Human Subject's identity, either alone or when combined with other personal or identifying information; or

   (B) The coded Human Material with the key to such information in 4(A) above; or

   (C) Identifiable, sensitive information ("ISI"), as defined in the Public Health Service Act at 42 U.S.C 241(d)(4), regarding the Human Material (see https://humansubjects.nih.gov/coc/faqs);

   Then Recipient agrees to:

   (a) Abide by all applicable human subjects and other regulations and guidance, which may include:

(i) The Privacy Act of 1974, as amended, at 5 U.S.C. §552a ("Privacy Act"), the Health Insurance Portability and Accountability Act of 1996 (HIPAA) or other equivalent privacy regulations; and

(ii) 45 C.F.R. Part 46, 21 C.F.R. Parts 50 and 56, and FDA Good Clinical Practice Guidelines (ICH E6 Good Clinical Practice: Consolidated Guidance, 62 FR 25692 (1997)); and

(iii) A certificate of confidentiality issued by NIH in accordance with 42 U.S.C 241(d) of the Public Health Service Act.

(b) Maintain any transferred information in a secure manner that restricts access by any individual not involved in the Research Project (e.g., for paper records – locked file cabinets or continual physical presence in a room that locks, or for electronic records – encryption and password protection); and

(c) Remove or destroy any information that may be used to identify the Human Subject at the earliest time at which removal or destruction can be accomplished consistent with the purpose of the Research Project; and

(d) Make no further use or disclosure of the information unless approved by the Provider or required by Federal, State, or local laws (e.g., as required by the Federal Food, Drug, and Cosmetic Act, or state laws requiring the reporting of communicable diseases to State and local health departments). Notwithstanding the foregoing, ISI is immune from the legal process, and will not, without the consent of the Human Subject, be admissible as evidence or used for any purpose in any action, suit, or other judicial, legislative, or administrative proceeding.

5. Recipient agrees not to contact or make any effort to identify Human Subjects, without specific written approval from Provider.

6. Recipient represents that it has obtained Institutional Review Board approval, as appropriate, to use Human Material.

7. All information to be deemed confidential that is transferred between the Parties under this Agreement will be clearly marked "CONFIDENTIAL" by the disclosing Party ("Confidential Information") and maintained in confidence by the receiving Party for a period of three (3) years from the date of receipt. Any Confidential Information that is orally disclosed must be reduced to writing and marked "CONFIDENTIAL" by the providing Party and such notice must be provided to the receiving Party within thirty (30) days of the oral disclosure. Notwithstanding any other provision of this Agreement, the obligation to not disclose ISI to any other party will extend indefinitely.

8. For the purposes of this Agreement, Confidential Information will not include information that:

(a) Has been published or is otherwise publicly available at the time of disclosure to the receiving Party or was in the possession of or readily available to the receiving Party without being subject to a confidentiality obligation from another source prior to the disclosure;

(b) Has become publicly known, by publication or otherwise, not due to any unauthorized act of the receiving Party; or

(c) The receiving Party can demonstrate it developed independently, or acquired without reference to, or reliance upon, such Confidential Information.

9. If the receiving Party becomes legally required to disclose any of the Confidential Information, the receiving Party will take all reasonable measures to disclose only that Confidential Information legally required and will notify the disclosing Party as soon as practicable. In all instances, the receiving Party will only disclose that portion of the disclosing Party's Confidential Information which is obliged to be disclosed. The disclosing Party is free to seek any remedies at law or in equity to limit or prevent the disclosure of the disclosing Party's Confidential Information.

10. Recipient will comply with all laws, rules, regulations and policies applicable to the handling, use and disposal of the Human Material.

11. When the Research Project is completed or upon the termination of this Agreement, whichever comes first, any unused Human Material will be destroyed unless the Provider gives Recipient directions for disposing of the Human Material by another means.

12. Either Party may terminate this Agreement by providing sixty (60) days prior written notice to the other Party, subject to the terms of Articles 10 and 11, above.

13. In all oral presentations or written publications concerning the use of Human Material, Recipient will acknowledge Provider's contribution of Human Material, unless requested otherwise by Provider.

14. Any Human Material delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties. **Provider makes no representations and extends no expressed or implied warranties of any kind, including warranties of merchantability, quality, or fitness for a particular purpose, or that the use of Human Material will not infringe any patent or other proprietary rights**.

15. Provider will not be liable for any loss, harm, illness or other damage or injury arising from Recipient's handling, use or disposal of the Human Material. No indemnification for third party claims is intended, implied, or provided by either Party.

16. This Agreement will be construed in accordance with United States Federal law as applied by the Federal courts in the District of Columbia.

17. This Agreement may be executed in one or more counterparts, each of which together will be deemed original but all of which together shall constitute one and the same document. A Portable Document Format (PDF) or other common format electronic file or electronic signature will constitute valid execution and delivery of this Agreement. Any communication or notice to be given will be emailed via the contact information listed below.

Signatures Appear on the Next Page

SIGNATURE PAGE

FOR PROVIDER:

_____

(Signature of Authorized Official) Date

Name:

Title:

(Signature of NIH Technology Development Coordinator) Date

Name:

Title:

Address:

Phone:

Email:

Provider Investigator:

I represent that the Human Material (including any data) that I am providing under this Agreement has all the necessary approvals required (including informed consent forms, Institutional Review Board etc.) to be transferred to Recipient for the uses contemplated in the Research Project.

(Signature of Providing Investigator) Date

Name:

Title:

Address:

Phone:

Email:

(Note: this signature block may or may not be included.)

FOR RECIPIENT:

(Signature of Authorized Official) Date

Name:

Title:

Address:

Phone:

Email:

Recipient Investigator:

I have read and understood the terms and conditions of this Agreement, and I will abide by them in the receipt and use of the Human Material.

_____

(Signature of Investigator) Date

Name:

Title:

Address:

Phone:

Email:

Appendix 5. Example of an NCI Biospecimen Evidence-Based Practice (NCI BEBP)

Download this NCI BEBP

View All Available NCI BEBPs

1. PURPOSE

The purpose of this document is to provide evidence-based guidance for the proper collection and processing of cell-free microRNA (cfmiRNA) from human plasma and serum. This guidance is intended to support the development and execution of evidence-based Standard Operating Procedures (SOPs) for human biospecimen collection, processing, and storage.

2. SCOPE

This evidence-based best practice document is applicable to the collection, processing, isolation, and storage of circulating cfmiRNA from plasma or serum that is intended for analysis in clinical and/or research settings; it does not include the requisite processing for analysis of exosomal miRNA. The ISO 21899.2020 standard (Reference 9.1.9) may be useful as a reference for validation and verification of SOPs produced using this BEBP.

3. DEFINITIONS

3.1. Anticoagulant: A substance that is used to prevent and treat blood clots in blood vessels and the heart; also called blood thinner; any agent capable of preventing blood clot formation

3.2. Serum: The clear liquid portion of the blood that remains after blood cells and clotting proteins have been removed

3.3. Plasma: The clear, yellowish, fluid portion of the blood that carries blood cells; the proteins that form blood clots are in plasma

3.4. Aliquot: A portion of the total amount of the biospecimen collected

3.5. cell-free microRNA (cfmiRNA): microRNA found in the bloodstream and generally measured in serum or plasma

3.6. Processing delay: The time between venipuncture and centrifugation of blood to obtain plasma

3.7. Clot time: The time between venipuncture and centrifugation of blood to obtain serum

3.8. Extraction delay: The time between the completion of plasma or serum processing and RNA extraction

3.9. Interim plasma/serum storage: The duration between the transfer of plasma (or serum) to new tube(s) and analysis

3.10. Freeze-thaw cycles: The number of times a biospecimen or sample has been frozen and then thawed

4. ENVIRONMENTAL HEALTH & SAFETY

4.1. Universal Precautions (CDC-2007) and guidelines associated with Coronavirus Disease 2019 (COVID-19) and Ebola should be used for all phases of blood collection and processing, and cfmiRNA processing (Reference 9.1.1, 9.1.2 and 9.1.3).

5. RECOMMENDED MATERIALS/EQUIPMENT

5.1. Appropriate safety equipment as described in published guidelines (References 9.1.1, 9.1.2 and 9.1.5).

5.2. Plastic-backed absorbent bench paper

5.3. Blood collection tube of choice (See 7.1 and 7.2)

5.4. Antiseptic wipes

5.5. Vacutainer needle (21-23 gauge) with hub or butterfly needle with Luer adapter

5.6. Tourniquet

5.7. Phlebotomy chair

5.8. Refrigerator (4°C)

5.9. Hi-speed centrifuge

5.10. Falcon tubes

5.11. Storage tubes, such as cryotubes, suitable for centrifugation, storage at -80°C and amenable to waterproof labeling using barcodes or with unique identifiers. RNase-free tubes are suggested.

5.12. LoBind tubes

5.13. Pipettes and sterile RNase-free tips for transfer

5.14. Freezer (≤ -80°C) for long-term storage. A -30°C freezer is acceptable if the anticipated duration of frozen storage is ≤ 1 year.

6. PROCEDURAL GUIDELINES

6.1. Recording of biospecimen pre-acquisition data

6.1.1. Whenever possible, extensive data relating to preacquisition conditions that may affect the integrity of the biospecimen should be recorded. Such data must include patient information (including age, gender, fasting status, diagnosis, physical activity level, and treatment type(s) and date(s)) as well as details relating to biospecimen acquisition (including number of venipuncture attempts, patient position, tourniquet usage, and date and time of blood collection) (Reference 9.1.4). When possible, the complete blood count of the blood specimen should be recorded as it may be informative in identifying potential sources of bias. Appropriate authorization by HIPAA or another pertinent regulatory agency and informed patient consent must be obtained prior to the collection of patient blood and data for research purposes (Reference 9.1.7).

6.1.2. Label each collection tube with unique unambiguous identifiers, such that the tube can be readily matched to all relevant patient and specimen handling data (Reference 9.1.4). Ensure that all labels are robust to all handling steps including but not limited to frozen storage, water, and commonly used solvents. The labeling scheme should accommodate the real-time documentation/recording of pre-analytical conditions (See 6.5.5 for additional details).

6.2. Collection tube considerations

6.2.1. Collection tubes containing ethylenediaminetetraacetic acid (EDTA), sodium citrate, acid citrate dextrose (ACD), citrate-theophylline-adenosine-dipyridamole (CTAD), or sodium-fluoride/ potassium-oxalate (NaF/KOx) are acceptable for blood collection as are select proprietary tubes containing a preservative such as Streck cfDNA BCT, Roche Cell-Free tubes, or Norgen cfDNA/cfRNA tubes)(See 7.3). When specimen processing within 1-2 h of collection is not possible, then use of preservative-containing collection tubes is preferred. Use of serum tubes may also be acceptable if processing is well-controlled (See 7.2 and 8.3.2). Use of heparin, Streck RNA BCT, or PAXgene tubes is not advisable (See 7.3). Optimally, tube choice should be validated during study design and remain consistent throughout the duration of the study. If changes to tube type are necessary, they must be validated in a pilot study as miRNA levels may differ between serum and plasma or among different anticoagulants (See 7.3, and 8.3.2).

6.2.2. Blood collection tube choice may also be guided by specimen availability and the need to assess multiple analytes (cfmiRNA, cfDNA, etc.) from a single collection tube (See 8.3.1).

6.2.3. To ensure sufficient yield, ideally a minimum of 10 mL of blood per tube should be collected (Reference 9.1.6). However, the total volume of blood required will depend on both the analytical method and the anticipated abundance of the cfmiRNA of interest. Consequently, smaller volumes may be sufficient. Care should be taken to avoid underfilling blood collection tubes containing an anticoagulant or preservative (See 8.3.3).

6.3. Blood Collection

6.3.1. Blood collection after the subject has fasted for > 6 h is preferred, as eating prior to venipuncture may result in a diet-specific increase in fatty acids in plasma/serum (See 8.3.3).

6.3.2. Blood collection tubes should be stored at room temperature (20-24°C) unless otherwise indicated by the manufacturer (See 8.3.2).

6.3.3. The patient must be seated for at least 5 minutes before venipuncture with the arm positioned on a slanting armrest such that there is a straight line from the shoulder to the wrist (Reference 9.1.6).

6.3.4. Apply a tourniquet 3-4 inches above the venipuncture site (Reference 9.1.5) with enough pressure to provide adequate vein visibility. Have the patient form a fist. Select the median, cubital, basilic, or cephalic veins for venipuncture (References 9.1.4 and 9.1.6). Collection from a port should be avoided (References 9.1.5 and 9.1.6). A vein imager should be used when available to improve venipuncture technique.

6.3.5. Clean the venipuncture site with an antiseptic wipe in a circular motion beginning at the insertion site (References 9.1.5 and 9.1.6) and allow to air dry. Once the skin is completely dried, anchor the vein by placing your thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving (References 9.1.5 and 9.1.6).

6.3.6. Insert the 21-23 gauge butterfly needle (See 8.3.3) with Luer adapter into the vein at 30° angle and then push the evacuated tube into the hub or adapter (References 9.1.5 and 9.1.6).

6.3.7. Once blood flow is established, release the tourniquet (total elapsed tourniquet time should be < 1 min) (References 9.1.5 and 9.1.6) and ask the patient to open their hand.

6.3.8. Make sure that tube additives do not touch the stopper or the end of the needle during venipuncture (Reference 9.1.6).

6.3.9. Non-cfmiRNA clinical specimens should be collected first (See 8.3.3). If no other blood specimens are being collected, discard the first 2-3 mL of blood prior to collecting blood specimens for cfmiRNA analysis (Reference 9.1.6; See 8.3.3).

6.3.10. After completely filling the tube, immediately remove the tube leaving the needle inserted and slowly and gently invert the tube as recommended by the manufacturer for the tube type of choice (Reference 9.1.6).

6.3.11. After filling the last collection tube, place gauze over the puncture site and remove the needle (Reference 9.1.5).

6.3.12. Store anticoagulant and serum tubes containing blood specimens upright (Reference 9.1.6) at room temperature (20-25°C) (See 7.4, 7.5, 8.3.4 and 8.3.5).

6.4. Processing Delay

6.4.1. In most instances, blood specimens should be centrifuged as soon as possible, optimally within 2 h of venipuncture (See 7.4, 7.5, 8.3.4 and 8.3.5). However, if proprietary tubes containing a preservative are used, then a pre-centrifugation delay of 24-48 h at room temperature is acceptable (See 7.3 and 8.3.4). Serum separator tubes should be processed immediately, but serum tubes relying on natural clot formation should be processed after 1 h at room temperature (See 8.3.5).

6.4.2. Regardless of tube type, agitation of blood should be minimized during a processing delay as hemolysis alters cfmiRNA levels (See 7.6).

6.5. Blood Processing

6.5.1. Centrifuge blood collection tubes using a protocol validated for the tube type (See 8.3.6). Acceptable centrifugation speeds and durations include the following ranges, 820-3,500 x *g* for 1-20 min at 4°C or room temperature (See 7.7).

6.5.2. If a two-step centrifugation is desired transfer plasma or serum to a new Falcon tube (or an equivalent container), carefully leaving the buffy coat behind.

6.5.3. Further removal of platelets, white blood cells, and cellular debris can be achieved through a second centrifugation at 10,000-16,000 x *g* for 15 min (See 7.8) or filtration (See 7.9 and 8.3.6). While the suitability of including a filtration or second centrifugation step will depend on the abundance of the miRNA of interest, a secondary processing step is generally recommended with the notable exceptions of miRNAs with low levels of expression and discovery-based studies (See 7.9, 7.8 and 8.3.6).

6.5.4. Serum and plasma specimens with evidence of hemolysis must be excluded from cfmiRNA analysis (See 7.6). Hemolysis should be quantified by measurement of hemoglobin concentration or when possible, using the ratio of miR-23a to miR-452 or miR-451a (See 7.6). Use of a spectrophotometer or other visual method is not recommended due to low detection sensitivity (See 7.6). Residual platelet count should be enumerated by flow cytometry or an impedance-based method (See 7.7 and 11.3).

6.5.5. Plasma or serum should be aliquoted into multiple tubes suitable for cryostorage at ≤ -80°C. Generally, a plasma or serum aliquot of 100-1,000 µL is adequate for a single extraction using a proprietary kit; however, the volume of plasma or serum required will depend on several factors that include extraction method, assay, and anticipated cfmiRNA abundance. Aliquot volume should be chosen to optimize cfmiRNA detection and avoid multiple freeze-thaw cycles. Optimally tubes should be RNase-free. While a barcode labeling system is recommended, any clear and robust labeling system that can withstand frozen storage and common solvents is acceptable (See 8.3.7). The labeling system should accommodate annotation of the preanalytical conditions experienced by each aliquot; please consult the International Society for Biological and Environmental Repositories (ISBER)'s Sample PREanalytical Code (SPREC) as a comprehensive example of biospecimen documentation (Reference 9.1.8).

6.6. Interim Plasma/Serum Storage (Note: not applicable for exosome analysis)

6.6.1. Optimally, cfmiRNA analysis should be conducted immediately, whether after extraction or by direct analysis of plasma or serum (See 7.10, 7.11 and 8.3.7). However, if immediate analysis is not possible, storage of plasma at room temperature or 4°C for up to 3 h, at -20°C for several months, or -80°C

for years is acceptable for most cfmiRNA endpoints. Potential effects of each storage temperature should be carefully considered for the targeted miRNA(s); for example, effects associated with freeze-thaw cycling may be more severe than a pre-extraction delay at 4°C. Freeze-thaw cycling should be avoided (See 7.12 and 8.3.8). Interim storage of serum should be avoided (See 7.10 and 7.11).

6.6.2. Frozen serum and plasma aliquots should be thawed on wet ice with occasional gentle mixing for the minimum time necessary (See 8.3.8).

6.6.3. Thawed serum and plasma should be thoroughly mixed and centrifuged at 1,000-1,600 x *g* for 1-2 min to pellet cryoglobulins immediately prior to miRNA extraction or analysis (See 7.12 and 8.3.8).

6.7. miRNA Extraction and Quantification

6.7.1. For analytical methods requiring extraction (See 8.3.9), a miRNA-specific commercial extraction kit is preferred, but phenol-chloroform based methods are acceptable (See 7.13). The expert panel advises against the use of column-based extraction kits for miRNA discovery studies (See 8.3.9).

6.7.2. Optimally, analysis should be performed immediately following extraction (See 7.14 and 8.3.10). Based on reports in the literature, interim storage of extracted miRNA or cDNA in low bind tubes at or below -80°C may be acceptable for several months (See 7.14). However, the expert panel advises that long-term storage should be limited to plasma/serum specimens, and if short-term storage of extracted miRNA is required then Tris buffer or ethanol should be used as the medium (See 8.3.10).

6.7.3. cfmiRNA levels quantified by real-time PCR should be expressed as an average quantification cycle (Cq) value relative to the Cq values of two or more constitutively expressed miRNA transcripts that display stable expression across the experimental conditions and disease states anticipated during the study (See 7.15 and 8.3.11). Additionally, miRNA/RNA used in any assay can be quantified using capillary electrophoresis and/or fluorometric methods, but these methods are unacceptable if extraction included carrier RNA.

6.7.4. All analytical methods used should be evaluated for reproducibility and standardized to minimize platform-specific effects (See 7.15 and 8.3.11). The ISO 21899.2020 standard (Reference 9.1.9) may be useful as a reference for further validation and verification.

7. SUMMARIES OF LITERATURE EVIDENCE

An inclusive approach was applied when composing the Summaries of Literature Evidence section; therefore, all studies that were identified and reviewed are presented and cited when relevant. Critical review of individual studies was limited; for example, larger well-controlled studies may be highlighted within the text, but smaller and/or flawed studies are also discussed. This approach was applied so that individual readers of the BEBP can assess and evaluate the evidence presented and its impact on their particular work.

7.1. Preacquisition:

Variables such as patient age, gender, fasting status, and exercise as well as the time of blood collection may each affect miRNA expression and thus must be carefully recorded and considered in any analysis. Many cfmiRNA levels show extremely high intra-individual variation in both serum and plasma and this variation must be considered in any assay [1, 2]. Patient age and gender are known to influence the levels of some [3, 4] but not all [3-6] miRNAs. The effects of fasting status on cfmiRNA levels are unclear, with some studies reporting significant differences [7-9] while others observed nonsignificant fluctuations [10, 11]. Although evidence is limited, one study reported exercise significantly affected levels of several miRNAs [12], while another reported an interaction between the timing of exercise relative to when the individual last ate [8]. Further complicating analysis, some but not all miRNAs display a rhythmic fluctuation in levels based on the time of day the specimen was sampled [13].

7.2. Serum versus Plasma:

The literature supports use of either serum or plasma for cfmiRNA analysis; however they are not interchangeable specimen types as the levels and stability of individual cfmiRNAs can differ between serum and plasma [6, 14-21](See Tables 10.1 and 10.2). The magnitude of differences between serum and plasma have been attributed in large part to differences in preanalytical handling [22], patient disease state [23], and clot time (See 7.4). In the absence of extensive processing delays, plasma generally displays a greater diversity of miRNA species than serum [15, 16], but clotting time is a confounding factor (See 7.4). Although 94% of the miRNAs identified in serum were also present in plasma/platelet poor plasma, only 35-78% of the miRNAs identified in plasma were present in serum [15, 16]. cfmiRNA from clotted blood samples can be deceiving as PBMCs and platelets broken up during clotting can release miRNA. With a few notable exceptions [19, 20], serum tends to have levels of individual miRNAs that are higher or comparable to those in plasma

specimens [6, 16-18, 24], including those indicative of hemolysis [17]. Differences between plasma and serum may also include the coefficient of variation and disease state-induced alterations in cfmiRNA levels [23]. Differences in serum and plasma processing may be partially responsible for the reported dissimilarities in miRNA detection. Hemolysis has been shown to increase levels of miR-15b, miR-16 and miR-24 [19]; and centrifugation speed affects the ratio of individual miRNA levels in serum versus plasma [22]. In conclusion, plasma and serum may not be used interchangeably and the optimal specimen type will depend on the cfmiRNA targeted, workflow constraints such as processing delays, and the assay used for quantification.

7.3. Anticoagulant/ Tube Type:

Anticoagulation with K_2EDTA [5, 7, 25-30], sodium citrate [7, 25-27, 29, 30], ACD [25], CTAD [29], NaF [30] and NaF/KOx [26] or cell stabilization tubes by Streck, Norgen, and Roche are all acceptable for cfmiRNA detection analysis; however, heparin should be avoided due to potential interference in enzyme-based molecular assays [7, 20, 25-27]. Notably, anticoagulant-specific differences in individual cfmiRNA levels have been reported. Mean levels of miR-16 and miR-223 were slightly higher when quantified in sodium fluoride (NaF)/potassium oxalate (KOx) plasma than in EDTA or citrate plasma [26]. Further, the addition of NaF/KOx to EDTA plasma resulted in a doubling of miR-16 levels [26]. Although plasma collected in platelet preparation tubes (PPT) had more cfmiRNA species detected by real-time PCR than K_2EDTA plasma [31], differences in cfmiRNA stability may be a confounding factor [31]. Similarly, alpha diversity was found to be significantly lower in specimens anticoagulated with K_2EDTA than ACD, citrate or CTAD [32]. When plasma is destined for cfmiRNA analysis, heparin should be avoided as it interfered with miRNA quantification by PCR [25, 26]. While treating plasma with heparinase rescued miR-16 detection [26], the extent of heparin-mediated enzyme inhibition may depend on the details and conditions of cfmiRNA extraction. Pre-analytical factors may also confound analysis of anticoagulant-specific effects on cfmiRNA levels. For example, compared to citrated plasma, K_3EDTA plasma had higher levels of miR-451a [20, 32] and other hemolysis-associated miRNAs [32] and lower levels of platelet-derived miRNA [32], which may be due to differences in either anticoagulant or the degree of hemolysis. The effects of pre-centrifugation storage on cfmiRNA levels in plasma was dependent on anticoagulant [25].

If processing delays > 6 h are anticipated, collection tubes containing a preservative have been reliably used for miRNA analysis, although the stability of miRNA differs between tube types (See Tables 10.1 and 10.2). Total cfmiRNA levels in plasma

remained unaffected when blood was stored at room temperature in Streck, Norgen, and Roche cell-free DNA tubes for up to 1 week [28]. However, blood collected in PAXgene Blood tubes displayed an increase in cfmiRNA levels following a pre-centrifugation delay of 24 h at room temperature [28], and blood collected in Streck RNA BCT had significant declines in individual miRNA levels following storage at room temperature for 24 h or more [33]. Blood collection tubes containing preservatives also differed in their ability to prevent contamination introduced by red blood cell (RBC) lysis. Hemolysis was first observed among specimens stored for 6 days in Streck cfDNA tubes or 7 days in Roche Cell-Free DNA Collection Tubes but was not observed in specimens stored in Norgen cfDNA/cfRNA tubes for up to 7 days [28]. Although additional study is required, one report observed less variability in quantified cfmiRNA levels among blood specimens collected in Streck cfDNA tubes compared to those collected in Streck RNA tubes [17].

7.4. Clot time for Serum:

Blood collected into serum separator tubes should be processed immediately (within minutes) and those relying on natural clot formation should be processed within 1 h. Processing delays result in rapid increases in levels of select miRNAs in serum specimens due to hemolysis (See 7.6) and/or high expression in RBCs and platelets (See Table 10.3). Although refrigerated or room temperature storage of blood in SSTMII Advance tubes for up to 9 h did not alter miRNA concentration [25], an increase in hemolysis was observed when blood collected in plain tubes was stored on ice for 2 h [34]. Altered abundance of individual cfmiRNAs assayed by real-time PCR were also observed after 24 h [34] or 4 days [27]. The abundance of miR-21 (or miR-21-5p) increased significantly in some specimens after a clot time of up to 24 h [34] or 4 days [27], while levels of miR-142-3p declined in 2 of 5 specimens that had a clot time of up to 24 h [34]. Importantly, more effects were attributed to the temperature (room temperature versus refrigerated) than the clot time, as less variability in cfmiRNA yield was observed after refrigerated storage than room temperature storage [25].

7.5. Delayed Centrifugation for Plasma Isolation:

When cfmiRNA analysis is anticipated, a room temperature delay to plasma processing by centrifugation should be limited to 2 h or less. Effects observed after a room temperature delay to centrifugation included changes in cfmiRNA yield [25, 28] or cfmiRNA profile in plasma specimens. Storage of EDTA blood at 4°C for > 2 h led to progressive increases in hemolysis [35] and lower library concentrations, a lower percentage of reads mapped, and changes in levels of some miRNAs after 3 days [30] although the window of stability was influenced by tube type and included

miRNA-specific differences in sensitivity [29, 36]. Effects attributable to a centrifugation delay were anticoagulant- and preservative-specific as total cfmiRNA yield was altered beginning after a delay of 9 h when specimens were stored in tubes containing EDTA or ACD, although no such effects were observed with citrate tubes [25]. Among tubes containing preservatives, miRNA yield was affected beginning after a 24 h delay to centrifugation for PAXgene tubes [28] while no such effects were observed among Streck, Roche, or Norgen cfDNA tubes when specimens were stored for up to 1 week [28]. Individual studies reported different windows of stability even when the same anticoagulant was used; for example, altered cfmiRNA profiles in K_2EDTA plasma were reported beginning after a centrifugation delay of 2 h [34, 37], 3 h [38], 12 h [36] or 72 h [27] compared to those processed with 30 min. Although principal component analysis (PCA) distinguished plasma specimens stored for 24 h at room temperature before centrifugation from those stored for shorter durations based on the cfmiRNA transcriptome, specimens stored for 24 h at 4°C before centrifugation co-clustered with those stored for shorter durations [39]. Notably, several miRNAs remained stable after a centrifugation delay of 24 h [34] or 72 h [33, 36]. Altered levels of individual miRNAs were first reported after an 18 h delay for plasma collected in PAXgene tubes [40], and after 24 h for plasma collected in Streck cfDNA BCT tubes [33]. Confounding analysis, many storage-associated effects are miRNA-specific and detectability of changes will depend on expression and the analytical platform sensitivity. The following miRNAs are reported to be stable after a delay of ≥ 24 h at 4°C when compared to < 1 h: miR-21 [36], miR-27a [36], miR-142-3p [36], miR-218 [36], miR-374-5p [36], miR-376c [36], miR-485-3p [36], miR-520d-5p [36], miR-523 [36]. The following cfmiRNAs were shown to be stable after a delay of ≥ 12 h at room temperature compared to < 1 h: miR-1 [38], miR-16 [41], miR-23 [38], miR-223 [41] and miR-423-5p [38]. Levels of cfmiRNAs expressed in blood cells (See Table 10.3) may increase rapidly and concomitantly during a delay to centrifugation, as hemolysis increases with K_2EDTA blood storage [17, 28, 38] (See 7.6). The window of stability for some cfmiRNAs may be extended if hemolyzed specimens are excluded from further analysis, or if the cfmiRNA of interest is not present in blood cells. Further confounding analysis, windows of stability can differ between cohorts within a study, as an effect of room temperature storage in K_2EDTA tubes was observed in one cohort after 3 days while no effect was observed in a second cohort after 4 days of storage [27].

7.6. Hemolysis:

Specimens with visual evidence of hemolysis should be excluded from analysis and the addition of a real-time PCR based assay for hemolysis is recommended.

Hemolysis was associated with increased levels of RBC-specific miRNAs in serum [19] and plasma [42-44] (See Table 10.3 for a list of affected miRNAs) and a decrease in the number of miRNAs detected at a given read depth [45], while other miRNAs remained unaffected [19, 42-44]. Interestingly, a single study found hemolysis may have a stabilizing effect on select cfmiRNAs in serum [46]. Importantly, the cfmiRNAs detected in hemolyzed and non-hemolyzed serum were different, with 231 miRNA having a difference of 3-fold or greater when case-matched severely hemolyzed and non-hemolyzed specimens were compared [10]. Imposing cut-off values of < 0.2 absorbance units at 414 nm has been shown to effectively eliminate RBC-contaminated specimens and hemolysis-associated miRNAs artifacts [17, 44]. Alternatively, a threshold difference of five or eight cycles for miR-23a and miR-452 has been successfully used to identify a moderate or severe risk of hemolysis, respectively [47]; while, a threshold difference > 8 cycles for miR-23a-3p and miR-451a successfully detected hemolyzed specimens [17].

7.7. Initial Centrifugation:

Centrifugation speed affects both the number of miRNAs detected as well as levels of individual cfmiRNAs; speeds of 820-3,500 x g for 1-20 min are considered acceptable for cfmiRNA analysis of plasma/serum. Faster centrifugation speeds led to the detection of fewer cfmiRNAs [14, 19, 48], as well as reduced levels of some but not all cfmiRNAs [17, 19, 48, 49], although the magnitude of decline (range: 0-8 cycles) was dependent upon the cfmiRNA species [17]. Collectively, evidence supports that effects of centrifugation speed are rooted in cellular contamination. Given that the cfmiRNAs most affected by centrifugation speed include those commonly detected in platelets (See Table 10.3)[14, 48, 50] and/or RBCs (See Table 10.3)[19], centrifugation induced cell lysis is a plausible source of the increase. Further, platelet-rich plasma obtained by a single centrifugation at 600 x *g* had 1.17-fold more detectable miRNAs than standard plasma obtained by centrifugation at 3,400 x *g* (325 versus 277 miRNAs)[14]. While only four miRNAs were investigated, a faster centrifugation speed (10,000 x *g* versus 1,900 x *g*) led to fewer differences between serum and plasma specimens [22]. The literature supports centrifugation of blood at 820-3,000 x *g* [2, 7, 16-18, 20, 23, 25, 28, 31, 33, 37, 38, 41, 48-64] to obtain plasma and centrifugation at 1,500-3,500 x *g* [16-18, 20, 23, 34, 56, 65-70] to obtain serum. While duration is wed to speed, the majority of the studies surveyed centrifuged specimens for 10-20 min to obtain either serum or plasma for cfmiRNA analysis [2, 7, 16-18, 20, 23, 25, 28, 31, 33, 34, 37, 38, 41, 48-50, 54-70], but centrifugation for 30 min at < 2,000 x *g* has also been reported [52, 53]. Experimental comparisons of centrifugation temperatures are lacking, however centrifugation at 4°C [2, 7, 16, 20,

23, 38, 41, 48, 52, 54, 59, 65, 67], 20°C [66], and at room temperature [31, 63] have been reported in the literature for subsequent cfmiRNA analysis.

7.8. Second Centrifugation:

A second centrifugation step at 10,000-16,000 x g for 15 min is acceptable for the adequate removal of platelets from plasma/serum, although loss of low abundance cfmiRNA is an associated risk. A second centrifugation step has been shown to decrease the number of cfmiRNAs detected [62] and levels of individual cfmiRNAs [17, 19, 25, 60]. As centrifugation is used to remove contaminating platelets and cells, costs and benefits of a second centrifugation step may be dependent on centrifugation speeds. Levels of several individual cfmiRNA were lower among plasma specimens centrifuged at 14,400-16,000 x *g* after initial centrifugation at 795-2,500 x *g* [17, 19, 25, 62], but not among specimens centrifuged at slower speeds (3400 x *g* after 1,900 x *g)* [14]. Further, while increasing the speed of the second centrifugation from 1,000 to 2,000 x *g* had little effect on the cfmiRNAs detected [48], fewer platelet cfmiRNAs were detected when the speed was increased to 10,000 x *g* [48] and less rRNA contamination was observed after centrifugation at 16,000 x *g* compared to 3,500 x *g* [71]. Notably, any effects attributable to platelet contamination will compound during storage, as a smaller percentage of cfmiRNAs were affected by storage of platelet poor plasma compared to standard K_2EDTA plasma [31]. Importantly, a third centrifugation step had little [19] or no [72] effect on miRNA abundance in plasma [19, 72] and serum [19]. Notably, effects associated with centrifugation workflow are cfmiRNA transcript-specific. A study investigating the effects of two centrifugations at 3,000 x *g* for 15 min versus a single centrifugation at 3,000 x *g* for 30 min observed higher cfmiR-126 detection but no change in the three other cfmiRNAs investigated after two-step centrifugation; however, significance was dependent on the method of miRNA normalization [60].

7.9. Filtration:

Filtration can be an effective alternative to a second centrifugation step for reducing platelet and cellular contamination. The abundance of several miRNAs, particularly those highly expressed in RBCs or platelets (See Table 10.3), was altered when plasma [14, 22] or serum [14] was passed through a 0.1 or 0.22 µm filter. However, filter size should be carefully considered, as levels of the hemolysis marker miRNA-451 were non-significantly lower when one-step centrifugation plasma was passed through a 0.1 or 0.2 µm filter, but higher when passed through a 1 µm filter [22].

7.10. Extraction Delay ≥ 4°C:

Storage of plasma at 4°C prior to miRNA extraction should be limited to < 3 h. The number of cfmiRNAs identified [31] and levels of individual cfmiRNA in plasma and serum were influenced by the temperature [19, 30, 31, 73] and duration [19, 30, 46] of a delay to miRNA extraction. However, numerous miRNA-specific effects [19, 27, 46, 65, 69, 74] confounded the identification of acceptable durations and temperatures of a delay to extraction. While a 24 h delay to extraction at either room temperature or 4°C led to an increase in the number of detectable miRNAs in EDTA plasma, the same delay led to a decline in detectable cfmiRNAs in PPT plasma and EDTA plasma centrifuged a second time to produce platelet poor EDTA plasma [31]. Acceptable delays will ultimately depend upon the initial level of the cfmiRNAs of interest, the specificity of the assay used for quantification, and the magnitude of changes that are anticipated due to the pathology of the patient. Significant changes in individual cfmiRNAs were reported in plasma specimens following an extraction delay of 24 h at room temperature [27, 31] or 2 weeks at 4°C [74], and in serum specimens after an extraction delay of 1 h [69] or 3 h [46] at room temperature or 6 h at 4°C [65]. A single study investigating post-processing storage of serum at 37°C observed no effect on miR-25, miR-221, or miR-222 levels after 3 h [73]. Compared to a room temperature delay, fewer miRNAs were affected when a 24 h delay to extraction occurred at 4°C [31], although another study reported larger average changes in Cq values when a ≤ 2 h delay occurred at 4°C [37]. The presence of platelets was identified as a confounding factor when exploring effects of a delay to extraction, as platelets store and release miRNA the magnitude of delay-induced changes were smaller in platelet poor plasma than standard plasma [31, 64].

7.11. Extraction Delay ≤ -20°C:

The majority of miRNAs evaluated remain stable in plasma and serum when stored at -20°C for several months, or -80°C for several years. Nevertheless, frozen storage of plasma and serum specimens prior to extraction altered some [11, 30, 64, 73-75] but not all cfmiRNAs evaluated [30, 64, 73, 74, 76, 77]; and, the magnitude of change was influenced by storage temperature [78]. While levels of several cfmiRNAs differed between serum specimens stored at -20°C and those that were stored at -80°C for 20 months [78], the majority of cfmiRNAs evaluated had comparable levels [78]. Storage of plasma for up to 30 days at -20°C led to

-80°C [77].

The duration of frozen storage is also an important preanalytical factor as the number of cfmiRNAs detected [48] and levels of cfmiRNAs [74, 75] are altered over the course of storage. Effects attributable to the duration of pre-extraction frozen storage include fewer detectable cfmiRNAs (177 versus 202) when K_2EDTA plasma

was stored at -80°C for > 12 y compared to < 1 y [48], and altered levels of a small number of individual cfmiRNAs when K_2EDTA plasma was stored at -80°C beginning after 1 month [75] or 2 months [74]. Nevertheless, only 1 miRNA was found to differ by > 2-fold between K_2EDTA plasma stored for 3 versus 5 years at -80°C [30]. Similar effects were observed when plasma was stored at -20°C, as declines in both individual miRNA levels and total miRNA levels were observed beginning after 1 month [75] or 5 years [74]. Unfortunately, reports evaluating serum conflict, with some cfmiRNAs reported stable at -20°C for 72 h [69] or 7 days [79] and others affected following -20°C storage for 72 h [19] or 6 y [78]. Potentially confounding variables in the identification of acceptable temperatures and durations of frozen storage include cfmiRNA-specific effects [64, 73, 74]; the presence of residual platelets, RBCs, and white blood cells [50, 64]; and post-thaw mixing [20].

7.12. Thawing and Freeze-Thaw Cycling:

Given the literature conflicts as to the sensitivity of cfmiRNAs in plasma and serum to freeze-thaw cycling, as well as the magnitude and direction of changes when observed, multiple freeze-thaw events should be avoided. Several studies examining either serum or plasma reported increases [78, 80, 81], decreases [74, 79], or no effect [24, 64, 73, 82, 83] on cfmiRNA endpoints following multiple freeze-thaw events. Results conflict even when the same miRNA target is quantified in specimens collected using the same anticoagulant with an overlapping number of freeze-thaw events. For example, in EDTA plasma specimens miR-16 remained stable for up to 8 freeze-thaw cycles in one study [24], but levels were reduced after ≥ 3 freeze-thaw cycles in another [79]. In serum, miR-16 levels remained unchanged in specimens that experienced 2 [82] or 4 [83] freeze-thaw events compared to controls that were thawed once [82, 83], although effects of freeze-thaw cycling will depend upon the miRNAs targeted, their physiological level, and the detection assay used. Potential factors that may have confounded investigations of freeze-thaw cycling on cfmiRNA endpoints include the presence of precipitates in thawed specimens, whether thawed specimens were mixed prior to extraction [20], temperature during thaw [80], the presence of platelets [50], and the anticoagulant used during blood collection [50]. In K_2EDTA and citrate plasma, miR-146a-5p and miR-382-5p levels were altered when the precipitate observed in thawed plasma was removed compared to specimens that were thoroughly mixed; however, no such differences were observed between separated and thoroughly mixed serum specimens [20]. Some effects of freeze-thaw cycling are influenced by anticoagulant and the presence of platelets, as effects of a single freeze-thaw event before versus after a second centrifugation differed among EDTA and citrate plasma [50]. A single study reports that sensitivity to thaw temperature was cfmiRNA-specific in serum; when

serum specimens thawed on wet ice were compared to those thawed at 37°C, differences ranged from severe (miR-93-5p, miR451a) to minute or absent (miR-21-5p, miR-23-3p) [80].

7.13. miRNA Extraction:

Sufficient cfmiRNA yields (as determined by real-time PCR) for downstream analysis were reported using a wide variety of specialty extraction kits as well as an in-house phenol-chloroform based method. Inclusion of an extraction step led to lower Cq values in TaqMan assays [56], although direct analysis of plasma has been successfully employed for next-generation sequencing (NGS) [84, 85], as well as for real-time PCR when samples underwent purification after reverse transcription [86] or when serum was treated with Tween-20 [87]. Tween can be used to dissolve circulating blood exosomes which often contain miRNA cargo [87]. However, extraction was found to be the source of the majority of inter-assay variability [9]. Proprietary kits for miRNA extraction from plasma recommended in the literature surveyed include miRCURY [55, 70, 88], QiAamp CNA [48], miRNeasy [5, 9, 36, 48, 52, 55, 89, 90], mirVana PARIS [19, 75, 91], Norgen miRNA purification kit [17], NucleoSpin [56], EasyPure [7] and RNAdvance [89]. Phenol chloroform-based extraction methods [18, 20] and guanidium thiocyanate and octanoic acid (Gu/OcA) extractions have also been recommended. Reported effects attributable to extraction kit choice included altered between-sample variance in Cq values [17-19, 92], and differences in NGS-generated cfmiRNA profiles of plasma specimens extracted with different proprietary kits (although differences were smaller than those introduced by library preparation) [93]. The studies that investigated potential effects of miRNA extraction method on serum specimens reported successful cfmiRNA analysis after extraction with the following proprietary kits: miRNeasy [19, 94], mirVana PARIS [19], Trizol-LS [19], Qiazol in combination with dr. GenTLE, precipitation [94], Norgen Total RNA isolation kit [94], and miRCURY RNA isolation kit -Biofluids [94]; and while differences between kits were identified [19], the optimal choice was dependent on the miRNA evaluated [94] and post-extraction storage [19]. When serum-derived miRNA samples were analyzed immediately, extraction with miRNeasy or miRVANA kits led to lower Cq variance compared to Trizol LS; but, when miRNA samples were stored for 6 months at -20°C, extraction with Trizol LS led to lower Cq variance than the mirVana PARIS kit [19]. Additional confounding factors that affected evaluation of extraction techniques included the use of carrier RNA such as MS2 [52, 92] or yeast [52, 91] and preheating steps [73]. When MS2 and glycogen were used in combination as an RNA carrier, higher cfmiRNA yields [34] and a lower standard error of the mean were observed than when no carrier was used [34], but excessive amounts of carrier RNA altered

cfmiRNA profiles [91]. While preheating serum to 75°C for 5 min before RNA extraction lowered mean Cq values [73], incubation at 60°C for 2 h was detrimental.

7.14. Post-Extraction Storage:

Given the need for high powered studies on stability, the conservative recommendation is that frozen storage of isolated cfmiRNA and cDNA samples should be limited to ≤ -80°C for the shortest duration required. In one study, frozen storage of extracted miRNA specimens from the blood of *one* volunteer resulted in a decline in the level of miR-451a, but not miR-155-5p, after ≥ 1 day at -80°C [20] and non-significant changes in miR-155-5p and miR-451a after storage of cDNA at -20°C for up to 120 days [20]. Conversely, in a single study

Importantly, for cfmiRNAs with low expression even a small decrease may lead to loss of detection.

7.15. Quantification:

The method used for miRNA normalization greatly affects the miRNA profile and should be carefully selected; acceptable strategies include normalization to input amount and two or more constitutively and stably expressed miRNA transcripts. General guidance on the adequate validation of a real-time PCR assay can be found in de Gonzalo-Calvo (2022)[96]. Generally, low coefficient of variation (CV)s and a low median CV value were associated with normalization to the global mean Cq [63, 97], quantile [97] or normalization to the three [16] or eight [63] most stable cfmiRNAs in a dataset; however, one study concluded that normalization via geNorm or NormFinder was superior to normalization to the global mean [51]. Others have reported that normalization to a single miRNA increased sensitivity [63] or resulted in a lower CV [16]. Importantly, the best miRNA for normalization may depend on a specific dataset and the miRNA(s) of interest [20]. One study suggests that assays for cfmiRNA in plasma should have a PCR efficiency of 100±10%, an intra- and inter-day precision of < 25% and < 35%, respectively, and a calibration curve with an $R^2 \geq 0.98$ [20].

8. EXPERT-VETTING

8.1. Details of Expert Review:

Three experts were identified and invited to review the document based on their contributions to the literature regarding the study of cfmiRNAs (See 8.2). Feedback from participants was collected and documented following initial review of the draft BEBP. Final thoughts and recommendations were captured from the expert panel during a scheduled teleconference after review of the BEBP document. Participating individuals did so voluntarily and without compensation.

8.2. Participating Experts:

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8.3. Expert Recommendations

8.3.1. General Considerations:

The expert panel agreed that effects of pre-analytical steps on the quantifiable levels of miRNAs will be transcript-specific. Thus, the panel advised that the complete workflow (from collection to analysis) be validated for each miRNA of interest. Further, the workflow should remain consistent for a given study and any necessary changes to the protocol require validation. Additionally, if differences in workflow and protocols are necessary and/or anticipated (such as due to different collection sites or use of retrospectively collected specimens), then it is crucial to assess their impacts. The panel advised that differentially processed specimens be run and analyzed together to verify that they fall within an acceptable reference range and have acceptable measures of integrity.

In consideration of the donating patient and the low volume of blood that may be available for research purposes after clinical chemistry, the panel recommended designing collection and processing workflows such that multiple analytes can be assessed from a single blood puncture and blood collection tube.

8.3.2. Blood Tube Type:

While the expert panel agreed that a variety of blood collection tubes are suitable for cfmiRNA detection and quantification, they cautioned that optimal processing and extraction protocols will depend on the tube type selected. Experts agreed that plasma is the optimal biospecimen type for miRNA analysis. They advised that while serum specimens are not preferred for cfmiRNA analysis, data from serum may still be informative with proper validation. Of note, all FDA approved cfDNA assays specify that plasma be used for analysis. The expert panel emphasized that collection and processing protocols (including collection tube type) must remain constant for the duration of the study. If, for example, a change in tube type is unavoidable then the change must be carefully validated, which could include re-running specimens collected in both types of blood collection tubes simultaneously to verify that the resultant data fall within the same reference range and have similar measures of integrity. The expert panel emphasized that serum and plasma specimens are not interchangeable. All experts agreed that blood collection tubes should not be pre-chilled prior to collection, noting that pre-chilling tubes is not common phlebotomy practice and doing so would result in additional and unnecessary variability. They also noted that chilling tubes containing preservatives is against manufacturer recommendations and could result in detrimental effects.

8.3.3. Blood Collection:

The expert panel agreed that blood should be collected with a 21-23 gauge needle, and that needles with a higher gauge (25 gauge) should be avoided given an increased risk of hemolysis. The experts agreed that the first tube of blood collected from a patient should not be used for cfmiRNA analysis due to potential contamination from the skin plug. Clinical chemistry analysis of the first collection tube is acceptable and routine practice; however, if cfmiRNA is the only analyte investigated then the first tube of blood should be discarded. One expert specified that all alcohol should be allowed to evaporate completely from the skin prior to venipuncture to reduce the likelihood of alcohol interference. Another expert advised that blood for cfmiRNA analysis should be collected from fasting patients (> 6 h prior to collection), as eating immediately prior to venipuncture can increase fatty acids in collected plasma/serum. It is also important that the

tube be filled to the proper volume to ensure the correct anticoagulant/preservative concentration. Under- or over- filling of the tube may influence subsequent downstream analysis.

8.3.4. Processing Delay of Blood for Plasma:

The expert panel agreed that in the absence of stabilizers and preservatives plasma should be isolated within 2 h of blood collection. However, specimens stored for longer durations (up to 18-24 h) may still contain stable cfmiRNAs and provide informative data, but modest changes in some cfmiRNAs are likely due to platelet contamination. In instances when long processing delays (> 2 h) are anticipated, such as when blood specimens will be sent to a centralized laboratory for processing, experts advised that preservative tubes should be used, which will extend the acceptable processing delay to 48 h.

8.3.5. Processing Delay of Blood for Serum:

The expert panel agreed that plasma is preferred over serum for cfmiRNA analysis. Two of the three experts noted a lack of control in the release of miRNA from the clot when serum is used. When cfmiRNA analysis of serum is necessary, one expert advised that specimens be placed on ice and processed immediately if serum separator tubes are used but stored for 1 h (but not > 2 h) at room temperature if a natural clot formation is needed.

8.3.6. Blood Processing:

The expert panel noted that each processing step results in miRNA loss, and that sequential and/or high-speed centrifugation also shears nucleic acids within the sample. Thus, the number and conditions (speed, temperature, duration) of centrifugation and filtration will depend on the abundance of the miRNA of interest. For example, the experts advised limiting centrifugation to a single spin for cfmiRNAs with low abundance. The panel noted that it is essential that the number and conditions of centrifugations be standardized and remain consistent within a study, and that processing workflow be tailored to the properties of the cfmiRNAs of interest during study design. While two experts have not explored filtration due to concerns with loss of small or low abundance cfmiRNAs, one expert found filtration prior to freezing beneficial in the removal of platelets, cells, and cellular debris.

8.3.7. Plasma/Serum Storage:

The expert panel recommended that under ideal circumstances plasma or serum should be aliquoted and stored in barcoded low bind RNase-free cryotubes (or a comparable tube) at -80°C or colder. An expert noted that the effects of storage are dependent on the amount of platelet and cellular contamination in the plasma. Use of other labeling systems are also acceptable provided labels are unambiguous, permanent, and waterproof. One expert noted that while use of RNase-free tubes is optimal, it is not necessary in all cases. However, vials should be sterile at best.

8.3.8. Thawing of Plasma:

All experts agreed that plasma aliquots should be thawed on ice with gentle and occasional swirling to ensure rapid thawing. Each of the experts noted that immediately after thawing, aliquots are centrifuged at low-speed (between 1,000-1,600 x *g*) for 1-2 min to pellet any cryoglobulins that may have precipitated during freezing. Multiple freeze-thaw cycles should be avoided. If non soluble particles are observed in the specimen after thawing, it should not be used.

8.3.9. Extraction:

The expert panel agreed that the need for cfmiRNA extraction is dependent on the platform used for analysis. Extraction steps can lead to loss of detection of low level or small miRNAs, and different extraction methods (such as column-based extraction) can potentially introduce bias. As extraction kits will affect each miRNA differently, it is important that the extraction method remains consistent within a study and that any necessary changes to methodology be experimentally validated. Screening studies by directly assaying a panel of miRNA without extraction is preferable if cfmiRNA abundance is low.

8.3.10. Storage of Extracted cfmiRNA:

The expert panel unanimously agreed that extracted cfmiRNA is very labile and recommends analyzing miRNA samples immediately after extraction. The expert panel recommended plasma aliquots be used for long-term storage at -80°C, as opposed to extracted cfmiRNA samples. Short-term storage of extracted cfmiRNA at -80°C is acceptable, when necessary, although the acceptable duration of short-term miRNA storage is dependent on the buffer used. One expert noted that miRNA stored in Tris buffer will be stable for several months, and miRNA stored in ethanol will be stable for up to a year at -80°C. It is advisable to validate the effects of storage on each targeted cfmiRNA panel.

8.3.11. Quality Assessment/Validation:

The expert panel was in agreement that reporting and quality standards are necessary for cfmiRNA endpoints. The standards specified by the expert panel include (i) the quantity of total cfmiRNA extracted to assess whether recovery is acceptable and to mitigate false negatives due to loss; (ii) evaluation of hemolysis markers as indicators of sample quality; and (iii) for real-time RT-PCR based analysis, identification of two or more reference miRNAs that have demonstrated stability across both the collection/processing workflow and patient disease state(s) that can be used for normalization, as well as to define an acceptable level of variability. One expert suggested defining a stable amount of variation as within 0.5 of a Cq value. For NGS, it is critical to include counts of both positive and negative controls.

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10. Figures and Tables

10.1. Blood collection tube-specific recommendations for plasma

TUBE TYPE	PRECENTRIFUGATION DELAY	CENTRIFUGATION	SHORT-TERM STORAGE	LONG-TERM STORAGE	FREEZE-THAW CYCLING
EDTA TUBE	< 1 h at RT (storage at 4°C may decrease stability)	2 steps required, Second step must be ≥ 10,000 *g*	< 24 h at RT or 4°C	< 30 days at -20, -70 or -80°C	< 1 cycle, Post-thaw mixing critical
SODIUM CITRATE	Limited evidence suggests < 9 h at RT	2 steps	Insufficient literature evidence	Insufficient literature evidence	More stable than EDTA plasma, Post-thaw mixing critical
ACD	≤ 3 h at RT	2 steps	No reported effects of 24 h at RT	Insufficient literature evidence	No reported effects ≤ 6 cycles
PPT	Insufficient literature evidence	Insufficient literature evidence	Small differences observed when stored for 24 h at 4°C or RT	Insufficient literature evidence	Insufficient literature evidence
STRECK cfDNA BCT	< 24 h, but other reports extend stability	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence
ROCHE	≤ 5 days	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence
NORGEN	≤ 5 days	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence
PAXGENE	< 24 h	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence

10.2. Blood collection tube-specific recommendations for serum

TUBE TYPE	PRECENTRIFUGATION DELAY	CENTRIFUGATION	SHORT-TERM STORAGE	LONG-TERM STORAGE	FREEZE-THAW CYCLING
SERUM GEL TUBE	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence	Insufficient literature evidence	Post-thaw mixing is not critical
SERUM SEPARATOR TUBE	< 3 h at RT or 4°C	2 steps	< 24 h at RT or 4°C	< 24 h at -20°C	Insufficient literature evidence
SERUM CLOT ACTIVATOR TUBE	Insufficient literature evidence	Insufficient literature evidence	< 1 h at RT, ≤ 72 h at 4°C	< 72 h at -20°C or -80°C	≤ 4 cycles
PLAIN SERUM TUBE	< 2 h	Insufficient literature evidence	< 6 h at 4°C	Insufficient literature evidence	< 2 cycles, temperature of thaw is a confounding factor
SERUM UNSPECIFIED TUBE	Insufficient literature evidence	Insufficient literature evidence	≤ 3 h at 4°C, ≤ 24 h at RT	< 20 months at -20°C or -80°C	< 10 cycles

RT, room temperature

10.3. miRNA species highly expressed in red blood cells (RBC) or platelets and their reported sensitivity to hemolysis.

miRNA	RBC expression	Platelet expression	Sensitivity to hemolysis
let-7a	[42]	[14, 15, 42, 98]	[42]
let-7b			[42]
let-7c		[98]	[42]
let-7d		[15]	[42]
let-7e		[15]	[42]
let-7f	[42]	[42, 99]	[42]
let-7g		[15]	[42]
let-7i		[98]	[42]
miR-103	[42]	[15, 42, 98, 100]	[42]
miR-106a	[42]	[15, 42, 98, 100, 101]	[42]
miR-106b			[42]
miR-107	[42]	[42, 98]	[42]
miR-125a		[15]	
miR-1255B	[43]		[43]
miR-126	[42]	[15, 42, 98, 99, 101]	[42]
miR-126-3p	[29]		
miR-127-3p		[15]	
miR-130a		[98]	
miR-130b		[100]	
miR-139-5p		[15]	
miR-141		[14]	
miR-142-3p	[42]	[14, 15, 42, 98, 99, 101]	[42]
miR-142-5p		[98, 99]	
miR-145		[15]	
miR-146a		[15, 98, 101]	
miR-146b		[15]	

miR-150		[15]	
miR-150-5p	[29]		
miR-151-3p		[101]	
miR-151-5p	[42]	[42]	[42]
miR-15b	[19, 42, 43]	[15, 42, 98]	[19, 42, 43]
miR-16	[42-44]	[14, 15, 42, 99-101]	[19, 42-44]
miR-17	[42]	[15, 42, 100, 101]	[42]
miR-185		[98]	
miR-186		[15, 101]	
miR-19a	[42]		[42]
miR-19b	[42]		[42]
miR-191		[15, 98, 99, 101]	
miR-191-5p	[29]		[66]
miR-197	[29]	[15]	
miR-199a-3p		[101]	
miR-199a		[15]	
miR-19a		[15, 42, 98]	
miR-19b		[15, 42]	
miR-20a		[15, 98, 101]	
miR-20a-5p			[66]
miR-20b	[42]	[15]	[42]
miR-21	[42]	[15, 42, 98, 99]	[42]
miR-21-5p	[29]		
miR-210		[14]	
miR-22		[99]	
miR-221		[15, 42]	
miR-222		[15, 101]	
miR-223	[29, 42]	[14, 15, 42, 98, 99, 101]	[42]
miR-23a		[98, 99]	

miR-23a-3p	[29]		
miR-23b		[98-100]	
miR-24	[42]	[15, 42, 99-101]	[19, 42]
miR-24-3p	[29]		
miR-26a		[15, 98-101]	
miR-26b		[15, 99]	
miR-27-3p	[29]		
miR-28-3p	[29]	[15]	
miR-30b	[42]	[15, 42, 98, 101]	[42]
miR-30b-5p			[66]
miR-30c	[42]	[15, 42, 98, 101]	[42]
miR-30d		[42]	
miR-30e	[42]	[15, 98]	[42]
miR-320a	[29]		
miR-328		[15]	
miR-331-3p		[15]	
miR-342-3p		[15]	
miR-374a		[15]	
miR-374b		[15]	
miR-383		[15]	
miR-423-5p		[15]	
miR-451	[42-44]		[42-44]
miR-451a	[29]		[37]
miR-484		[101]	
miR-486-3p	[43]		[43]
miR-486-5p	[42]		[42]
miR-486b	[42]		[42]
miR-532-3p	[43]		[43]
miR-574-3p		[15]	

miR-636	[43]		[43]
miR-652		[15]	
miR-720		[99]	
miR-744		[15]	
miR-886-5p	[43]		[43]
miR-92a	[42-44]	[14, 15, 42]	[42-44]
miR-93	[42]	[15, 42]	[42]

11. REVISION HISTORY

11.1. Revision 1.0 (2/5/2025): 508 Compliance

To achieve 508 compliance, formatting changes were applied, tables were edited, and alternative text was added to the original version (5/1/2023).

Appendix 6: CAP Biorepository Accreditation Program Checklist

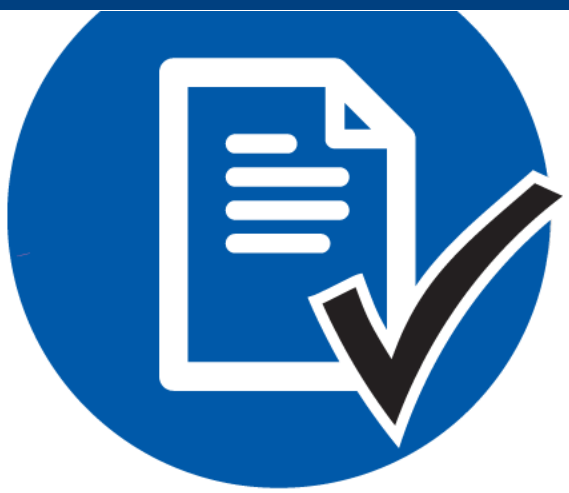
The CAP Biorepository Checklist presented here is for reference only and not definitive for accreditation purposes, nor is it the current CAP version. All CAP checklists are reviewed and updated regularly. The CAP Biorepository Accreditation Program includes additional checklists (Director Assessment, Lab General, All Common) which cover a substantial amount of general laboratory quality assessment. These additional checklists are not posted here but can be obtained through CAP.

COLLEGE of AMERICAN PATHOLOGISTS

Master

Biorepository Checklist

CAP Accreditation Program



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Biorepository Checklist

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ON-LINE CHECKLIST DOWNLOAD OPTIONS

Participants of the CAP accreditation programs may download the checklists from the CAP website (cap.org) by logging into e-LAB Solutions Suite. They are available in different checklist types and formatting options, including:

- Master — contains ALL of the requirements and instructions available in PDF, Word/XML or Excel formats
- Custom — customized based on the laboratory's activity (test) menu; available in PDF, Word/XML or Excel formats
- Changes Only — contains only those requirements with significant changes since the previous checklist edition in a track changes format to show the differences; in PDF version only. Requirements that have been moved or merged appear in a table at the end of the file.

CHECKLIST ACCREDITATION RESOURCES

CAP accredited laboratories have access to additional checklist accreditation tools and resources found on the CAP website (cap.org) by logging into e-LAB Solutions Suite - Accreditation Resources. Content found in Accreditation Resources includes:

- A library of past Focus on Compliance webinars and laboratory inspection preparation videos
- Answers to the most common checklist questions
- Customizable templates and forms (eg, competency assessment, personnel, validation/verification, quality management)
- Proficiency testing (PT) frequently asked questions, forms, and troubleshooting guides
- IQCP eligibility, frequently asked questions, forms, templates, and examples
- Laboratory director education and resources
- Quality management resources
- Inspector training and inspection tip sheets
- Self and post inspection toolbox

SUMMARY OF CHECKLIST EDITION CHANGES
Biorepository Checklist
08/24/2023 Edition

The information below includes a listing of checklist requirements with significant changes in the current edition and previous edition of this checklist. The list is separated into three categories:

1. New
2. Revised:
   - Modifications that may require a change in policy, procedure, or process for continued compliance; or
   - A change to the Phase
3. Deleted/Moved/Merged:
   - Deleted
   - Moved — Relocation of a requirement into a different checklist (requirements that have been resequenced within the same checklist are not listed)
   - Merged — The combining of similar requirements

NOTE: The requirements listed below are from the Master version of the checklist. The customized checklist version created for inspections and self-evaluations may not list all of these requirements.

Previously Cited Checklist Requirements

- The **inspector's version** of the checklist contains a listing of previously cited checklist requirements. Specific information on those citations, including the inspection date and inspector comments, is included following each related requirement within the checklist.

- Laboratories can access data on previously cited deficiencies by logging into e-LAB Solutions Suite on cap.org and going to Accreditation Reports - Inspection Summation Report.

NEW Checklist Requirements
None

REVISED Checklist Requirements

Requirement	Effective Date
BAP.06856	10/24/2022
BAP.06858	10/24/2022
BAP.06865	10/24/2022
BAP.07110	10/24/2022
BAP.08600	08/24/2023
BAP.09600	08/24/2023

DELETED/MOVED/MERGED Checklist Requirements

Requirement	Effective Date
BAP.07610	08/23/2023
BAP.07620	08/23/2023

INTRODUCTION

A biorepository is defined as an entity that collects, processes, stores, manages, and/or distributes biospecimens, their derivatives and relevant data, as needed, for research purposes. It encompasses the physical location as well as the full range of activities associated with its operation. The term biorepository used within the checklist may be considered synonymous with biobank and repository.

The term laboratory may also be used to describe a biorepository. When the term "patient" is used within the checklist, it may also refer to donors, clients, and study participants.

This checklist covers a broad range of activities that occur in biorepositories. Not all checklist requirements will apply to every biorepository.

The scope of services of the biorepository must be clearly recorded.

***Laboratories not subject to US regulations:** Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist. When the phrase "FDA-cleared/approved test (or assay)" is used within the checklist, it also applies to tests approved by an internationally recognized regulatory authority (eg, CE-marking).*

References used in the development of this checklist were the CAP Accreditation Checklists, 2018 Best Practices for Repositories (ISBER), and the NCI Best Practices for Biospecimen Resources.*

*ISBER — International Society for Biological and Environmental Repositories is an international forum that addresses the technical, legal, ethical, and managerial issues relevant to repositories of biological and environmental specimens.

DEFINITION OF TERMS

Aliquot - Process wherein a specimen is divided into separate parts which are typically stored in separate containers as individual samples. The term aliquot may also be used as a noun to denote a single sample.

Anonymization - The process of removing particulars from samples, test results, or records to prevent traceability to the original patient

Blinding - An action taken to prevent access to information that might affect the outcome of an observation

Coded specimen - Identifying information (such as name or social security number) that would enable the investigator to ascertain the identity of the individual to whom the private information or specimens pertain has been replaced with a number, letter, symbol, or combination thereof (ie, the code); and a key to decipher the code exists, enabling linkage of the identifying information to the private information of specimens

De-identify - The removal from a specimen of all 18 elements that could be used to identify the individual or the individual's relatives, employers, or household members; these elements are enumerated in the HIPAA Privacy Rule

Derivative - A substance that can be made from another substance

Function check - The set of routines that show an instrument to be ready for operation

Legacy specimen - Biospecimens available for research once all protocol-specified endpoints, including clinical and biorepository studies, have been completed. These remaining biospecimens could be made available by the biorepository for correlative studies (subject to application, scientific review, and approval).

Material Transfer Agreement (MTA) - An agreement that governs the transfer of tangible research material and associated clinical data between two organizations, when the recipient intends to use it for his/her own research purposes

Pathologist - A physician who has successfully completed an approved graduate medical education program in pathology.

In the US, a physician is defined as a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine who is licensed by the state to practice medicine, osteopathy, or podiatry within the state in which the laboratory is located. In jurisdictions not subject to US regulations, a physician is defined as an individual who has a primary medical school degree (eg, MBBS, MBChB, MD, DO) in keeping with the standards of that particular jurisdiction.

Qualified pathologist - A pathologist who has training in the specific functions to be performed (eg, an anatomic pathologist for anatomic pathology functions, a clinical pathologist for clinical pathology functions, or an anatomic pathologist or dermatopathologist for skin biopsies).

Quality assurance - The systematic monitoring and evaluation of the various aspects of a project, process, service or facility to maximize the probability that minimum standards of quality are being attained

Quality control - An integral component of *quality management* composed of the aggregate of processes and techniques used to detect, reduce, and correct deficiencies in an analytical process

Quality control (QC) is a surveillance process in which the actions of people and performance of equipment and materials are observed in some systematic, periodic way that provides a record of consistency of performance and action taken when performance does not conform to standards set by the biorepository. QC is a set of procedures designed to monitor the test method and the results to assure test system performance; QC includes testing control materials, charting the results and analyzing them to identify sources of error, and determining, performing and recording any corrective action taken as a result of this analysis.

Remnant specimens - Remaining portion of a specimen obtained for clinical purposes that is no longer needed for its original purpose and that would otherwise be discarded

Sample - A single unit containing material derived from one specimen

Source Facility - Those sites that contribute specimens to the biobank. The source facility may be a clinic, hospital or individual investigator, and, in some instances, the biorepository may be the source facility, (eg, when the biorepository does blood or specimen collections for normal controls).

Specimen - A specific tissue, blood sample, etc. taken from a single subject or donor at a specific time

Sponsor - The person, organization or biorepository that seeks and is responsible for the initiation, maintenance, and governance of the biospecimen collection. The sponsor typically provides the financial support to create and maintain the collection.

NOTE: This could include: 1) a sponsor-investigator (such as a pharmaceutical company seeking samples for an internal research project or as part of a multi-site clinical trial); 2) a biobank seeking biosamples to fulfill the needs of its research clients; 3) a cooperative oncology group that sets criteria (such as disease type, specific samples required, accompanying medical data, informed consent specifications) for inclusion into a biobank and that cooperative oncology group confirms all criteria have been met (directly or through a contracted biobank) before submitted samples are accepted into the biobank.

BIOSPECIMEN COLLECTION AND HANDLING

SPECIMEN COLLECTION AND HANDLING

The collection and handling for all biospecimens is critical to the overall quality and diversity of the sample inventory.

Inspector Instructions:

	• Sampling of policies and procedures for sample collection and handling, including sample types, samples with potentially infectious materials, preservation, de-identifying or anonymizing, aliquoting, specimen storage conditions, and chain-of-custody • Policy for the type of samples suitable for submission to the biorepository • Storage temperature records • Sampling of biospecimen QA reports for key elements of processing and preservation of solid and fluid specimens • Records of informed consent and IRB releases
	• Sampling of stored specimens for temperatures required by protocols • If collection occurs on-site, observe the processing/preservation procedure • Specimen storage conditions during sample receipt
	• How does your biorepository capture variables that could impact biospecimen usage? • How/when would the biorepository communicate pre-analytic variables to researchers? • How do you ensure accuracy of pre-analytic data capture? • What is your specimen coding system for sample identification? • How do you confirm patient consent prior to processing and banking? • What do you do if the sample size is too small relative to the requirements or it does not meet researchers' needs? • Do you receive specimens considered infectious biological agents from outside the United States?
	• Follow a tissue sample released for research from the pathologist to storage

BAP.01600 Specimen Types Submission Criteria **Phase II**

The biorepository defines the types of specimens submitted based on the following:

1. **Purpose (intended use of specimen)**

2. **Required specimen data**
3. **Biosafety/risk level (laboratories are suitable for the type of specimen/ pathogen requiring processing)**
4. **Duration of storage (may be indefinite)**

NOTE: This may be an overarching statement that defines the criteria required for all collections held in the biorepository. This may include the receipt or transfer of an entire collection.

REFERENCES

1) Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, HHS Publication No. (CDC) 21-1112 Revised December 2009. https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.pdf

BAP.01700 Collection/Processing Oversight — Phase II

A pathologist or designee assigned to the management of the biospecimens ensures that collection policies and processes reflect published best practices.

NOTE: 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.

If samples are acquired according to sponsor-driven protocols, the sponsor makes all decisions about sample usability. The biorepository carries out the instructions provided by the sponsor. In this instance BAP.01700 is not applicable.

REFERENCES

1) Best Practices: Recommendations for Repositories. Fourth Edition. Section L: Legal and Ethical Issues for Biospecimen Collection. ISBR. 2018.

BAP.01703 Disease Control Import Permit — Phase II

If the biorepository receives specimens that are considered infectious biological agents imported from outside of the United States and its territories, the biorepository has obtained the Centers for Disease Control Import Permit.

NOTE: The Office of Public Health Preparedness and Response CDC Import Permit Program regulates the importation of the following into the United States:

- *Naturally occurring or bioengineered infectious biological agents capable of causing disease in a human;*
- *Any material that is known or reasonably expected to contain an infectious biological agent;*
- *Vectors, including animals/animal products that are known to transfer or are capable of transferring an infectious biological agent to a human.*

If the material being imported is rendered sterile (eg, thermal, chemical or irradiation treatment) or it has been confirmed not to contain infectious agents for humans, a CDC-issued import permit is not required for importation. Information, guidance documents, and resource materials may be found on the following website: http://www.cdc.gov/od/eaipp/. The application may be obtained from http://www.cdc.gov/od/eaipp/importApplication/.

BAP.01704 Chain-of-Custody Procedures — Phase II

The biorepository follows a defined process for chain-of-custody specimen collection, accessioning, and handling.

NOTE: If specimens are referred to another laboratory, the collection site must follow chain-of-custody instructions provided by the referral laboratory.

REFERENCES

1) Clinical and Laboratory Standards Institute. *Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline.* 3rd ed. CLSI Document C52-ED3. Clinical and Laboratory Standards Institute, Wayne, PA; 2017.

BAP.01706 Biospecimen Chain of Custody **Phase II**

The biorepository has a system to track biospecimen chain of custody.

NOTE: Chain of custody is used to maintain the integrity of the biospecimen by providing records of the control, transfer, and analysis of biospecimens.

The intent of this requirement is to have a system in place to ensure adequate records of the "life history" of the biospecimen. Chain of custody provides a traceable record that guarantees unbroken control over biospecimens and its containers from initial collection to final disposition. This is achieved with accurate and effective labeling, tracking and reporting.

Chain of custody requires that from the moment the biospecimen is received every transfer between departments be recorded.

Evidence of Compliance:

- ✓ Logs or message boards showing specimen movement through biorepository **AND**
- ✓ Work flow diagrams

BAP.01709 Surgical Pathology Specimens Release for Research **Phase II**

A sample of a surgical pathology gross specimen 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. **For laboratories subject to US regulations, formal written authorization is obtained in accordance with the requirements of HIPAA if identifiable patient information is released.**
3. **The biorepository meets other relevant requirements, including but not limited to, the requirements of the institution, the directives of any applicable institutional review board (IRB) or similar entity, and national, federal, state (or provincial), and local laws and regulations.**
4. **De-identified/anonymized sample of a surgical pathology gross specimen may be submitted for research if a waiver of consent has been obtained.**

BAP.01712 De-identification for Research **Phase II**

For specimens that are released for research, the biorepository follows a defined process for de-identifying/blinding or anonymizing specimens without compromise to research-related demographic information, when required.

BAP.01715 Coding **Phase II**

There is a defined coding system for sample identification.

BAP.01718 Participation/Donor Informed Consent **Phase II**

For specimens that are released to a biorepository, appropriate participant/donor informed consent is secured.

NOTE: This is not applicable when specimens are obtained under waiver of consent.

BAP.01721 IRB Release **Phase II**

For specimens that are released to a biorepository, an appropriate IRB release is in place.

BAP.01722 Specimen Handling **Phase II**

Specimens are handled in a manner that prevents specimen loss, alteration, or contamination.

NOTE: Because of the high sensitivity and potential for contamination in molecular testing involving amplification of DNA, the laboratory must be alert to the possibility of commingled specimens. An example of a potentially commingled specimen is one that is received after the specimen container was entered by a sampling device that enters multiple samples, albeit with rinses in between specimens. If such samples must be tested by molecular methods, the results should be interpreted with caution, considering the potential for contamination.

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.
2) Compton CC, Robb JA, Anderson MW, et al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Pathol Lab Med.* 2019;143(11)1346-63.

BAP.01724 Specimen Collection/Handling Protocol **Phase II**

Collection, processing, and storage times are recorded, as required by the biorepository protocol in place at the time of biospecimen procurement.

NOTE: Time is kept to a minimum between when a specimen is removed from its site of origin and when it is preserved (eg, fixed, cooled, or frozen).

BAP.01727 Pre-Analytic Variables **Phase II**

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

NOTE: While intended use of specimens is not always known, the specimens 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 optimized for the greatest number of molecular analytes/analysis platforms.

REFERENCES
1) Standard Preanalytical Coding for Biospecimens: Defining the Sample PREanalytical Code, Betsou, *et al*, *Cancer Epidemiol Biomarkers Prev* April 2010 19; 1004.

BAP.01730 Processing/Preservation - Solid Specimens **Phase II**

The key elements related to the processing and preservation of solid specimens are recorded in the biospecimen QA report, when available.

NOTE: 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 tumor/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 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 biorepository for all biospecimen collections, especially those that were procured before recent best practices for biorepositories were published.

BAP.01733 Processing/Preservation - Fluid Biospecimens **Phase II**

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

NOTE: Key elements may include, but are not limited to:

1. *Collection preservative*
2. *Original volume received*
3. *Temperature and duration of specimen 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. *Tumor content (%), if applicable*

The 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 biorepository encourage their source sites to gather/provide as much information as possible.

REFERENCES
1) Standard Preanalytical Coding for Biospecimens: Defining the Sample PREanalytical Code, Betsou, *et al*, *Cancer Epidemiol Biomarkers Prev* April 2010 19; 1004.

BAP.01734 Specimen Processing/Storage **Phase II**

Specimens are processed promptly or stored appropriately to minimize degradation of nucleic acids.

REFERENCES
1) Farkas DH, Kaul KL, Wiedbrauk DL, *et al*. Specimen Collection and Storage for Diagnostic Molecular Pathology Investigation. *Arch Pathol Lab Med*. 1996;120:591-596
2) Kiechle FL, Kaul KL, Farkas DH. Mitochondrial Disorders: Methods and Specimen Selection for Diagnostic Molecular Pathology. *Arch Pathol Lab Med*. 1996;120:597-603
3) Farkas DH, Drevon AM, Kiechle FL, *et al*. Specimen Stability for DNA-based Diagnostic Testing. *Diag Molec Pathol*. 1996;5(4):227-235
4) Rainen L, *et al.* Stabilization of mRNA expression in whole blood samples. *Clin Chem.* 2002;48:1883-1890
5) Pahl A, Brune K. Stabilization of gene expression profiles in blood after phlebotomy. *Clin Chem.* 2002;48:2251-2253
6) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods.* 2nd ed. CLSI guideline MM13. Clinical and Laboratory Standards Institute, Wayne, PA; 2020.
7) Compton CC, Robb JA, Anderson MW, et al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Pathol Lab Med.* 2019;143(11)1346-63.

BAP.01736 Specimen Storage Conditions **Phase II**

The biorepository has defined storage conditions for the different specimens handled and a protocol for the return of each specimen type to storage after issuance for use, as appropriate.

Evidence of Compliance:

- ✓ Records of storage conditions **AND**
- ✓ Records of return of specimens to storage

BAP.01739 Specimen Storage Temperature **Phase II**

Records show that specimens were stored at the protocol-required temperatures.

NOTE: Storage of specimens must be appropriate for the type of specimens and its means of preservation. Failure to adhere to requirements could result in a specimen not being suitable for the purpose for which it was intended.

INFORMED CONSENT AND INSTITUTIONAL REVIEW BOARD

This section applies to human subjects research only.

Inspector Instructions:

READ	• Privacy and confidentiality policies and procedures • Informed consent criteria • Waiver of Consent criteria
ASK	• What action is taken if a sample is received without the records of proper informed consent? • How do you ensure that the proposed use of human tissue is consistent with the informed consent?
DISCOVER	• Select a specimen in storage and review that the proper informed consent records are complete

BAP.01742 Informed Consent Criteria **Phase II**

The biorepository ensures that the proposed uses of human tissue with or without data shared for research purposes are consistent with the informed consent and scope of services, when applicable.

NOTE: There are some instances when informed consent and/or waiver of consent are not applicable (eg, non-human specimens).

BAP.01745 Required Approval(s) Records **Phase II**

When human specimens are to be collected, all of the required approvals (eg, IRB or other ethics committees) have been recorded and appropriate patient consent processes are complete.

NOTE: The only exception to this is when there has been a waiver of consent.

BAP.01748 Informed Consent Records **Phase II**

Informed consent records are obtained for the collection, storage, distribution, and use of identifiable human specimens and data.

NOTE: The only exception to this is when there has been a waiver of consent.

BAP.01751 Waiver of Consent **Phase II**

A waiver of consent, in accordance with applicable laws and/or requirement and approved by the institution's ethics review committee, is obtained when informed consent is not obtained/required.

BAP.01754 Biospecimen/Data Usage **Phase II**

The biorepository ensures that the proposed use of the biospecimen/data is within the guidelines of the project and of the informed consent, when applicable.

BAP.01757 Privacy/Confidentiality **Phase II**

The biorepository ensures the privacy and confidentiality of patients/donors and their data.

BAP.01760 Procedures Available for Review **Phase II**

The biorepository's procedures for human specimen collection, processing, storage, and dissemination are available for ethics committee and/or IRB review, as needed.

SOURCE FACILITY

If the biorepository is not the source, the requirements under the Source Facility section are not applicable.

Inspector Instructions:

READ	• Sampling of protocol procedures • Sampling of record content when the biorepository is the sponsor • Sampling of source facility procedures • Sampling of collection site audits when the biorepository is the sponsor
OBSERVE	• The QC process for specimens received from collection sites not under the control of the biorepository
ASK	• How do you ensure the quality of specimens from collection sites not under the control of the biorepository? • When the biorepository is the collection sponsor, who conducts the audits, how are the audits recorded, and who ensures corrective action is appropriate when needed?

BAP.01763 Biorepository/Source Facility Responsibilities **Phase II**

The responsibilities between the facility(ies) and its sponsor are clearly defined in writing, reviewed by the biorepository within the last 24 months, and available during the inspection.

BAP.01766 Protocols **Phase II**

The biorepository follows protocols describing methods for participant identification, participant education, specimen collection and labeling, specimen preservation, and conditions for transportation, and storage before testing, consistent with good clinical practice and good laboratory practice, when applicable.

NOTE: All specimens must be labeled with a unique identifier and sufficient quality control practices must be in place to ensure appropriate linkage of that identifier to the participant. Protocols may be separate documents or included in the procedure manual.

BAP.01769 Source Facility Procedure Manual **Phase II**

The procedure manual is comprehensive and includes information on the following elements, as applicable to the scope of the biorepository.

1. **Informed consent**
2. **Equipment monitoring, calibration, maintenance, and repair**
3. **Control of biospecimen collection supplies (disposable and reagents)**
4. **Biospecimen identification and labeling conventions**
5. **Biospecimen collection and processing methods**
6. **Storage and retrieval**
7. **Shipping and receiving**
8. **Laboratory tests performed in-house including biospecimen QC**
9. **Biospecimen data collection and management (informatics)**
10. **Biosafety**
11. **Training**
12. **Security**

NOTE: A copy of the procedure manual would enable the sponsor to ensure that best practices are being followed.

BAP.01772 Off-site Contact Information **Phase I**

Contact information for off-site collection sites is readily available to personnel at all times to resolve discrepancies or other issues that may arise.

NOTE: This may include active phone numbers, email, etc.

SPONSOR FACILITY

The requirements under the Sponsor Facility section are applicable only if the biorepository is the sponsor.

If the biorepository initiated the collection, the biorepository is the sponsor and the following requirements are applicable. If an entity other than the biorepository initiated the collection, the biorepository is not the sponsor and the requirements below do not apply to those collections. It is possible that the biorepository will be the sponsor for some collections, but not others.

BAP.01775 Registration/License **Phase I**

If the biorepository is the primary requestor/sponsor for the specimen collection, the biorepository ensures that all source facilities are registered, licensed, and accredited as required by national, federal, state (or provincial), and local regulations, and appropriate for the study.

BAP.01778 Record Content for Sponsor Facility **Phase II**

If the biorepository is the sponsor for collections, the biorepository keeps a record of the following for each contributing site, as applicable.

1. **Principal investigator (PI)**
2. **Protocol number**
3. **Protocol title**
4. **Protocol version date**
5. **Informed consent**
6. **Informed consent version date**
7. **Study expiration date**
8. **Approval of the above by Institutional Review Board**
9. **Principal investigator signature for Protocol and version against approval letter**
10. **Signature and delegation list for employees responsible for obtaining consent from patients, sample transport, clinical data, sample processing, manifesting of samples, and coordination of shipments**
11. **Curriculum vitae of principle investigator**
12. **License or diploma (for non-US sites) of PI**
13. **Governmental approval as required for each participating site**

BAP.01781 Off-site Collection Sites QC **Phase I**

The biorepository monitors the quality of specimens and associated records received from off-site collection sites not under the direct control of the sponsor facility following a defined process approved by the biorepository director.

NOTE: The sponsor facility should perform an annual review of off-site collection QA/QC data as part of their quality management system.

BAP.01784 Contributing Sites Audits **Phase II**

If the biorepository is the sponsor for collections, the biorepository performs audits of contributing sites at defined frequencies.

NOTE: The scope of the audit is defined by the activities of the contributing facility. The type of audit (onsite, paper, etc.) and the timeframe are determined by the biorepository.

The audit is part of the sponsor facility's QC procedures to ensure contributing/collection sites are following protocols and procedures appropriately. Records required to ensure protocols and procedures are being followed should be checked and those checks recorded as part of the audit. CAP inspectors should be able to understand from audit records that policies and procedures are being followed by the contributing/collection site and monitored by the sponsor biorepository. If the contributing/collection sites are located outside of the United States, audit records should be in English and also in the official native language(s) of the contributing/ collection site country.

Evidence of Compliance:

- ✓ Written results of each audit **AND**
- ✓ Corrective action plans for issues of non-compliance and follow up on each plan

BIOSPECIMEN PROCESSING AND QUALITY

BIOSPECIMEN QUALITY

The biorepository must have a written quality assessment process applicable to the scope of activities performed. This quality process should be capable of detecting, reducing and correcting any deviation from acceptable standards set by the biorepository. Examples may include enrollment in a proficiency testing program or using sets of testing control materials to check the biorepository samples over time.

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

Inspector Instructions:

	• Sampling of policies and procedures for specimen processing including aliquoting, relabeling, and specimen retrieval • Sampling of records for the assessment of the quality of stored specimens • Specimen rejection criteria policy and records of rejection
	• Specimen processing area for clean environment • Aliquot sizes of specimens • Specimen identifiers • Specimen storage conditions during sample processing • Tracking of samples as they move from one station to another • Sampling of reagents (expiration date)
	• How does your biorepository maintain and track temperature excursion information? • Explain your quality assessment process for stored specimens • How is the risk of specimen misidentification monitored and the process improved? • What do you do if the sample size is too small relative to the requirements or it does not meet researchers' needs?
	• Follow a tissue sample released for research from the pathologist to storage, verifying specimen identification throughout the process • Select several specimens and follow their tracking throughout the life of the specimen, including from parent to child, etc.

BAP.01800 Quality Assessment of Stored Specimens **Phase II**

The biorepository periodically assesses the quality of stored specimens for each class of biospecimens in the biorepository.

NOTE: The frequency of the checks may be determined by the following:

1. *Type of specimens being stored*
2. *Preservation method*
3. *Turnover of the material*

The form and frequency for the periodic assessment is to be defined by the biorepository. The assessment may take a variety of forms including direct observation of materials, sampling, integrity of records, enrollment in proficiency testing, or other alternate performance assessment.

The quality of stored specimens may be assessed at the time of disbursement.

Evidence of Compliance:
- ✓ Records of inventory sampling **OR**
- ✓ Records of unsuitable specimens by collection, as applicable **OR**
- ✓ Records of inventory QA/QC processes **OR**
- ✓ Assessment from researchers using the specimens

BAP.01900 Aliquot Size **Phase II**

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

NOTE: 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:
- ✓ Records of sample size stated in protocols

BAP.02000 Temperature Excursions **Phase II**

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

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

BAP.02100 Clean Environment **Phase II**

Specimens are processed in a clean environment, when required.

*NOTE**:** RNA is particularly sensitive to RNases that may be present on tools and surfaces that have not been sterilized.*

BAP.02200 Biological Safety Cabinet **Phase II**

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

BAP.02300 Safe Handling of Specimens for Infectious Diseases **Phase II**

The biorepository follows a defined process for receipt and management of potentially infectious material that includes application of standard precautions.

NOTE: Elements of the procedure must include proper handling of specimens for biohazard protection. The procedure may include information about prior testing for infectious hazards.

REFERENCES
1) OSHA regulation 29CFR1910.1020.
2) Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline.* 4th ed. CLSI document M29-A4. Clinical and Laboratory Standards Institute, Wayne, PA; 2014.

BAP.02500 Histological Characteristic Review **Phase II**

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

NOTE: Histologic review of banked solid tissue biospecimens is important for the following reasons: 1) the review of banked solid tissue biospecimens ensures that well-annotated, high quality biospecimens will be utilized in downstream testing; and 2) the review of banked solid tissue biospecimens may be used to confirm diagnostic findings. The timing of the pathologists' histologic review is at the discretion of the biorepository director. There may be situations where the sponsor of the collection or the user arranges for pathology review outside of the biorepository. This should be recorded by the biorepository.

BAP.02600 Specimen Identity **Phase II**

The identity of every specimen is maintained through each step of processing and slide preparation.

NOTE: An unambiguous system of unique specimen identification coupled with a legible, sequential container labeling system that withstands exposure to anticipated reagents and temperature extremes are essential to fulfill 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 specimen and how it will be used/stored.

BAP.02700 Misidentification Risk **Phase II**

The biorepository monitors the risk of misidentification and subjects the related processes to continual process improvement.

NOTE: The 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) QC and application of a barcode or other identifier, and 3) record of the number of sample derivatives prepared.

Evidence of Compliance:

- ✓ Occurrence records/error logs demonstrating appropriate review and follow-up of significant errors and patterns of errors in identification and other processes

BAP.02800 Unique Identifier **Phase II**

Each specimen received into the biorepository receives a unique identifier.

BAP.02900 Specimen Tracking Mechanism **Phase II**

The biorepository maintains and tracks the identity of every specimen throughout the life of the specimen and its derivatives (eg, parent to children to grandchildren, etc.).

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

BAP.03000 Specimen Rejection Criteria **Phase II**

The biorepository follows defined criteria for specimen condition exceptions to be recorded and communicated to researchers regarding conditions that may impact research results.

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

BAP.03100 Relabeling **Phase II**

The biorepository has a defined process for relabeling of a biospecimen and/or aliquots.

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

Evidence of Compliance:
- ✓ Records, including reason for relabeling

BAP.03700 Retrieval Procedures **Phase II**

All specimen retrieval procedures ensure specimen integrity.

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

BAP.03800 Paraffin Embedding and/or Fixation QC **Phase II**

The biorepository has a process for paraffin embedding and/or fixation that includes quality checks at a defined frequency (eg, 24 hours/48 hours).

NOTE: This requirement applies only to biorepositories that perform their own fixation and embedding and are not a part of a CAP-accredited laboratory.

Evidence of Compliance:
- ✓ Records of quality checks

DNA/RNA EXTRACTION/AMPLIFICATION

Inspector Instructions:

	• Sampling of nucleic acid extraction and amplification policies and procedures • Sampling of nucleic acid measurement records • Records of nucleic acid integrity and purity assessment • Records of internal controls • Sampling of specimen processing, handling, aliquoting, and storage policies and procedures
	• Nucleic acid amplification procedures for proper physical containment and procedural controls to prevent carryover • Observe quantitation and quality control assessments

	• How is adequacy of nucleic acid isolation and preparation evaluated? How often is this done? • How does your laboratory ensure RNase-free conditions are maintained?
	• Follow a sample from extraction through storage

BAP.04500 Specimen Identification **Phase II**

There is a system to positively identify all participant specimens, specimen types, and aliquots through all phases of the analysis, including specimen receipt, nucleic acid extraction, nucleic acid quantification, hybridization, detection, preparation of records, and storage.

BAP.04525 Extracted Nucleic Acid Specimens **Phase II**

If extracted nucleic acid is accepted as a specimen type, the biorepository ensures that isolation of nucleic acids for clinical testing occurs in a CLIA-certified laboratory or a laboratory meeting equivalent requirements as determined by the CAP and/or the CMS. This policy is clearly displayed to ordering clients.

NOTE: All clinical testing must be performed in CLIA-certified laboratories or laboratories meeting equivalent requirements (refer to GEN.41350). This includes all components of testing that may impact the quality of the test result, including isolation or extraction of nucleic acids. Laboratories may choose to have referring clients formally attest that extracted nucleic acid submitted for testing has been isolated or extracted in an appropriately qualified laboratory.

Evidence of Compliance:

✓ Written statement on the test requisition, test catalog, or policy available to referring clients stating that the laboratory only accepts isolated or extracted nucleic acids for which extraction or isolation is performed in an appropriately qualified laboratory

BAP.04700 Nucleic Acid Extraction/Isolation/Purification **Phase II**

Nucleic acids are extracted, isolated, and purified by methods reported in the literature, by an established commercially available kit or instrument, or by a validated method developed by the laboratory.

NOTE: The method should be assessed for its suitability for each source type that requires extraction. Any modification to established procedures must be recorded, as well as variations to procedures depending on anatomic site and biospecimen preservation format (eg, fresh frozen vs. OCT-embedded). Extraction procedures may combine purification or isolation of nucleic acids according to the level of purity needed for downstream applications.

Evidence of Compliance:

✓ Records to support nucleic acid extraction/isolation/purification is performed by a validated method

REFERENCES

1) Clinical and Laboratory Standards Institute. *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI Document MM19-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
2) Clinical and Laboratory Standards Institute. *Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications. 1st ed*. CLSI guideline MM21-ED1. Clinical and Laboratory Standards Institute, Wayne, PA, 2015.

BAP.04800 Nucleic Acid Quantity and Quality Determination **Phase II**

The quantity and quality of nucleic acids are determined, when appropriate.

NOTE: The quantity and quality of nucleic acids (DNA or RNA) must be measured prior to use in a procedure whose success depends on accurately determining the quantity, concentration, integrity, and/or purity of the nucleic acids. Techniques commonly used to assess nucleic acid quantity and/or quality include electrophoresis, UV/VIS spectrophotometry, and fluorescence spectroscopy.

Standard measure for DNA purity is A260/280 ratio of 1.6 to 2.0. Values less than 1.6 are indicative of protein contamination and values of >2.0 are indicative of RNA contamination, RNA should have A260/280 ratio of greater than 2.0. Analytical measures of nucleic acids include, but are not limited to: A260/280 spectrophotometric ratio, RNA-specific measures, double-stranded DNA (dsDNA), or integrity by agarose gel electrophoresis. RNA integrity assessments should be determined if such a quality indicator would exclude samples from specific downstream methodologies.

RNA in specimens is highly labile because RNase is ubiquitous and difficult to inhibit. For human RNA targets, RNA quality must be assessed. However, depending on the target, it may not be necessary for all specimens to be assessed for RNA quality. RNA quality is not assessed, for example, for many types of viral RNA targets; however, the false negative rate must be recorded.

Evidence of Compliance:

✓ Records of nucleic acid quantity and/or quality determination

REFERENCES

1) Tsui NBY, Ng EKO, Lo YMD. Stability of Endogenous and Added RNA in Blood Specimens, Serum and Plasma. *Clin Chem* 48:1647-1653, 2002
2) Farrell R. Gel electrophoresis based assessment of cellular RNA quality may also be used (RNA Isolation Strategies). In: RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, 1998
3) Clinical and Laboratory Standards Institute. *Diagnostic Nucleic Acid Microarrays: Approved Guideline*; CLSI Document MM12-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2006.

BAP.04900 Human/Non-Human DNA **Phase I**

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

BAP.05100 Neoplastic Cell Content **Phase II**

For paraffin-embedded tumor specimens from which DNA or RNA is extracted for analysis (eg, microsatellite instability, *KRAS* or *KIT* analysis), there is a record of histological assessment of neoplastic cell content.

NOTE: In addition to confirming the presence or absence of neoplastic cells by a qualified pathologist, it may be necessary for some assays to estimate and consider neoplastic cellularity in relation to the lower limit of detection of the assay.

A corresponding H&E section from the same tissue block used for nucleic acid extraction may be used to assess sample adequacy. Alternatively, a stain such as toluidine blue may be used to stain the slide that is being used for nucleic acid extraction. When assessment of sample adequacy is performed outside of the testing laboratory, a record of such assessment must accompany the sample.

This requirement is applicable to all molecular methods for the detection of sequence variants (eg, Sanger sequencing, NGS, PCR).

BAP.05125 Ribonuclease-Free Conditions **Phase I**

Ribonuclease-free conditions are maintained for all assays that detect RNA or use an RNA probe.

NOTE: RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.

Evidence of Compliance:

- ✓ Records that RNase-free conditions are maintained (ie, wipe test in event of contamination incident) with corrective action if conditions are not met

REFERENCES
1) Gulley ML, *et al*. Guidelines for interpreting EBER *in situ* hybridization and LMPI immunohistochemical tests for detecting Epstein-Barr virus in Hodgkin lymphoma. *Am J Clin Pathol. 2002*;117:259-267

BAP.05200 Carryover - Nucleic Acid Amplification **Phase II**

Nucleic acid amplification procedures (eg, PCR) use appropriate physical containment and procedural controls to minimize carryover (false positive results).

NOTE: 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 minimize aerosolization. Enzymatic destruction of amplification products is often helpful, as is real-time measurement of products to avoid manual manipulation of amplification products.

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A. Clinical and Laboratory Standards Institute, Wayne, PA, 2011.

BAP.05300 Internal Controls Nucleic Acid Amplification **Phase II**

In 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.

NOTE: The facility 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 specimen 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.

Evidence of Compliance:

- ✓ Records of assay validation and monitoring statistics for test result trends

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.

Inspector Instructions:

	• Sampling of cell fractionation policies and procedures
	• System to maintain the identification of the derivatives to the parent biospecimen • Cell fractionation process follows the steps in the procedure
	• How is the quality of the cell fractionation process ensured?

BAP.05303 Specimen Identification **Phase II**

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

NOTE: Records of specimen 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.

BAP.05306 Cell Fractionation **Phase II**

The biorepository follows a defined process for all steps in the cell fractionation process.

NOTE: Deviations from the manufacturer instructions must be validated and recorded.

BAP.05309 Quality Control/Quality Assurance **Phase II**

Biorepositories performing cell fractionation record all quality control and quality assurance measures.

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

CELL AND TISSUE CULTURE

Inspector Instructions:

	• Sampling of cell and tissue culture policies and procedures • Sampling of records of microbial contamination and other cell line testing
	• How does the biorepository ensure that the quality of cell lines is maintained? • How do you define and monitor maximum cell line passage?

BAP.05312 Culturing Environment **Phase II**

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

BAP.05315 Cell Line Loss **Phase I**

There is a system in place to prevent loss of the cell line in case of culture failure, contamination or other problems.

NOTE: Potential systems include duplicate or independently established cultures, harvesting in duplicate or at different times, or other control processes.

BAP.05318 Monitoring of Passage Numbers **Phase I**

The biorepository defines the maximum number of passages for each cell line by either reference or laboratory method.

NOTE: 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:
- ✓ Records of tracking of cell line passages **OR**
- ✓ Records of growth curves

BAP.05321 Testing for Microbial Contamination **Phase I**

The biorepository tests cell lines for microbial contamination at intervals defined by the biorepository director.

Evidence of Compliance:
- ✓ Records detailing the type(s) of tests and test outcomes

BAP.05324 Testing for Functionality and/or Unique Characteristics **Phase I**

Cell lines are tested for functionality or unique characteristics.

NOTE: Such testing may be performed by analyzing aspects of the phenotype (eg, expression patterns), genotype or morphology. The biorepository should have a policy 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

BAP.05327 Recording of Failures **Phase I**

Culture failures are recorded.

NOTE: Records must indicate corrective actions.

Evidence of Compliance:

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

HISTOLOGY

Inspector Instructions:

READ	• Sampling of histology policies and procedures • Sampling of specimen preparation records • Sampling of histology QC policies and procedures • Sampling of QC records (histochemical) • Sampling of records of daily review of histologic slide quality • Sampling of immunofluorescence QC records • Sampling of IHC policies and procedures • Sampling of new antibody validation/verification records • Sampling of new reagent/shipment confirmation of acceptability records • Sampling of antibody QC records • Sampling of buffer pH records • Sampling of batch control records
OBSERVE	• Sampling of tissue blocks (identification) • Sampling of slides (labeling, quality)
ASK	• How does the histology section ensure specimen identity throughout processing? • How does your biorepository validate/verify new antibodies? • How does your biorepository confirm the acceptability of new reagent lots? • How does your biorepository distinguish non-specific false-positive staining from endogenous biotin?
DISCOVER	• If problems are identified during the review of histology procedures, further evaluate the responses, corrective actions and resolutions • Select a representative specimen and follow from receipt in the department through accessioning, grossing, processing, time reported and availability in the LIS

BAP.05330 Specimen Preparation Records **Phase I**

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

BAP.05332 Cross-Contamination - Histology **Phase II**

The biorepository prevents cross-contamination of specimens in the histology section.

NOTE: The process must address steps to prevent cross-contamination during the various phases of tissue handling including: processing, embedding, microtomy, and slide preparation. Problems with cross-contamination must be addressed in the biorepository quality management system.

Instruments must be clean and well-maintained (eg, tissue processors, embedding centers, dispensers, floatation baths, staining and coverslipping equipment).

At the embedding station, cleaning or wiping of forceps between cases is required. Only one cassette should be handled at a time.

For microtomy, there must be a clear process for handling of blocks and labeling of slides to prevent specimen mix-ups. Floatation baths require periodic water changes or blotting of the water surface so that sections from one patient block are not inadvertently carried over to another case or block (so-called "floaters" or "extraneous tissue").

REFERENCES
1) Lott R, Tunnicliffe J, Sheppard E, et al. Practical Guide to Specimen Handling in Surgical Pathology. College of American Pathologists, April 2020. Accessed May 4, 2022. Available at https://documents.cap.org/documents/practical-guide-specimen-handling.pdf.
2) Gephardt GN, Zarbo RJ. Extraneous tissue in surgical pathology: A College of American Pathologists study of 275 laboratories. *Arch Pathol Lab Med.* 1996; 120:1009-14.

BAP.05336 Special Stains/Studies **Phase II**

For special stains, including histochemical stains, and studies using immunologic and ISH methodology, positive and negative controls are verified and recorded as acceptable prior to or concurrent with the reporting of patient results and records retained.

NOTE: Controls must be verified and recorded as acceptable by a pathologist or designee (provided the designee meets high complexity testing qualifications).

Positive tissue controls must contain the component specific to the special stain that is being applied to the specimen.

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 laboratory director following appropriate validation. The Centers for Medicare and Medicaid Services (CMS) recognizes the use of polymer-based detection systems (biotin free) may preclude the use of a negative reagent control. However, there have been no changes to the histopathology regulations. The CMS will be looking into an alternate QC method for these types of stains.

If interpretation of the special stain or study is performed by a different laboratory, there must be a procedure for the laboratory performing the stain or study to verify the acceptability of the controls before transfer, if the controls are not sent with the patient slides (regardless of the outside laboratory's accrediting organization). Records of this verification must be readily available to the laboratory performing the interpretation.

Evidence of Compliance:

✓ Records for verification of control acceptability (prior to completion of associated cases)

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7166 [42CFR493.1256(e)(2)] and [42CFR493.1273(a)]

BAP.05337 Paraffin Microtomy **Phase II**

The appropriate thickness of paraffin embedded tissue for various tissue types and procedures is defined.

NOTE: Paraffin embedded sections are routinely sectioned at 4-5 microns. Some tissues (eg, renal biopsy) may require thinner sections, while some special stain techniques (eg, Congo red stain) may require thicker sections. Use of the recommendations in the table below is at the discretion of the laboratory director.

Tissue	Thickness
Routine Paraffin	**4 to 5 microns**
Renal Sections	**1 to 3 microns**
Bone Marrow	**2 to 3 microns**
Nerve histochemical staining	**6 to 15 microns**
Amyloid demonstration	**6 to 12 microns**

BAP.05338 Slide Quality **Phase II**

Slides are of sufficient quality for diagnosis.

NOTE: Histopathology slides must be of adequate technical quality to be diagnostically useful. Criteria to evaluate include adequate tissue fixation, processing, thickness of sections, absence of interfering tissue folds and tears, and good staining technique and cover slipping. For hematoxylin and eosin and other routine stains, the patient slide serves as the internal control to ensure adequate staining technique. The sections must be cut from sufficient depth in the block to include the entire tissue plane.

BAP.05342 Specimen Modification **Phase II**

If the biorepository performs immunohistochemical staining on specimens other than formalin-fixed, paraffin-embedded tissue, the written procedure defines appropriate modifications, if any, for specimen types.

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

REFERENCES
1) Perkins SL, Kjeldsberg CR. Immunophenotyping of lymphomas and leukemias in paraffin-embedded tissues. *Am J Clin Pathol* 1993:99(4):362-373
2) Clinical and Laboratory Standards Institute. *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline.* 2nd ed. CLSI Document I/LA28-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.

BAP.05345 Buffer pH **Phase II**

The pH of the buffers used in immunohistochemistry is monitored at defined intervals.

NOTE: pH must be tested when a new batch is prepared or received.

Evidence of Compliance:
- ✓ Records of initial and subsequent QC on each buffer

BAP.05348 QC - Antibodies **Phase II**

Positive tissue controls are used for each antibody.

NOTE: 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 recorded, either in internal 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 specimen type as the donor test specimen (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 donor specimen. However, for most biorepositories, it is not practical to maintain separate positive control samples to cover every possible combination of fixation, processing and specimen type. Thus, it is reasonable for a biorepository to maintain a bank of formalin-fixed tissue samples as its positive controls; these controls can be used for donor specimens that are of different type, or fixed/processed differently, providing that the biorepository can show that these donor specimens exhibit equivalent immunoreactivity. This can be accomplished by parallel testing a small panel of common markers to show that specimens of different type, or processed in a different way (eg, alcohol-fixed cytology specimens, 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 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 donor tissue is optimal practice because it helps identify failure to apply primary antibody or other critical reagent to the donor 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 scrutinized 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 failure to identify assays of insufficient sensitivity, leading to false-negative results.

Evidence of Compliance:

- ✓ Donor reports or worksheet with control results **AND**
- ✓ Immunohistochemical-stained slides with positive tissue controls

REFERENCES

1) O'Leary TJ. Standardization in immunohistochemistry. *Appl Immunohistochem Molecul Morphol* 2001;9:3-8
2) Clinical and Laboratory Standards Institute. *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline - Second Edition*. CLSI document I/LA28-A2. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 2011.
3) Allen M. Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med*. 2016;140(9):893-898.
4) Cheung CC, D'Arrigo C, Dietel M, et al; From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path). Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4: Tissue Tools for Quality Assurance in Immunohistochemistry. *Appl Immunohistochem Mol Morphol.* 2017;25(4):227-230.
5) Cheung CC, Taylor CR, Torlakovic EE. An Audit of Failed Immunohistochemical Slides in a Clinical Laboratory: The Role of On-Slide Controls. *Appl Immunohistochem Mol Morphol.* 2017;25(5):308-312.
6) Torlakovic EE, Nielsen S, Francis G, et al. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. *Appl Immunohistochem Mol Morphol.* 2015;23(1):1-18.

BAP.05351 QC - Antibodies **Phase II**

Appropriate negative controls are used.

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

For biorepositories using older biotin-based detection systems, it is important to use a negative reagent control to assess nonspecific 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 specimen 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 biorepository director must determine which cases will have only one negative reagent control, and this must be specified in the department's procedure 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 biorepository director, 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 labeling.

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. *Multitissue blocks. These can provide simultaneous positive and negative tissue controls, and are considered “good 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 multitissue blocks) must be specified in the biorepository manual.

Multitissue blocks or tissue microarray (TMA) 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 of the sensitivity and specificity of every stain, particularly when mounted on the same slide as the donor tissue. When the components are chosen appropriately, multitissue blocks may be used for many different primary antibodies, decreasing the number of different control blocks needed by the biorepository. Multitissue 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:

- ✓ Donor reports or worksheet with control results **AND**
- ✓ Immunohistochemical-stained slides with appropriate negative controls

REFERENCES

1) Leong AS-Y, Cooper K, Leong FJW-M. Manual of Diagnostic Antibodies for Immunohistology. 2nd ed. London: Greenwich Medical Media; 2003
2) Dabbs DJ, ed. Diagnostic Immunohistochemistry: Theranostic and Genomic Applications. Philadelphia: Saunders/Elsevier; 2010
3) Burry RW. Specificity controls for immunocytochemical methods. *J Histochem Cytochem* 2000;48:163-166
4) Weirauch M. Multitissue control block for immunohistochemistry. *Lab Med.* 1999;30:448-449
5) Miller RT. Multitumor "sandwich" blocks in immunohistochemistry. Simplified method and preparation and practical uses. *Appl Immunohistochem* 1993;1: 156-159
6) Chan JKC, Wong CSC, Ku WT, Kwan MY. Reflections on the use of controls in immunohistochemistry and proposal for application of a multitissue spring-roll control block. *Ann Diagn Pathol* 2000;4: 329-336
7) Clinical and Laboratory Standards Institute. *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline - Second Edition*. CLSI document I/LA28-A2. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 2011.
8) Torlakovic EE, Francis G, Garratt J, et al. International Ad Hoc Expert Panel. Standardization of negative controls in diagnostic immunohistochemistry: recommendations from the international ad hoc expert panel. *Appl Immunohistochem Mol Morphol.* 2014;22(4):241-52.

BAP.05354 Endogenous Biotin — Phase I

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

NOTE: Biotin is a coenzyme present in mitochondria, and cells that have abundant mitochondria such as hepatocytes, kidney tubules and many tumors (particularly carcinomas) are rich in endogenous biotin. Biotin-rich intranuclear inclusions are also seen in gestational endometrium and in some tumors 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 artifact is often localized to tumor 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.

REFERENCES

1) Miller RT, Kubier P. Blocking of endogenous avidin-binding activity in immunohistochemistry: the use of egg whites. *Appl Immunohistochem* 1997; 5: 63-66
2) Miller RT, Kubier P, Reynolds B, Henry T. Blocking of endogenous avidin-binding activity in immunohistochemistry: the use of skim milk as an economical and effective substitute for commercial biotin solutions. *Appl Immunohistochem & Molec Morphol* 1999;7:63-65
3) Clinical and Laboratory Standards Institute. *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline.* 2nd ed. CLSI Document I/LA28-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
4) Allen M. Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med*. 2016;140(9):893-898.

BAP.05357 Control Slide Review — Phase II

The biorepository director or designee reviews all control slides each day specimens are stained.

NOTE: Records of this review must be retained and clearly show that positive and negative controls for all antibodies stain appropriately. Control records must be retained for two years.

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

Evidence of Compliance:

✓ Records of worksheets with control results

REFERENCES
1) Shellhorn N. IHC troubleshooting tips. Advance/Lab. 2000;9(1):33-37

BAP.05360 Antibody Validation/Verification — Phase II

The biorepository has records of validation/verification of new antibodies, prior to sample characterization, including appropriate positive and negative controls.

NOTE: The performance characteristics of each assay must be appropriately validated/verified before being made available as characterization 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 optimized, a panel of tissues must be tested to determine the assay's sensitivity and specificity. The scope of the validation/verification is at the discretion of the biorepository director and will vary with the antibody. For a well-characterized antibody with a limited spectrum of antigenic targets, like chromogranin or prostate specific antigen, the validation/verification 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 characterized and/or has a wide range of reported reactivity, a more extensive validation/verification 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/verification. In the exceptional case where only limited control tissue is available (fewer than 10 cases), the biorepository director should alert the investigator of this limitation.

Evidence of Compliance:

✓ Records of validation/verification, if applicable

REFERENCES
1) Hsi ED. A practical approach for evaluating new antibodies in the clinical immunohistochemistry laboratory. *Arch Pathol Lab Med.* 2001;125:289-294
2) Clinical and Laboratory Standards Institute. *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline - Second Edition*. CLSI document I/LA28-A2. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 2011.
3) Allen M. Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med*. 2016;140(9):893-898.
4) Uhlen M, Bandrowski A, Carr S, et al. A proposal for validation of antibodies. *Nat Methods*. 2016; 13(10):838-7.

BAP.05361 IHC Assay Performance — Phase I

Laboratories confirm assay performance when conditions change that may affect performance.

NOTE: A change in antibody clone requires full revalidation/verification of the assay (equivalent to initial analytic validation/verification - see BAP.05360).

Laboratories must confirm assay performance with at least two known positive and two known negative cases when an existing validated/verified assay has changed in any of the following ways: antibody dilution, antibody vendor (same clone), or the incubation or retrieval times (same method).

A more extensive study to confirm acceptable assay performance in accordance with published guidelines must be performed when any of the following have changed: fixative type, antigen retrieval protocol (eg, change in pH, different buffer, different heat platform), antigen detection system, tissue processing or testing equipment, environmental conditions of testing (eg, laboratory relocation), or laboratory water supply. This study must include a representative sampling of the assays affected by the change and an appropriate number of positive and

negative cases per assay, sufficient to confirm acceptable assay performance. The laboratory director is responsible for determining the extent of the study. The rationale for the assays selected and number of positive and negative cases checked per assay must be recorded.

REFERENCES
1) Fitzgibbons PL, Bradley LA, Fatheree LA, et al. College of American Pathologists Pathology and Laboratory Quality Center. Principles of analytic validation of immunohistochemical assays. Guideline from the Pathology and Laboratory Quality Center. *Arch Pathol Lab Med.* 2014; 138(11):1432-43.

BAP.05363 New Reagent Lot Confirmation of Acceptability **Phase II**

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

NOTE: 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:
✓ Records of confirmation of new reagent lots

BAP.05366 Slide Quality **Phase II**

The immunohistochemical stains produced are of acceptable technical quality.

NOTE: The biorepository director 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.

REFERENCES
1) Shellhorn N. IHC troubleshooting tips. *Advance/Lab*. 2000;9(1):33-37

BAP.05367 QC - Immunofluorescence **Phase II**

For immunofluorescence microscopy, appropriate positive and negative controls are performed.

NOTE: Internal antigens serve as positive controls (eg, IgA in tubular casts, IgG in protein droplets and C3 in blood vessels). When internal positive controls are absent, daily external positive controls are required. Non-reactive elements in the patient specimen may serve as a negative tissue control. A negative reagent control in which the patient tissue is processed in an identical manner to the test specimen, but with the primary antibody omitted, should be performed for each patient test specimen at the discretion of the laboratory director.

Evidence of Compliance:
✓ Records of immunofluorescence QC

REFERENCES
1) Walker PD, *et al.* Practice guidelines for the renal biopsy. *Mod. Pathol.* 2004;17:1555-1563
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 2003(Jan 24): [42CFR493.1273(a)]

BAP.05369 Special Handling of Creutzfeldt-Jakob Disease (CJD) **Phase II**

The biorepository handles tissues from cases of suspected transmissible spongiform encephalopathies (TSE), including Creutzfeldt-Jakob disease (CJD), using procedures that minimize the risk of transmission.

NOTE: Specialized handling instructions and an appropriate process for intra-laboratory communication must be addressed in the written procedure.

Neuropathology tissues from suspected cases of Creutzfeldt-Jakob disease should be treated with formic acid. Paraffin blocks and slides prepared from formic-acid-treated tissue may be handled routinely.

If tissue has not been treated with formic acid, it must be hand-processed and treated as containing potentially transmissible prions. Double gloves must be worn at all times when handling such tissue. All solutions, including water washes, must be collected and treated with equal volumes of fresh undiluted household bleach for 60 minutes before disposal. All scraps of paraffin and unused sections should be collected on a disposable sheet. The microtome may be wiped with bleach or NaOH solution. No special precautions are needed in handling intact glass slides once they have been coverslipped. Broken slides should be decontaminated and discarded. Paraffin blocks should be stored in a bag or box and labeled as infectious. Alternatively, the biorepository may reseal the cut surface of the blocks with paraffin.

REFERENCES

1) Brown W, et al. A simple and effective method for inactivating virus activity in formalin-fixed tissue samples from patients with Creutzfeldt-Jakob disease. *Neurology.* 1990; 40:887-890.
2) Brown P. Guidelines for high risk autopsy cases: special precautions for Creutzfeldt-Jakob disease. In: Hutchins G, ed. Autopsy performance and reporting. Northfield, IL: College of American Pathologists. 1990:68-74.
3) Greenblatt M. Q&A. Northfield, IL: College of American Pathologists. *CAP Today.* 1993(March): 7(3):69-70.
4) Crain BJ. Safety tips for anatomic studies of possible CJD. Northfield, IL: College of American Pathologists. *CAP Today.* 1996(Jan); 10(1):56.
5) Rank JP. How can histotechnologists protect themselves from Creutzfeldt-Jakob disease. *Lab Med.* 1999; 30:305.
6) Nixon RR. Prions and prion diseases. *Lab Med.* 1999; 30:335-338.
7) Collins KA, ed. *Autopsy Performance & Reporting.* 3rd ed. Northfield, IL: CAP Press; 2017.

SPECIALIZED TECHNIQUES

WHOLE SLIDE IMAGING

Inspector Instructions:

READ	• Sampling of training records • System validation records
ASK	• How are the images generated used?

BAP.05375 Whole Slide Imaging User Training **Phase I**

There are records showing that all users of the whole slide imaging system have been trained.

NOTE: Users of the whole slide imaging system include individuals responsible for slide scanning and digital slide quality assessment, as well as pathologists. The training procedure should

include role-specific training, as defined in the approved laboratory procedures. Retraining may be required when significant system changes are made.

Evidence of Compliance:

✓ Records for whole slide image training in personnel files

BAP.05400 System Qualification - Whole Slide Imaging **Phase II**

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

DIGITAL IMAGE ANALYSIS (DIA)

This section applies to laboratories using digital image analysis to evaluate specific features in a tissue section image following enhancement and processing of that image, including but not limited to, IHC, morphometric analysis, and ISH. This checklist section does not apply to laboratories that are imaging slides for manual scoring or review by an individual.

VALIDATION AND CALIBRATION (DIA)

Inspector Instructions:

	• Sampling of validation and calibration policies and procedures • Sampling of validation/calibration records
	• What is your course of action if calibration is unacceptable?

BAP.05410 Preanalytic Testing Phase Validation **Phase II**

There are records showing that the preanalytic phase of the test system has been validated for each assay, including fixation and processing.

NOTE: Applicable requirements under the "Test Method Validation and Verification-Nonwaived Tests" of the All Common Checklist must be followed.

REFERENCES

1) Hipp J, Bauer TW, Bui MM, et al. CAP Pathology Resource Guide: Digital Pathology. Version 5.0(1). Northfield, IL: College of American Pathologists; 2014.

BAP.05415 Calibration **Phase II**

Each instrument is calibrated in accordance with the specifications of the instrument.

REFERENCES

1) Hipp J, Bauer TW, Bui MM, et al. CAP Pathology Resource Guide: Digital Pathology. Version 5.0(1). Northfield, IL: College of American Pathologists; 2014.

QUALITY CONTROL

Inspector Instructions:

	• Sampling of QC policies and procedures • Sampling of QC records
	• How do you determine when QC is unacceptable and corrective actions are needed?

BAP.05420 Quality Control - Digital Image Analysis — Phase II

Control materials are run concurrently with patient specimens to ensure appropriate functionality of the digital image system.

NOTE: Controls are samples that act as surrogates for patient/client specimens. They are periodically processed like a patient/client sample to monitor the ongoing performance of the analytic process. Controls should check test performance at relevant decision points for the digital image analysis system.

For qualitative tests, a positive and a negative control may be sufficient. For quantitative or semiquantitative tests, controls at more than one level should be used.

Evidence of Compliance:

✓ Records of QC results

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 2003(Jan 24):5232 [42CFR493.1256(d)(3)(i)]
2) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

BAP.05425 QC Handling — Phase II

The biorepository tests control specimens in the same manner and by the same personnel as patient/client samples.

NOTE: Personnel who routinely perform patient/client testing must analyze QC specimens; however, this does not imply that each operator must perform QC daily. Personnel must participate in QC on a regular basis. To the extent possible, all steps of the testing process must be controlled.

Evidence of Compliance:

✓ Records reflecting that QC is run by the same personnel performing patient testing

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7166 [42CFR493.1256(d)(8)]; 2) *ibid*, 2003(Jan 24):3708[42CFR493.1256(d)(7-8)

BAP.05430 QC Confirmation of Acceptability — Phase II

Personnel review control results for acceptability before reporting results.

NOTE: Control results must be reviewed before reporting patient/client results.

Evidence of Compliance:
- ✓ Records of control result approval

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7166 [42CFR493.1256(f)]
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3708 [42CFR493.1256(d)(6)]

BAP.05435 Monthly QC Review **Phase II**

The biorepository director or designee reviews and assesses quality control data at least monthly.

NOTE: The reviewer must record follow-up for outliers, trends, or omissions that were not previously addressed.

The QC data for tests performed less frequently than once per month may be reviewed when the tests are performed.

Evidence of Compliance:
- ✓ Records of QC review with recorded follow-up for outliers, trends or omissions

SPECIMEN ANALYSIS

Inspector Instructions:

- Sampling of specimen analysis policies and procedures

BAP.05440 Area of Analysis **Phase II**

A qualified pathologist selects or confirms the appropriate areas for analysis prior to reporting the results, as applicable.

NOTE: Specimens that do not represent "in situ" samples embedded in paraffin may not require pathologist review. Examples include cultured preparations and direct preparations of liquid specimens including blood, urine, pleural fluid, etc.

BAP.05445 Analysis Guidelines **Phase II**

There are written guidelines for identification of appropriate areas and cells for analysis.

NOTE: Evaluation of heterogeneous cell populations requires use of specific guidelines and procedures to ensure analysis of the appropriate areas and/or cells, particularly if there is background or nonspecific staining, or if there is cell debris, endogenous pigment, and/or artifacts of aging, sectioning or preparation.

Test results may be affected by fixation parameters, including time of fixation, type of fixative used, hemorrhage, necrosis, and autolysis of tissue.

PERSONNEL

Inspector Instructions:

READ	• Records of personnel education and experience

BAP.05450 Testing Personnel Qualifications **Phase II**

Personnel who are responsible for evaluating the imaging system data are qualified as high-complexity testing personnel.

NOTE: Refer to the Laboratory General Checklist for high complexity testing personnel (GEN.54750) and general supervisor (GEN.53600) qualifications. A detailed listing of personnel qualifications can be found in e-LAB Solutions Suite on cap.org (log-in required) under Accreditation Resources.

Evidence of Compliance:

- ✓ Records of qualifications including diploma, transcript(s), primary source verification report, equivalency evaluation, or current license (if required) **AND**
- ✓ Work history in related field

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Oct 1):1070-1071 [42CFR493.1489]

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 hybridization, and fluorescent in situ hybridization (FISH) analyses. (Reference: https://ccrod.cancer.gov/confluence/display/CCRTARP/About)

Inspector Instructions:

READ	• Sampling of tissue microarray policies and procedures • Records of methods selected for region of interest of tissue and communication with the microarray technologist
OBSERVE	• System to positively identify specimens, specimen types and aliquots throughout the process

	• Who is responsible for selecting tissues and performing analysis for tissue microarray? • How are the selection and number of cores determined?
	• Follow a tissue specimen for TMA from processing to final analysis. Observe specimen identification, core selection and analysis.

BAP.05500 Specimen Identification - Tissue Microarray **Phase II**

There is a system to positively identify all participant specimens, specimen types, and aliquots through all phases of the analysis.

NOTE: The phases include, but are not limited to:

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

BAP.05600 Preparation - Tissue Microarray **Phase II**

There are records describing the tissue types and purpose for the tissue microarray (TMA), including the size and placement of the tissue cores as well as control tissue cores.

NOTE: Criteria for selection and records of the tissue cases are required. The usefulness and analysis of tissue microarray cores can be affected by the location (edges versus center) and loss of tissue cores as the tissue microarray block is thin sectioned. Consideration of size, frequency, and location of cores therefore, should be considered and recorded 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.

BAP.05700 Original Paraffin Tissue Block - Tissue Microarray **Phase II**

The biorepository has criteria for determining the extent to which the original paraffin tissue block lesion can be removed.

BAP.05800 Tissue Core Selection - Tissue Microarray **Phase II**

A qualified anatomic pathologist selects the appropriate tissues (paraffin block and tissue region of interest) to make a tissue microarray.

BAP.05900 Core Selection - Tissue Microarray **Phase II**

There is a defined process for selecting the regions of interest in the tissue and clearly communicating the instructions to the tissue microarray technologist.

BAP.06000 Number of Cores - Tissue Microarray **Phase II**

The methods for determining the relevant number of cores to accurately represent the parent tissue block are recorded.

NOTE: The biorepository must follow a written procedure to determine the optimum number of cores required per tissue microarray, as dictated by each study protocol.

BAP.06100 Tissue Placement - Tissue Microarray **Phase II**

Personnel follow a defined process for ensuring that the correct tissue is placed in the correct location of the tissue microarray (TMA) (eg, a TMA map identifying tissue type, key ID, and location in the TMA).

NOTE: This includes 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.

REFERENCES
1) Clinical and Laboratory Standards Institute. *Fluorescence in Situ Hybridization Methods for Clinical Laboratories; Approved Guideline.* 2nd ed. CLSI Document MM07-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.

BAP.06200 Analysis - Tissue Microarray **Phase I**

Analysis of tissue microarrays is performed by an anatomic pathologist.

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

Evidence of Compliance:
- ✓ Records of tissue microarray analysis

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 ionization (SELDI) protein profiling. Commercial kits for the isolation of RNA and DNA are available and adaptable to the micro samples obtained by LCM.

Inspector Instructions:

	• Sampling of LCM policies and procedures • Records of LCM laser focus and alignment
	• System to positively identify specimens, specimen types and aliquots throughout the process
	• How is the quality of LCM tissue material ensured?

BAP.06300 Specimen Identification - LCM **Phase II**

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

BAP.06400 LCM Process **Phase II**

The biorepository monitors and records laser capture microdissection (LCM) following a defined process.

NOTE: 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.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, Preparations, and Storage of Specimens for Molecular Methods.* 2nd ed. CLSI guideline MM13. Clinical and Laboratory Standards Institute, Wayne, PA; 2020.

BAP.06500 LCM Equipment **Phase II**

The laser capture microdissection (LCM) laser focus and alignment is maintained and recorded to ensure optimal performance.

NOTE: Maintenance records related to the critical components of the LCM as noted by the manufacturer are required.

MOLECULAR METHODS

ELECTROPHORESIS

Inspector Instructions:

	• Sampling of electrophoresis policies and procedures
	• Autoradiographs/gel photographs (sufficient resolution/quality)
	• How does your laboratory prevent degradation of the nucleic acid sample used for electrophoresis?

BAP.06510 Loading Analytical Gels **Phase I**

Standard amounts of nucleic acid are loaded on analytical gels, when possible.

BAP.06520 Molecular Weight Markers **Phase II**

Known molecular weight markers that span the range of expected bands are used for each electrophoretic run.

Evidence of Compliance:

✓ Records of appropriate markers with each run

BAP.06530 Visual/Fluorescent Markers **Phase II**

Visual or fluorescent markers are used to determine the endpoint of gel electrophoresis.

TARGET AMPLIFICATION/POLYMERASE CHAIN REACTION (PCR)

Inspector Instructions:

	• Sampling of amplification/PCR policies and procedures
	• Physical containment practices (frequent glove change, separate manipulation of pre- and post-specimens, dedicated pipettes)
	• How does your laboratory distinguish a true negative from a false negative result?

BAP.06610 Carryover - Nucleic Acid Amplification **Phase II**

Nucleic acid amplification procedures (eg, PCR) use appropriate physical containment and procedural controls to minimize carryover (false positive results).

NOTE: 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 the laboratory take special precautions. 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 minimize aerosolization. Enzymatic destruction of amplification products is often helpful, as is real-time measurement of products to avoid manual manipulation of amplification products.

REFERENCES

1) Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;339:237-238
2) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

BAP.06620 Internal Controls - Nucleic Acid Amplification **Phase II**

In 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.

NOTE: The laboratory 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 specimen 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.

BAP.06630 Melting Temperature **Phase I**

For tests that generate a result based on a T_m, appropriately narrow temperature ranges (+/- 2.5°C) are defined and recorded each day of use.

IN SITU HYBRIDIZATION (ISH)

The use of the term in situ hybridization (ISH) in this section applies to all ISH methods, including fluorescence (FISH), chromogenic (CISH), silver (SISH), and brightfield (BRISH) in situ hybridization.

Please refer to the Definition of Terms section in the All Common (COM) Checklist for definitions of analytical validation and analytical verification.

Inspector Instructions:

	• Sampling of ISH policies and procedures • Sampling of probe validation/verification records • Sampling of QC records • Sampling of patient test reports
	• How are ISH cut-off values established? • How does your laboratory validate/verify assay performance prior to test implementation? • What is your course of action when a probe does not produce an internal control signal?

BAP.06710 ISH Probe Validation/Verification **Phase II**

All in situ hybridization (ISH) probes are validated/verified.

NOTE: Additional requirements for test method validation/verification are in the All Common Checklist.

Evidence of Compliance:

✓ Records of ISH probe validation/verification

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.
3) Lawrence Jennings, Vivianna M. Van Deerlin, Margaret L. Gulley (2009) Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests. *Archives of Pathology & Laboratory Medicine*: Vol. 133, No. 5, pp. 743-755
4) Wiktor AE, Van Dyke DL, Stupca PJ, et al. Preclinical validation of fluorescence in situ hybridization assays for clinical practice. *Genetics in Medicine* 8:16-23, 2006.
5) Weremowicz S, Sandstrom DJ, Morton CC, Miron PM. Validation of DNA probes for preimplantation genetic diagnosis (PGD) by fluorescence in situ hybridization (FISH) R1. *Prenat Diagn.* 2006 Nov;26(11):1042-50.
6) Saxe DF, Persons DL, Wolff DJ, Theil, KS; Cytogenetics Resource Committee of the College of American Pathologists. Validation of fluorescence in situ hybridization using an analyte-specific reagent for detection of abnormalities involving the mixed lineage leukemia gene. *Arch Pathol Lab Med.* 2012; 138(1):47-52.

BAP.06720 Interphase ISH - Cut-off Value **Phase II**

For interphase in situ hybridization (ISH), the laboratory establishes a normal cut-off value for results for each probe used, when applicable.

NOTE: Refer to the All Common Checklist for specific test method validation/verification requirements. Cut-off values are usually required when ISH testing uses locus-specific probes against nuclear DNA.

Evidence of Compliance:
- ✓ Records from cut-off value studies

REFERENCES
1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) Clinical and Laboratory Standards Institute. *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline.* 2nd ed. CLSI Document MM07-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.

BAP.06740 ISH Assay Performance — Phase I

There are records of in situ hybridization (ISH) performance for each assay.

NOTE: Assay performance should include monitoring hybridization efficiency, probe signal intensity and overall assay results, including controls, as applicable.

Evidence of Compliance:
- ✓ Records of QC monitoring of ISH assay performance at defined frequency

BAP.06750 ISH Probe Intended Target — Phase I

A system is used to ensure that the in situ hybridization (ISH) probe used is for the intended target.

NOTE: Examples can include (but may not be limited to): 1) concurrent analysis of any available metaphase cells in an interphase cell analysis; 2) inclusion of an internal or external target that results in a positive signal for each hybridization; 3) written protocols that ensure the respective probe is applied to the intended specimen.

Evidence of Compliance:
- ✓ Records confirming intended target

BAP.06760 ISH Scoring — Phase II

Scoring of in situ hybridization (ISH) assays, including the number of cells scored, is performed as defined in a written procedure.

REFERENCES
1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.

BAP.06770 ISH Controls — Phase II

The biorepository performs and records controls (internal and/or external) for each in situ hybridization (ISH) analysis.

NOTE: What functions as a control depends on the specific assay, signal pattern present, and sample type. For example, assays designed to detect deletions may use internal controls that include both the probe of interest and a control locus probe, both of which map to the same chromosome. In this situation, there are two internal controls, the signal for the probe of interest on the normal homolog and the control locus signals on both the normal and deleted homolog. For a dual fusion assay, the probe signals on each of the normal homologs function as internal

controls. If a probe is used that does not produce an internal control signal (eg, a Y chromosome probe in a female), another sample that is known to have the probe target must be run in parallel as an external control with the patient sample. In addition, many ISH assays use an external control(s). For FDA-cleared or approved ISH assays, laboratories must follow manufacturer's instructions for quality control at minimum.

Evidence of Compliance:

✓ Records of QC results

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.
3) Stupca P, Meyer RG, Dewald GW. Using controls for molecular cytogenetic testing in clinical practice. *J Assoc Genet Tech.* 2005;31:4-8.

BAP.06780 Image and Slide Retention - ISH **Phase II**

Photographic or digitized images or permanent slides are retained of all in situ hybridization (ISH) assays for an appropriate period.

NOTE: Images or permanent slides of ISH assays for neoplastic disorders must be retained for 10 years; images or permanent slides of ISH assays for constitutional disorders must be retained for 20 years. For an ISH assay with a normal result, retain an image of at least one cell illustrating the normal probe signal pattern. For an ISH assay with an abnormal result, retain images of at least two cells illustrating each relevant abnormal probe signal pattern.

There is no retention requirement for retaining images of slide preparations when the source slides remain readable for the required retention period.

REFERENCES

1) American College of Medical Genetics, Standards and guidelines for clinical genetics laboratories, 2021 edition.

BAP.06790 ISH Interpretation **Phase II**

If an in situ hybridization (ISH) study requires consultation with a qualified pathologist and/or a cytogeneticist for an accurate interpretation, the appropriate expert is consulted and their involvement is recorded.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline.* 2nd ed. CLSI document MM07-A2. Clinical and Laboratory Standards Institute, Wayne, PA, 2013.

INSTRUMENTS AND EQUIPMENT

A variety of instruments and equipment are used to support the biorepository. All instruments and equipment should be properly operated, maintained, serviced, and monitored to ensure proper performance. The procedures and schedules for instrument maintenance and function checks must be as thorough and as frequent as specified by the manufacturer. Examples of equipment include, but are not limited to centrifuges, microscopes, incubators, heat blocks, microwaves, etc.

The checklist requirements in this section should be used in conjunction with the requirements in the All Common Checklist relating to instruments and equipment.

Inspector Instructions:

	• Sampling of histology safety policies and procedures • Sampling of microwave reproducibility and ventilation checks • Sampling of thermocycler monitoring records
	• Location of automated tissue processor • Storage cabinets
	• How frequently do you change solutions in the tissue processor? How is the timeframe for changing solutions determined? • How does your laboratory prevent cross-contamination of paraffin sections in the flotation bath? • How often do you decontaminate your cryostat? • How does your laboratory ensure the individual wells of the thermocycler are maintaining accurate temperature?

BAP.06844 Automated Tissue Processor **Phase II**

Each open (ie, generative of flammable vapors into the ambient workspace) automated tissue processor is operated at least five feet (1.5 m) from the storage of combustible materials and from the paraffin dispenser.

NOTE: Tissue processors that operate as a closed system confine ignitable vapor hazards within the processor and thus do not pose a hazard requiring five feet of separation.

Each open (ie, generative of flammable vapors into the ambient workspace) automated tissue processor must be located at least five feet from the storage of combustible materials unless separated by one-hour fire-resistive construction. Flammable and combustible liquids must not be positioned near sources of heat or ignition. At least five feet must separate each open system tissue processor from the paraffin dispenser.

BAP.06846 Microtome Knife Storage **Phase II**

Microtome knives are stored in original containers or by some other means to avoid personnel injury or equipment damage.

BAP.06851 Microtome Maintenance **Phase I**

Microtomes and microtome knives are clean and well-maintained.

NOTE:

- *Microtomes must be clean, properly lubricated, and without excessive play in the advance mechanism*
- *Knives must be sharp and free of nicks*

NOTE: The following four requirements apply to microwave devices used in the histology section.

BAP.06854 Microwave Usage **Phase I**

Microwave devices are used in accordance with manufacturer's instructions.

***REVISED** 10/24/2022*

BAP.06856 Microwave Monitoring **Phase I**

Microwave devices are monitored for reproducibility at least annually.

NOTE: "Reproducibility" is defined as consistency in diagnostic quality obtained from microwave equipment and procedures. For some devices, reproducibility may be evaluated by monitoring the temperatures of identical samples after microwave processing. For those microwave devices (particularly those incorporated into histology processing equipment) that use temperature-independent methods to evaluate reproducibility, the reproducibility must be assessed following instrument manufacturer's instructions.

The microwave device must be tested for radiation leakage if there is visible damage to the device. A description of the specific damage along with the result of the test must be recorded.

Evidence of Compliance:

✓ Records of monitoring the diagnostic quality of specimens processed using microwaves

***REVISED** 10/24/2022*

BAP.06858 Microwave Container Venting **Phase I**

All containers used in microwave devices are vented or are used in compliance with manufacturer's instructions for the microwave instrumentation used.

NOTE: Venting of containers is necessary so that processing occurs at atmospheric pressure, to prevent explosion. For procedures using pressure above that of the atmosphere, specialized containers must be used, with strict adherence to manufacturer's instructions.

***REVISED** 10/24/2022*

BAP.06865 Microwave Venting **Phase I**

Microwave devices are properly vented and the effectiveness of ventilation is monitored at least annually.

NOTE: Some types of microwave devices need to be operated in an appropriate ventilation hood to contain airborne chemical contaminants and potentially infectious agents. Before operation of the microwave device, flammable and corrosive reagents must be removed from the hood to prevent fire or chemical damage to the electronic components of the device. Microwave devices used outside a fume hood must have an integral fume extractor certified by the manufacturer for use in a clinical laboratory.

This checklist item does not apply to microwave devices that are designed by the manufacturer to operate without venting. It also does not apply if non-hazardous reagents (as defined in the safety data sheets) and non-infectious specimens (eg, paraffin specimens) are used in the device.

Evidence of Compliance:

✓ Records of annual evaluation of ventilation effectiveness

***REVISED** 10/24/2022*

BAP.07110 Automated Stainer **Phase II**

The biorepository changes the solutions in automated stainers following a defined schedule.

NOTE: Solutions must be changed at intervals appropriate for the biorepository's workload. Cleaning of the stainers must be recorded when performed.

Evidence of Compliance:
- ✓ Records for solution changes

BAP.07120 Incubator QC **Phase II**

Incubators are monitored for temperature, CO_2 level, and humidity on each day of use.

NOTE: The procedure manual must specify the allowable limits for each type of culture. Readings must be recorded each day that cultures are incubated. There must be records of corrective action if the allowable limits are exceeded.

Evidence of Compliance:
- ✓ Instrument QC records

BAP.07200 Tissue Processor Solutions **Phase II**

Tissue processor solutions are changed at intervals appropriate for the workload.

NOTE: When solutions are changed, they must be entirely replaced with new solution and not just "topped off."

Evidence of Compliance:
- ✓ Records of solution changes at the defined frequency

REFERENCES
1) Compton CC, Robb JA, Anderson MW, et al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Pathol Lab Med.* 2019;143(11)1346-63.

BAP.07210 Tissue Processing Programs - Validation **Phase II**

Tissue processing programs are validated.

NOTE: To validate new processing programs, the biorepository should run tissue samples of the same size, thickness and fixation in duplicate. Reagents on the processor(s) should be comparable, eg, all fresh reagents. Process, embed, cut, and stain slides at the same time and evaluate the quality of the blocks, eg, firmness, ease of cutting. The slides should be evaluated by the pathologist without knowledge of which processing program was used and graded on quality of section and staining. The new processing program must be of equal or better quality before being put into use.

This method may also be used to verify a routine processing program before putting a new processor into production.

Evidence of Compliance:
- ✓ Records of validation

BAP.07220 Tissue Processing Programs **Phase I**

Specific tissue processing programs are available for different types and sizes of specimens.

NOTE: To achieve acceptable results for diagnostic purposes, processing programs may be needed for different sizes and types of specimens. Biopsy specimens may be processed on a shorter schedule than larger specimens; large, dense or fatty specimens and brain specimens will not process adequately on a shorter schedule. A variety of processing programs should be defined and used to achieve good processing results.

Evidence of Compliance:
- ✓ Defined processing programs for various types and sizes of specimen tissues

BAP.07400 Paraffin Baths, Flotation Baths, and Embedding Stations **Phase II**

Paraffin baths, flotation baths, and embedding stations are clean, controlled and well-maintained.

NOTE: Instruments must be clean and well-maintained (eg, tissue processors, embedding centers, dispensers, flotation baths, stain lines, coverslipping equipment).

The temperature of the paraffin dispenser and paraffin baths must be correct for the type of paraffin used. At a minimum, the equipment must be maintained according to the manufacturer's instructions and paraffin temperatures recorded.

The CAP recommends the use of high-quality paraffin with a melting point of <60°C. The benefit of low-melt paraffin is that it is removed more efficiently during de-paraffinization and/or antigen retrieval. Efficient paraffin removal is essential for all molecular analyses.

Written procedures must include required water type, fill volume, and optimal temperature range for the type of paraffin used for tissue blocks. Inappropriate temperatures may affect the downstream use of the biospecimen.

Evidence of Compliance:
- ✓ Records of maintenance **AND**
- ✓ Records of temperature checks

REFERENCES
1) Compton CC, Robb JA, Anderson MW, et al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Pathol Lab Med.* 2019;143(11)1346-63.

BAP.07600 Cryostat Decontamination **Phase II**

The cryostat is decontaminated at defined intervals and under defined circumstances.

NOTE: The cryostat must be defrosted and decontaminated by wiping all exposed surfaces with tuberculocidal disinfectant. The cryostat should be at room temperature during decontamination unless otherwise specified by the manufacturer.

Decontamination must be done at an interval appropriate for the institution; this must be weekly for instruments used daily. Trimmings and sections for tissue that accumulate inside the cryostat must be removed during decontamination. Although not a requirement, cut-resistant gloves should be worn when changing knife blades.

Evidence of Compliance:
- ✓ Records of cryostat decontamination

REFERENCES
1) Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline.* 4th ed. CLSI Document M29-A4. Clinical and Laboratory Standards Institute, Wayne, PA; 2014.
2) US Environmental Protection Agency: Antimicrobials Products Tested or Pending Testing. https://www.epa.gov/pesticide-registration/antimicrobials-products-tested-or-pending-testing Accessed April 19, 2018.

BAP.07630 Thermocycler Temperature Checks **Phase II**

Individual wells (or a representative sample thereof) of thermocyclers are checked for temperature accuracy before being placed in service and at least annually thereafter.

NOTE: A downstream measure of well-temperature accuracy (such as productivity of amplification) may be substituted to functionally meet this requirement. For closed systems this function should be performed as a component of the manufacturer-provided preventive maintenance.

Evidence of Compliance:

✓ Records of thermocycler verification

REFERENCES

1) Saunders GC, *et al.* Interlaboratory study on thermal cycler performance in controlled PCR and random amplified polymorphic DNA analyses. *Clin Chem.* 2001;47:47-55

2) Saunders GC, *et al.* Interlaboratory study on thermal cycler performance in controlled PCR and random amplified polymorphic DNA analyses. *Clin Chem.* 2001;47:47-55

STORAGE

This section of storage for a biorepository should be based on the type of equipment, the type of specimen(s) to be stored, the length of time in storage, and the intended use of the specimen(s).

TEMPERATURE DEPENDENT STORAGE EQUIPMENT

Inspector Instructions:

READ	• Sampling of specimen storage policies and procedures • Sampling of preventive and corrective maintenance procedures • Records of storage container calibrations and calibration verifications • Sampling of temperature monitoring records • Sampling of temperature set points
OBSERVE	• Adequate space for storage containers • Active alarm systems in place • Walk-in storage environment • Liquid nitrogen tanks usage monitoring and storage, if applicable
ASK	• What do you do in the event of freezer breakdown? • How do you prevent overflow of storage containers?
DISCOVER	• Have you ever suffered a significant loss of samples? How did you address this and what were the corrective actions that became policy as a result?

BAP.07800 Storage Equipment Calibration/Calibration Verification **Phase II**

The biorepository performs calibration and calibration verification for all applicable storage equipment.

NOTE: The records of calibration and calibration verification include:

1. *Date calibration was performed*
2. *Identity of person who ran the calibration*
3. *Records of results*
4. *Name of the device used against which instrument was calibrated*

Evidence of Compliance:

- ✓ Records of calibration/calibration verification **OR** manufacturers' certification of calibration

BAP.07900 Temperature Set Points **Phase I**

High and low temperature set-points have been established that are appropriate for each storage environment.

BAP.08000 Proper Temperature **Phase I**

There is evidence that all temperature-controlled storage units maintain the proper temperature throughout the unit.

NOTE: On all temperature-controlled storage units, temperature mapping must be performed on a periodic basis to ensure that the proper temperature is maintained throughout. There must be records that such readings have been taken. Unrestricted air circulation within the unit reduces the potential for warmer or colder areas that may have detrimental effects on blood/component units without detection by the monitoring system. This requirement also applies to liquid nitrogen (LN2) storage units (vapor phase only).

Temperature mapping must be performed and recorded for each new temperature controlled storage unit prior to being placed in service and periodically for freezers currently in service. The frequency of mapping is determined by the director/designee as well as the review of the data generated.

BAP.08100 Refrigerator/Freezer Temperature **Phase II**

The biorepository monitors and records refrigerator/freezer temperatures daily, as defined in written procedure.

NOTE: Storage temperature of biospecimens must be appropriate for the type of tissue and its means of preservation. Failure to adhere to requirements could result in a unit not being suitable for the purpose for which it was intended.

This checklist requirement applies to refrigerators/freezers containing reagents or biological specimens. "Daily" means every day (seven days per week, 52 weeks per year). The biorepository must define the acceptable temperature ranges for these units. If temperature(s) are found to be outside of the acceptable range, the biorepository must record appropriate corrective action, which may include evaluation of contents for adverse effects.

The two acceptable ways of recording temperatures are: 1) recording the numerical temperature, or 2) placing a mark on a graph that corresponds to a numerical temperature (either manually, or using a graphical recording device). If the records are manually obtained, the identity of the individual recording the temperature(s) must be recorded (recording the initials of the individual is adequate).

The use of automated (including remote) temperature monitoring systems is acceptable, providing that biorepository personnel have ongoing immediate access to the temperature data, so that appropriate corrective action can be taken if a temperature is out of the acceptable range. There must be records showing daily functionality of the system.

BAP.08200 Walk-in Storage Criteria **Phase II**

Walk-in storage systems have the following:

1. **Dual compressors**
2. **Internal safety release**
3. **Non-slip floor covering**

4. Interior oxygen and CO_2 monitoring system, when required

BAP.08300 Freezer Preventive Maintenance **Phase II**

The biorepository performs regular freezer preventive maintenance.

NOTE: Regular preventive maintenance is required to keep units functioning properly. Routine cleaning and maintenance should be done by assigned employees according to a Preventive Maintenance Schedule. Actions should be targeted at elimination of the causes of equipment failure and unscheduled interruptions. This activity involves regular, routine cleaning, lubricating, testing, calibrating and adjusting, checking for wear and tear and eventually replacing components to avoid breakdown.

Evidence of Compliance:
- ✓ Record of employees trained to perform preventive maintenance **AND**
- ✓ Results of all preventive maintenance will be recorded

BAP.08400 Emergency Response Plan **Phase II**

There is an emergency response plan if acceptable temperature ranges for refrigerators and/or freezers are exceeded.

BAP.08500 Specimen Transfer Process **Phase II**

The biorepository has a defined process for maintaining appropriate temperatures in the event of a system failure.

NOTE: There is a plan in place for transfer and back-up storage. For example, having 10% back-up storage containers would be considered best practices for each type of temperature-controlled unit should any one unit suffer an unrecoverable failure. Failure mode analysis should be performed to identify possible root causes of failure. Corrective actions should include service calls to providers for system repair, as applicable. Duration of failure should also be recorded, as well as any potential adverse effects to specimens.

Evidence of Compliance:
- ✓ Temperature and alarm records **AND**
- ✓ Updated specimen location records **AND**
- ✓ Corrective action and preventive action records

*****REVISED** 08/24/2023***
BAP.08600 Liquid Nitrogen Supplies **Phase II**

Adequate liquid nitrogen (LN2) supplies are maintained securely onsite if LN2 is used as refrigerant or coolant for a storage environment.

NOTE: In general, vapor phase storage is the preferred method over storage in the liquid phase of nitrogen because vapor phase provides sufficiently low temperatures to maintain temperatures below the Tg (glass transition temperature), while avoiding safety hazards inherent in liquid phase storage.

The biorepository must have sufficient LN2 supply to fill a spare storage vessel and/or to allow for freezing of specimens in an emergency.

Access to supply tanks stored outside of the laboratory must be limited to trained personnel and authorized individuals (eg, vendors).

Evidence of Compliance:

✓ LN2 supply storage within the restricted area of the laboratory **OR** locked supply storage area outside of the laboratory with limited key access

BAP.08700 LN2 Monitoring **Phase II**

LN2 daily usage and LN2 levels are monitored and recorded for each storage container.

NOTE: The interval for monitoring of usage must be based on the requirements of the instruments.

Evidence of Compliance:

✓ Records of usage monitoring, as applicable

BAP.08800 Storage Containers Approval **Phase II**

All specimen storage containers have been approved for use under intended storage conditions.

NOTE: Refer to contact supplier specification sheet for valid use conditions.

TEMPERATURE MONITORING AND ALARMS

Inspector Instructions:

	• Sampling of temperature logs • Sampling of records of alarm trigger response • Sampling of alarm system testing records
	• Active alarm systems in place • Availability of emergency power supply
	• What do you do when a storage container alarm triggers? • What is the biorepository's contingency plan if the alarm system fails? • What do you do if a unit cannot maintain appropriate temperature?
	• Select a storage container that has had a temperature failure and follow the process from notification to response and final corrective action

BAP.09100 Temperature Checks **Phase II**

Temperatures are checked and recorded on each day of use, specifying the unit and location for all temperature dependent instruments and equipment.

NOTE: Controlled-temperature devices used must have temperatures recorded at least daily for units that are within the prescribed temperature range, and at least every 15 minutes if outside of that range.

The two acceptable ways of recording temperatures are: 1) recording the numerical temperature, or 2) placing a mark on a graph that corresponds to a numerical temperature (either manually, or using a graphical recording device). The identity of the individual recording the temperature(s) must be recorded (recording the initials of the individual is adequate).

The use of automated (including remote) temperature monitoring systems is acceptable, providing that biorepository personnel have ongoing immediate access to the temperature data, so that appropriate corrective action can be taken if a temperature is out of the acceptable range. There must be records showing daily functionality of the system.

Evidence of Compliance:

- ✓ QC records for continuous temperature monitoring **OR** records of checks at defined frequency

BAP.09200 Alarm Response Time **Phase I**

Temperature limits for the alarm are established with consideration for anticipated response time.

BAP.09300 Storage Temperature Deviation Procedure **Phase II**

The biorepository follows a defined process for deviations in the storage temperature limits, with an impact assessment when required.

NOTE: Procedures for the handling of biological specimens if storage temperature limits cannot be maintained must be written and included in personnel training. The primary concern is the preservation of specimen. If there is a failure, arrangements must be made for service, and for alternative storage.

BAP.09400 Emergency Power Supply **Phase II**

Temperature controlled storage equipment have an emergency power supply.

BAP.09500 Storage Unit Alarms **Phase II**

There is an audible alarm for each component storage unit, the alarm is continuously monitored 24 hours per day (in biorepository or remote), and the response system to an alarm has been validated.

NOTE: The biorepository should be able to demonstrate how this system works, and that there is a process to ensure a timely response to an alarm.

Evidence of Compliance:

- ✓ Records of response time to the alarm

*****REVISED** 08/24/2023***

BAP.09600 Alarm System Checks **Phase II**

Alarm system functionality is tested at least semiannually (eg, alarm triggers, ability to communicate, etc.).

NOTE: The Biorepository Director may define policies for more frequent alarm system testing based on the level of risk associated with an alarm system and/or communication failure. Temperature controlled storage unit alarms should be tested without taking specimens outside

of their acceptable range. Some ways to perform this testing may include: 1) electronic manipulation of freezer set points to trigger the alarm system, 2) warming or cooling the probe using external measures that do not affect the operating temperature at which the specimens are held, and other acceptable processes. This includes both individual alarms and central monitoring systems.

Records of appropriate alarm triggering and notification of personnel during normal operations may also be used as evidence of functionality.

Evidence of Compliance:
- ✓ Records of alarm system testing

BAP.09700 Alarm Adjustment **Phase II**

Alarms are adjusted to be triggered before the temperature falls outside the acceptable temperature range.

NOTE: The biorepository defines the acceptable range for specimen storage.

Evidence of Compliance:
- ✓ Records of trigger temperatures during alarm checks **AND**
- ✓ Records of corrective action, when appropriate

BAP.09800 Power Failure Back-Up **Phase II**

The alarms will continue to function if the power is interrupted.

NOTE: Alarm systems must continue to function during a power failure. This may be accomplished by having the alarm on a separate circuit, installing battery power back-up, or having a power failure alarm.

BAP.09900 Off-Site Notification Process **Phase II**

If the monitoring system allows for off-site notification, there is a

1. **Trained person on-call (24/7) to respond to alarm conditions**
2. **List of phone numbers or alternate means of contact for trained personnel in case the on-call person fails to respond**

BAP.10000 Back-Up Alarm QC **Phase II**

There is a back-up alarm system in place with records of testing at defined intervals.

BAP.10100 Alarm System Monitoring **Phase II**

There is a mechanism for monitoring the alarm system.

BAP.10200 Alarm System Contingency Plan **Phase II**

There is a contingency plan in place for monitoring if the alarm system fails.

NOTE: Downtime procedures should exist and staff should be trained on these procedures. This contingency procedure should be periodically tested.

INVENTORY MANAGEMENT SYSTEM

INVENTORY

Inspector Instructions:

	• Records of inventory system privilege levels for employees • Records of inventory system audits • Inventory tracking criteria
	• Use of inventory tracking criteria • Sample being placed into inventory • Labeling of specimens with a unique identifier/code
	• How are privilege levels assigned for the inventory system?
	• Select a specimen in storage and review the audit trail for the specimen • Is there a system in place to identify the exact refrigerator/freezer where a sample is stored?

BAP.12500 Inventory Process **Phase II**

The biorepository follows a defined inventory management process.

NOTE: Privilege levels should be set for performing specific functions in the system and for access to specific data.

Evidence of Compliance:

✓ Records of each person's level of access

BAP.12600 Computer-Based Inventory System Privileges **Phase II**

If the inventory system is computer-based, the system is controlled by assigning privilege levels to the biorepository staff.

BAP.12700 Computer-Based Inventory System Verification/Audits **Phase II**

If a computer-based inventory system is used, it has been verified and is subject to quality assurance audits at intervals defined by the director.

BAP.12800 Inventory System Tracking Criteria **Phase II**

The inventory system tracks, as applicable:

1. **Unique identifier**
2. **Study and study participant identifier**
3. **Visit identifier, if applicable**
4. **Specimen material type**
5. **Preservatives/additives/preservation methods**
6. **Specimen *parent/child* relationship, if applicable**
7. **Specimen vial type**
8. **Specimen volume**
9. **Date/time of collection**
10. **Date/time of receipt into inventory**
11. **Date/time of processing**
12. **Date/time and location of distribution**
13. **Number of thaws**
14. **Number of times sent previously for testing, if applicable**
15. **Condition warnings (eg, partially frozen upon receipt, micro-clots present, frozen sideways, or any other relevant exceptions to the SOP)**
16. **Clinical data, as applicable**
17. **Biospecimen status (eg, reserved or available)**
18. **Clinical collection site identifier, if applicable**

NOTE: If clinical data is not stored at the biorepository in the inventory tracking system, there is a method for linking the physical spec with the clinical information, as needed.

Information regarding some of these elements may not be available to the biorepository for all biospecimen collections, especially those that were procured before recent best practices for biorepositories were published or for legacy collections.

BAP.12900 Inventory System Audit Trail Criteria **Phase II**

The inventory system includes a full audit trail of changes made to the database to include:

1. **Original date**
2. **Changed date**
3. **Identity of who made the change**
4. **Reason for change**
5. **What was changed**
6. **How the change was made**

BAP.12950 Specimen Quantity Warnings **Phase II**

If required by the sponsor, there is a mechanism in place to ensure minimum vial and minimum volume warnings are triggered before quantities fall below collection specified quantities.

NOTE: The warning mechanism may be either manual or automated. The intent of the requirement is inventory based.

BAP.13000 Inventory System Distribution Records **Phase II**

The inventory system keeps full records for specimens after distribution.

BAP.13100 Environmental Storage Areas Identifiers **Phase II**

Environmental storage areas (eg, freezers and refrigerators) have their own unique identifier that includes a defined convention for numbering shelves, racks, boxes, and the location within each container.

BAP.13150 Missing Specimen - Inventory Update **Phase II**

If a specimen is missing, inventory is updated to reflect that the specimen cannot be located.

SAMPLE MANAGEMENT

Inspector Instructions:

READ	• Sampling of sample distribution records
OBSERVE	• Sample being removed from inventory
ASK	• What is the process if a sample entered into the inventory system cannot be located? • What are you looking for when performing a sample pre-distribution quality check? • How do you ensure personnel are available to receive shipments?
DISCOVER	• Select a specimen that was shipped and review the audit trail for the specimen

BAP.13200 Shipment Acceptance Confirmation **Phase II**

Recipients are notified before shipping to ensure that appropriate personnel are available to receive the shipment.

BAP.13300 Shipping Tracking Criteria **Phase II**

Tracking information for shipment of specimens includes the following, as applicable.

1. **Invoice/tracking number**
2. **Recipient/source**
3. **Date of shipment or receipt**
4. **Courier name and ID# for each package**
5. **Sample description**
6. **Number of samples shipped/received**
7. **Study name/number**
8. **Shipping conditions (eg, dry ice, ambient temperature)**

9. **Key investigators identification**
10. **Confirmation of receipt**
11. **Any discrepancies from manifest and actual shipment**
12. **Specimen damage**

BAP.13400 Specimen/Shipping Manifest Linkage **Phase II**

Specimens are labeled with a unique identifier and/or code.

NOTE: The intent of this requirement is to ensure that specimens arrive with accurate manifest of the contents of the shipping container.

BAP.13500 Reconciliation of Discrepancies **Phase II**

When specimens are retrieved from storage, any discrepancies found are recorded and reconciled prior to distribution.

BAP.13600 Pre-Distribution QC **Phase II**

A quality check is performed prior to distribution.

NOTE: Quality checks may include, but are not limited to, gross observations, labeling accuracy, condition of specimens, weight, and verification that storage temperature is appropriate for the shipping temperature.

RECORDS

Inspector Instructions:

READ

- Policy for record retention
- Policy for disposition of specimen and data
- Sampling of disposition records from the last 2 year period

BAP.13740 Record Retention - Biorepository **Phase II**

The biorepository specifies the length of time in which all records, paper and/or electronic, are retained.

NOTE: The length of time will depend on the nature of the record and is determined by the biorepository. The records include, but are not limited to, equipment maintenance and repair records, clinical and patient information, and records pertaining to closed collections.

BAP.13750 Disposition of Specimens, Data and Regulatory Documents **Phase II**

The laboratory complies with the regulations that govern the biorepository for the disposition of specimens, data, and related regulatory documents.

NOTE: Reasons for disposition may include, but are not limited to:

1. *Transfer or termination of collection*
2. *End of funding period*
3. *Depletion of the biospecimen*

4. *Research participant's request for discontinuation*
5. *Informed consent issues*
6. *IRB issues*
7. *Discrepancies between any clinical data and specimens*
8. *Quality of the physical specimen (eg, insufficient fixation or processing, hemolysis)*

DISTRIBUTION POLICIES AND AGREEMENTS

Inspector Instructions:

	• Sampling of material transfer agreements (MTAs) • End-user distribution policy
	• Who ensures that the MTA includes all the required information? • Describe the MTA process

BAP.15300 Material Transfer Agreements Criteria **Phase II**

Material transfer agreements (MTAs) define the rights and obligations of the provider (biorepository) and recipient (researcher), including allowable uses for the specimen and/ or data once transferred.

BAP.15400 MTA Areas Covered **Phase II**

The material transfer agreement (MTA) addresses each of the following areas as applicable.

1. **Future distribution of modifications and derivations made by the recipient**
2. **Records of each participant's role in the modifications or derivations**
3. **Terms of confidentiality**

BAP.15500 End-User Distribution Policy Criteria **Phase II**

The distribution policy includes confirmation that the end-user has IRB approval or there is a material transfer agreement (MTA) in place that provides relevant assurance for the appropriate use of the specimen according to appropriate ethical and legal requirements.

Evidence of Compliance:

✓ Copies of IRB approvals from end-users **OR** copies of MTA agreements