Practical Guide to Specimen Handling in Surgical Pathology

Authors: Robert Lott, Janet Tunnicliffe, Elizabeth Sheppard, Jerry Santiago, Christa Hladik, Mansoor Nasim, Konnie Zeitner, Thomas Haas, Shane Kohl, Saeid Movahedi-Lankarani

COLLEGE of AMERICAN PATHOLOGISTS

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INTRODUCTION

In spite of the abundant guidelines and recommendations published for specimen handling and testing in a clinical pathology laboratory, relatively little literature is available for guidance of specimen handling in a surgical pathology laboratory. This document does not relate to cytologic or clinical pathology samples.

The following comprehensive table is intended to serve as a general guideline for proper specimen handling from the time it is taken from the patient to the time a completed slide of the specimen is given to a pathologist for interpretation.

DISCLAIMER:

This document was created by members of the CAP/NSH Histotechnology Committee and is intended to serve as a guideline ONLY and NOT AN absolute recommendation for specimen handling. Each laboratory is advised to use these guidelines as a starting point and modify certain parameters to fit state and local institutional requirements, as appropriate. Regulatory references, standards, and CAP checklist items cited in the guideline are current at the time of publication of this version of the guideline. It is recommended that the user confirm all references used are the latest version available. The use of the information contained in this guideline does not guarantee compliance with the CAP accreditation requirements or regulations from other accrediting organizations. Some information may be different or more stringent than the published CAP Checklists.

It is the intent of the CAP/NSH Histotechnology Committee to update this document every 2 years or when required and have the updated version of the document available to members on the College of American Pathologists (CAP) and National Society for Histotechnology (NSH) websites.

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Version: 10.0
Revised: October 2020

Table of Contents:

VERSION	REVISION DATE	REVISION
2.0	November, 2013	1. Addition of disclaimer on cover page 2. Addition of version control
3.0	November, 2014	1. Revised per comments received from CAP Chair review
4.0	January, 2015	1. Updated references – CAP Checklists: ANP, COM, GEN, 4-21-2014 2. All references reviewed 3. Table of contents added
5.0	September, 2015	1. Updated to reflect LAP Committee 2015 Checklist changes
6.0	November, 2015	1. Updated to reflect corrected formalin solution to tissue ratio with references
7.0	September, 2017	1. Updated to reflect August 21, 2017 CAP Checklist edition changes
8.0	September, 2018	1. Updated to reflect August 22, 2018 CAP Checklist edition changes 2. Updated to reflect review of all references
9.0	April, 2020	1. Updated to reflect June 4, 2020 CAP Checklist edition Changes 2. Updated to reflect review of all references 3. Updated Table of Contents 4. Updated Title Page and organizational logos
10.0	October, 2020	1. Updated organizational logos

PART I	I. SPECIMEN COLLECTION and HANDLING		
Guideline Section	Statement	Related CAP Checklist Requirements 2020 Edition	Additional References
Collection and Handling A. Patient Identification	• Patient is to be identified in a manner that respects patient privacy with respect to their medical records and medical data.	Laboratory General Checklist, GEN.41303 - Patient Confidentiality	
	• Patient's identity must be verified at the time of specimen collection.	Laboratory General Checklist, GEN.40490 - Patient Identification	
	• At least two acceptable patient-specific identifiers are required for patient identification: ○ Full name ○ Assigned identification number e.g. health record / master index number ○ Date of birth ○ Photo on government issued or other photo ID card, such as driver's license ○ Other specific personal identifiers	Laboratory General Checklist, GEN.40491 Primary Specimen Container Labeling	Health Insurance and Portability and Accountability Act (HIPAA). Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2011: Vol. 30 No7. International Standard ISO 15189:2012 - Medical Laboratories; section 5.4 - Pre-examination Processes

Collection and Handling
 	
B. Proper Labelling
	• Specimen is labeled in the presence of the patient
	
	• Specimen label must contain at least two patient-specific identifiers:
	  ○ Full patient name
	  ○ Assigned identification number e.g. health record / master index number
	  ○ Date of Birth
	
	• Customizable label elements – additional identifiers that are acceptable:
	  ○ Patient gender
	  ○ Accession or requisition number
	  ○ Ordering physician
	  ○ Source of specimen (e.g. skin)
	  ○ Site of specimen (e.g. left side of chest)
	
	• Standardized format for label information should be implemented.
	  ○ Last name, first name
	  ○ Date of Birth – DD –MMM- YYYY i.e. 12 MAR 1968
	  ○ Gender M, F, U (unknown), T (Transgender), I (Intersex)
	
	• Written documentation developed for the correct positioning of the label on the collection container.
	  ○ Do not attach label to the container lid (in whole or part)
		Laboratory General Checklist, GEN.40490 – Patient Identification
		
		Laboratory General Checklist, GEN. 40100 - Specimen Collection Manual Elements
		
		Laboratory General Checklist, GEN. 40491 - Primary Specimen Container Labeling
		
		All Common Checklist, COM.06100 – Primary Specimen Container Labeling
		
		All Common Checklist, COM.06200 - Secondary Specimen Container Labeling
			Clinical Laboratory Standards Institute CLSI – Auto12-A Specimen Labels: Content and Location, Fonts and Label Orientation: 2011: Vol. 31 No7.
			
			Brown RW, Della Speranza V, Alvarez JO, et al. Uniform labeling of blocks and slides in surgical pathology: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center and the National Society for Histotechnology. *Arch Pathol Lab Med*. 2015;139(12):1515-24.

	○ Do not overlap label resulting in patient data being covered • Written documentation for the correction of labelling errors – to be followed when specimens cannot be replaced • All subsequent labelling of patient samples (blocks and slides) must follow same patient-specific identifying process. • Submitted slides may be labeled with a single patient-specific identifier but two are preferred.	Laboratory General Checklist, GEN.40492 – Specimen Label Correction Laboratory General Checklist, GEN.40825 - Specimen ID Laboratory General Checklist, GEN.40491 - Primary Specimen Container Labeling	
Collection and Handling B. Proper Labelling i. Barcoding and/or Radio Frequency Identification (RFID)	• All parameters used for standard specimen labelling are to be followed. • The unique specimen bar code or RFID label must be consistent across all applications: specimen container, requisition label, cassette and slide labels. • Barcode and RIFD specifications within a failure rate established by your facility for patient care. • Barcode label stock or RFID chip validated to withstand chemicals and processing used for anatomic pathology specimens.	Laboratory General Checklist, GEN.40825 - Specimen ID	Zarbo RJ, Tuthill JM, D'Angelo R, et al. The Henry Ford Production System: reduction of surgical pathology in-process misidentification defects by bar code-specified work process standardization. *Am J Clin Pathol*. 2009; 131:469-477. Clinical Laboratory Standards Institute CSLI – Auto02-A2 Laboratory Automation: Bar Codes for Specimen Container Identification: 2006: Vol. 25 No 29.

	• Bar coding and/or RFID documentation must be validated and maintained. • Automatic identification scanning equipment is validated for accuracy and resistant to chemicals used for anatomic pathology handing. • If used for specimen chain of custody tracking, the barcode or RFID tracking system must have intelligent location capabilities.		
Collection and Handling C. Transport Media i. No media / saline	• Collection, handling and submission procedures must be made available to all health care workers involved in the collection, labeling, submission and transport of specimens to the pathology laboratory. • All specimens must be placed in leak proof container. • Specimens should be transported to the laboratory immediately after collection. • Specimens that cannot be immediately transferred must be refrigerated until transferred to the Pathology laboratory. • For specimens submitted to the laboratory from remote sites, there is a documented tracking system to ensure that all specimens are actually received. • Specimens transferred from distant referral site to pathology lab should be shipped under temperature-controlled conditions to avoid over heating or freezing	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements All Common Checklist, COM.06000, Specimen Collection Manual Laboratory General Checklist, GEN.74500 Specimen Transport Procedures Laboratory General Checklist, GEN.40125 – Handling of Referred Specimens Laboratory General Checklist, GEN.40511 - Specimen Tracking/Labeling Laboratory General Checklist, GEN.40535 - Specimen Transport QM	Clinical Laboratory Standards Institute CLSI – GP33A, Accuracy in Patient and Sample Identification; 2011: Vol 30 No7. International Standard ISO 15189:2012 - Medical Laboratories; section 16 Pre-examination.

	• Policies regarding courier service should be established		
	• All specimens must be properly packaged and labelled, indicating materials to be transported prior to shipping to a centralized or referral laboratory.	Laboratory General Checklist, GEN.40530 - Specimen Tracking	Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008
	• To avoid drying of tissues that are not immediately placed into formalin at time of procurement: ○ wrap solid tissue masses (i.e. lymph node or breast lump) in saline dampened gauze prior to placement in labelled container (certain biopsies may need special handling) ○ add a small volume of saline to tissue with insufficient naturally occurring fluids (i.e. conceptus for embryopathology/genetic studies)	Laboratory General Checklist, GEN.40535 - Specimen Transport QM	Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009

Collection and Handling C. Transport Media ii. Different fixatives	• Collection, handling and submission procedures must be made available to all health care workers involved in the collection, labelling, submission and transport of Specimens to the pathology laboratory.	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements All Common Checklist, COM.06000, Specimen Collection Manual	Clinical Laboratory Standards Institute CLSI - LIS09A, Standard guideline for coordination of clinical laboratory services within electronic health record environment and networked architectures; 2003: Vol. 23 No 15.
	• All specimens must be placed in leak proof container. • Specimens must be placed in appropriate fixative as specified in collection/handling and submission procedure. • Volume of fixative to tissue ratio must be included in the collection/handling and submission procedures. i.e. 10% neutral buffered formalin volume should be 15-20 times the volume of the specimen. • Safety Data Sheets (SDS) must be made available to all staff handling fixatives.	Laboratory General Checklist, GEN.74500 Specimen Transport Procedures Laboratory General Checklist, GEN.76100 Chemical Safety Document Access	International Standard ISO 15189:2012 - Medical Laboratories; section 5.4 - Pre-examination Processes. Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008 Carson F, Hladik-Cappellano C. Histotechnology A Self- Instructional Text, 4th ed. Chicago, IL: ASCP Press; 2014 Brown RW. et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009

	• All specimen containers containing fixatives must have appropriate OSHA Chemical labels attached. • Specimens transferred from distant referral site to Pathology lab should be shipped under temperature-controlled conditions to avoid over heating or freezing. Specimens containers should be shipped following appropriate regulations for the shipping and handling of formalin i.e. hard sided container with absorbent packing material.	Laboratory General Checklist, GEN.40125 – Handling of Referred Specimens Laboratory General Checklist, GEN.40511 - Specimen Tracking/Labeling Laboratory General Checklist, GEN.40535 - Specimen Transport QM	Clinical Laboratory Standards Institute CLSI – GP 17-A3, Clinical Laboratory Safety, 3rd edition; 2012: Vol 32 No 9. Occupational Health and Safety Administration. Occupational Safety & Health Standards 1910.1200 toxic and Hazardous Substances. http://www.osha.gov/dsg/hazcom/index.html
Collection and Handling D. Completion of requisition i. Patient identifiers	• Written procedures on how to properly complete a pathology requisition must be made available to all health care workers involved in the collection, labelling, submission and transport of specimens to the pathology laboratory. • Written or electronic request for patient testing from authorized person.	Laboratory General Checklist, GEN.40700 - Requisitions Laboratory General Checklist, GEN.40930 - Authorized Requestor	

	• Required patient identifiers to be included on the requisition / test order: ○ Patient's name ○ Unique identifier i.e. health record or master index number ○ Date of Birth ○ Sex	Laboratory General Checklist, GEN.40750 - Requisition Elements	Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2011: Vol 30 No7. International Standard ISO 15189:2012 - Medical Laboratories; section 5.4- Pre-examination Processes.
Collection and Handling D. Completion of requisition ii. Specimen name/type/site	• Written or electronic request for patient testing to include: ○ Patient identifiers as listed above ○ Name and address or other suitable identifiers of the authorized person requesting the test ○ Name and address or other suitable identifier for the individual responsible for receiving the test results ○ Name and address of the laboratory submitting the specimen ○ Test and or tests to be performed ○ Procedure performed ○ Specimen site – if more than one specimen is collected during a single procedure; each specimen should be individually identified by anatomic site and or specimen type ○ Date and time of procedure or specimen collection ○ Date specimen received	Laboratory General Checklist, GEN.40930 - Authorized Requestor Laboratory General Checklist, GEN.40750 - Requisition Elements Laboratory General Checklist, GEN.40900 - Specimen Date Received	Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2011: Vol 30 No7. International Standard ISO 15189:2012 - Medical Laboratories; section 5.4 - Pre-examination Processes

Collection and Handling D. Completion of requisition iii. Pertinent clinical history	• Written or electronic request for patient testing to include: ○ Clinical history – any additional information relevant or necessary for a specific test to ensure accurate and timely testing and reporting of results, including interpretation if required.	Laboratory General Checklist, GEN.40750 - Requisition Elements	Health Insurance and Portability and Accountability Act (HIPAA). Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2011: Vol 30 No7. International Standard ISO 15189:2012 - Medical Laboratories; section 5.4- Pre-examination Processes
Collection and Handling D. Completion of requisition iv. Procedure time/date a. Time removed from patient (Warm ischemic time)	• The procedure date should be indicated on the requisition following standardized format DD - MM - YYYY (i.e. 04 JAN 2012). • The requisition must have a space for the documentation of the warm ischemic time by the physician obtaining the specimen or designate. • Warm ischemic time: The time measured from the interruption of the blood supply to the tissue/tumor by the surgeon to the excision time of the tissue specimen. • Information should be available in the laboratory for review and/or appear on the patient accession.	Laboratory General Checklist, GEN.40750 - Requisition Elements	Allison KH, Hammond EH, Dowsett M, McKernin SE et al. Estrogen and Progesterone Receptor Testing in Breast Cancer American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Path Lab Med*. Early Online Release. doi: 10.5858/arpa.2019-0904-SA International Standard ISO 20166-4:2020 - Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue for – Part 4: In situ detection techniques: section 6 – Inside the laboratory.

Collection and Handling D. Completion of requisition iv. Procedure time/date b. Time fixative added (if required) (cold ischemic time)	• The requisition should have a space for the documentation of the cold ischemic time by the physician obtaining the specimen or designate. • Cold ischemic time: The time from excision of the specimen from the surgical field to the time the tissue is placed in fixative. • Information should be available in the laboratory for review and/or appear on the patient accession. • The requisition should have a space for the documentation of the date and time the specimen is placed in fixative by the physician obtaining the specimen or designate.	Anatomic Pathology Checklist, ANP.22983 – Fixation – HER2 and ER Predictive Marker Testing Laboratory General Checklist, GEN.40125 – Handling of Referred Specimens	Allison KH, Hammond EH, Dowsett M, McKernin SE et al. Estrogen and Progesterone Receptor Testing in Breast Cancer American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Path Lab Med*. Early Online Release. doi: 10.5858/arpa.2019-0904-SA Clinical Laboratory Standards Institute CLSI – MM13, Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: 2020. Compton CC, Robb JA, Anderson MW, Berry AB, et.al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Path Lab Med*. Nov 2019, Vol. 143, No. 11 (November 2019) pp. 1346-1363. International Standard ISO 20166-4:2020 - Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue for – Part 4: In situ detection techniques: section 6 – Inside the laboratory.

Collection and Handling D. Completion of requisition iv. Procedure time/date c. Time received in lab (Transport time)	• The requisition must have a space for documentation of the date and time of arrival of the specimen in the AP laboratory to allow for calculation of the transport time. • Transport time: The time tissue specimen was collected in the operating room/doctor's office/clinic until it is received in the pathology laboratory for processing (this is the time point when the specimen is going to be grossly assessed). • Information must be available in the laboratory for review and/or appear on the patient accession.	Laboratory General Checklist, GEN.40535 - Specimen Transport QM Laboratory General Checklist, GEN.40530 - Specimen Tracking	Allison KH, Hammond EH, Dowsett M, McKernin SE et al. Estrogen and Progesterone Receptor Testing in Breast Cancer American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Path Lab Med*. Early Online Release. doi: 10.5858/arpa.2019-0904-SA Clinical Laboratory Standards Institute CLSI – MM13, Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: 2020.
Collection and Handling D. Completion of requisition iv. Procedure time/date d. Calculation of total fixation time	• The laboratory has the responsibility to calculate and document total time the specimen was kept in fixative for required specimens (i.e. breast). To include: ○ Time specimen held in the operating room ○ Transport time from remote site to AP lab ○ Time the specimen was kept in fixative while in the lab (i.e. large specimens like colon, breast mastectomy were opened/cut to allow for penetration of fixative) ○ Time the specimen(s) are kept in cassettes after grossing ○ Time in fixative onboard the tissue processor	Anatomic Pathology Checklist, ANP.22983 – Fixation – HER2 and ER Breast Cancer Predictive Marker Testing	Allison KH, Hammond EH, Dowsett M, McKernin SE et al. Estrogen and Progesterone Receptor Testing in Breast Cancer American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Path Lab Med*. Early Online Release. doi: 10.5858/arpa.2019-0904-SA Wolff AC, Hammond EH, Hicks,DG, Dowsett,M, et al: American Society of Clinical Oncology/College of American Pathologists Guideline Update Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer,*Journal of Clinical Oncology*, Vol 31, No. 31, Nov1 2013: pp. 3997-4013.

<table>
<tr>
<td>Collection and Handling
D. Completion of requisition
iv. Procedure time/date
e. Fixation time for breast tissue specimens</td>
<td>

Tissue handling requirements should be standardized and reported on every specimen.
10 % neutral buffered formalin is the recommended fixative.
All samples must receive a minimum of six (6) hours of 10% neutral buffered formalin fixation
Recommended fixation time is 6-72 hrs. for estrogen and progesterone receptors.
Recommended fixation time is 6 to 72 hours for Her2neu receptors.
Fixation time must be documented, and the following is an example of how the data could be recorded on the requisition:

<table>
<tr><th>Time frame</th><th>Minutes</th><th>Hours</th></tr>
<tr><td>Warm ischemic time</td><td></td><td></td></tr>
<tr><td>Cold ischemic time</td><td></td><td></td></tr>
<tr><td>Transport time from OR /physician office /clinic to laboratory to time of primary examination</td><td></td><td></td></tr>
<tr><td>Time whole specimen held for additional fixation prior to placing in cassettes</td><td></td><td></td></tr>
<tr><td>Time cassettes are held prior to loading onto tissue processor</td><td></td><td></td></tr>
<tr><td>Fixation time on tissue processor (delay time plus processing time)</td><td></td><td></td></tr>
<tr><td>Total Fixation time</td><td></td><td></td></tr>
</table>
</td>
<td>Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements

Laboratory General Checklist, GEN.40125 – Handling of Referred Specimens

Anatomic Pathology Checklist, ANP.22983 – Fixation - HER2 and ER Predictive Marker Testing

Anatomic Pathology Checklist, ANP.23004 - Digital Imaging – Preanalytic Testing Phase Validation</td>
<td>Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Path Lab Med; Nov 2018, Vol. 142, No. 11. pp. 1364-1382

Allison KH, Hammond EH, Dowsett M, McKernin SE et al. Estrogen and Progesterone Receptor Testing in Breast Cancer American Society of Clinical Oncology/College of American Pathologists Guideline Update. Arch Path Lab Med. Early Online Release. doi: 10.5858/arpa.2019-0904-SA

Clinical Laboratory Standards Institute CLSI – MM13, Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: 2020.

Werner M, Chott A, Fabiano A, Battifora H. Effect of Formalin Tissue Fixation and Processing on Immunohistochemistry. American Journal of Surgical Pathology. 24. July 2000:1016-1019.

Spruessel A, Steimann G, Jung M, Lee SA, Carr T, Fentz AK, Spangenberg J, Zornig C, Juhl HH, David KA. Tissue ischemia time affects gene and protein expression patterns within minutes following surgical tumor excision BioTechniques, Vol. 36, No. 6, June 2004:1030–1037.</td>
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			Petersen BL, Sorensen MC, Pedersen S, Rasmussen M. Fluorescence In-situ Hybridization on Formalin-fixed and Paraffin-Embedded Tissue: Optimizing the Method. *Applied Immunohistochemistry & Molecular Morphology*. 12(3) September 2004:259-265. Tanney A, Kennedy RD. Developing mRNA-based biomarkers from formalin-fixed paraffin-embedded tissue. *Personalized Medicine* (2010) **7**(2), 205–211. Compton CC, Robb JA, Anderson MW, Berry AB, et.al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Path Lab Med.* Nov 2019, Vol. 143, No. 11 pp. 1346-1363.
Collection and Handling D. Completion of requisition iv. Procedure time/date f. Fixation time for NON-breast specimens	• Establish standardized fixation times for all routine and specialized biopsies. • Document the recommended fixative for routine and specialized biopsies. • Establish specimen acceptance and rejection policies related to specimen fixation.	All Common Checklist, COM.06300 – Specimen Rejection Criteria	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): [42CFR493.1283(a)(3)] Compton CC, Robb JA, Anderson MW, Berry AB, et.al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Path Lab Med.* Nov 2019, Vol. 143, No. 11 (November 2019) pp. 1346-1363.

Collection and Handling D. Completion of requisition v. Requesting physician a. contact information available in LIS	• When alternate identifier is used for authorized person requesting test or receiving test results (medical billing number, hospital ID number), the number must be unique and traceable in the LIS.	Laboratory General Checklist, GEN.40750 - Requisition Elements	Health Insurance and Portability and Accountability Act (HIPAA). Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2011: Vol 30 No7. International Standard ISO 15189:2012 - Medical Laboratories; section 5.4 - Pre-examination Processes.
Collection and Handling E. Recommendations for Tissue Collection and Handling i. Limiting Artifacts a. Thermal injury	• The use of surgical instruments driven by heat should be avoided or limited when possible. • Thermal injury has been known to interfere with diagnosis.	.	Association of Surgical Technologists (AST) Recommended Standards of Practice for Handling and Care of Surgical Specimens. www.ast.org
Collection and Handling E. Recommendations for Tissue Collection and Handling i. Limiting Artifacts b. Crush injury	• The use of surgical instruments should be avoided or limited as much as possible when handing the specimen to prevent crushing or damaging the tissue.		Association of Surgical Technologists (AST) Recommended Standards of Practice for Handling and Care of Surgical Specimens. http://www.ast.org

Collection and Handling E. Recommendations for Tissue Collection and Handling i. Limiting Artifacts c. Drying artifact	• All tissue should be placed in fixative as soon as possible after removal from the body, unless special studies are ordered that might be affected by the available fixative. • If fixative cannot be added in a timely manner, the specimen should be placed in a sterile basin and kept moist with sterile saline or wrapped in saline-dampened sponges until the specimen can be properly placed in fixative. • All unfixed specimens should be transported to the pathology laboratory as soon as possible and refrigerated until placed into appropriate fixative.	Anatomic Pathology Checklist, ANP.11250 - Adequate storage Laboratory General Checklist, GEN.40535 - Specimen Transport QM	Association of Surgical Technologists (AST) Recommended Standards of Practice for Handling and Care of Surgical Specimens. www.ast.org Makary MA, Epstein J, Pronovost PJ, Millman EA, Hartmann EC, Freischlag JA. Surgical specimen identification errors: A new measure of quality in surgical care. *Surgery*. 2007.141:450-455.
Collection and Handling E. Recommendations for Tissue Collection and Handling ii. Tissue Transport a. All fresh specimens	• Health care facility policy and procedure should be followed for the proper collection, labeling, and transportation of the specimen to the pathology department. • All fresh specimens are to be submitted to the pathology department as soon as possible with instructions for special testing or processes. • All unfixed specimens should be transported to the pathology laboratory as soon as possible and refrigerated until placed into appropriate fixative. • Specimens not in fixative should be placed in a sterile basin and kept moist with sterile saline or wrapped in saline-soaked sponges until the specimen can be properly placed in fixative. • Confirmation with surgeon on other types of diagnostic studies to be performed, including Gram stain, acid fast and mycological studies.		Clinical Laboratory Standards Institute CLSI – MM13, Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: 2020. Makary MA, Epstein J, Pronovost PJ, Millman EA, Hartmann EC, Freischlag JA. Surgical specimen identification errors: A new measure of quality in surgical care. Surgery. 2007.141:450-455. Slavin L, Best MA, Aron DC. Gone but not forgotten: The search for the lost surgical specimens: Application of quality improvement techniques for reducing medical error. Quality Management in Health Care. 2001. 10(1): 45-53.

	• Exceptions to immediate delivery of tissue specimen must be clearly described in the policies and procedures. (Example: Placentas must be refrigerated until delivery).	Anatomic Pathology Checklist, ANP 10016 - Surgical Pathology Exclusion	The Joint Commission. (2014). 2014 National Patient Safety Goals Hospital Program. US Dept of Health and Human Services. Summary of the HIPAA privacy rule. 2003. World Health Organization. Guidelines for the safe transport of infectious substances and diagnostic specimens. 1997. Carson F, Hladik C. Histotechnology A Self-Instructional Text, 3rd ed. Chicago, IL: ASCP Press 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008
Collection and Handling E. Recommendations for Tissue Collection and Handling ii. Tissue Transport b. Specimens in fixative	• Specimen in fixative must be delivered to the pathology laboratory according to the Health care facility policies and procedures. • Special guidelines are required for the handling of breast tissues to ensure fixation guidelines are met. (please see section D, iv, e for specific fixation times) • Containers should be rigid, impermeable, unbreakable and non-reactive to fixative solutions.	Laboratory General Checklist, GEN.40535 - Specimen Transport QM Anatomic Pathology Checklist, ANP.22983 – Fixation - HER2 and ER Predictive Marker Testing Laboratory General Checklist, GEN.40942 – Specimen Container Analytic Interference	Association of Surgical Technologists (AST) Recommended Standards of Practice for Handling and Care of Surgical Specimens. www.ast.org World Health Organization. Guidelines for the safe transport of infectious substances and diagnostic specimens. 1997.
Collection and Handling E. Recommendations for Tissue Collection and	• Documentation of fixation time for Breast specimens is required as outlined in section C.		Compton CC, Robb JA, Anderson MW, Berry AB, et.al. Preanalytics and Precision Pathology: Pathology

Handling ii. Tissue Transport c. Monitoring of time and environmental parameters during transport	• All specimens are received in the pathology laboratory according to the policies and procedures approved, to include the acceptance of specimen protocol as time received, accessioned and grossed. • Specimen placed in different environment, i.e. dry ice, must be recorded and delivered with specimen.	Anatomic Pathology Checklist, ANP.22983 – Fixation – HER2 and ER Predictive Marker Testing Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements Laboratory General Checklist, GEN.40125 – Handling of Referred Specimens Laboratory General Checklist, GEN.40535 - Specimen Transport QM	Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Path Lab Med.* Nov 2019, Vol. 143, No. 11 pp. 1346-1363. Allison KH, Hammond EH, Dowsett M, McKernin SE et al. Estrogen and Progesterone Receptor Testing in Breast Cancer American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Path Lab Med*. Early Online Release. doi: 10.5858/arpa.2019-0904-SA Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Path Lab Med; Nov 2018, Vol. 142, No. 11. pp. 1364-1382 AST Recommended Standards of Practice for Handling and Care of Surgical Specimens. The Joint Commission. (2014). 2014 National Patient Safety Goals Hospital Program.

Collection and Handling E. Recommendations for Tissue Collection and Handling ii. Tissue Transport d. Chain of custody 1. Specimen removal from origin of Collection (time/date)	• Chain of custody ensures continuity of quality care for the patient and provides a method to retrieve needed information. • All specimens must be recorded on a chain of custody form or log that includes dates and times, patient identification, specimen number, specimen description, and purpose for specimen delivery to the pathology department.		The Joint Commission. (2014). 2014 National Patient Safety Goals Hospital Program. US Dept of Health and Human Services. Summary of the HIPAA privacy rule. 2003. World Health Organization. Guidelines for the safe transport of infectious substances and diagnostic specimens. 1997.
Collection and Handling E. Recommendation for tissue collection and handling ii. Tissue Transport d. Chain of custody 2. Personnel transporting specimen (name/title/date)	• It is advisable that chain of custody include the personnel involved in the handling and transportation of the specimen to the pathology lab and within the pathology lab during testing procedures. ○ Name of transporter ○ Title (i.e. RN, Surgical Tech, MD) ○ Dates: Collection, transported and received		The Joint Commission. (2014). 2014 National Patient Safety Goals Hospital Program. US Dept of Health and Human Services. Summary of the HIPAA privacy rule. 2003. World Health Organization. Guidelines for the safe transport of infectious substances and diagnostic specimens. 1997.

Collection and Handling E. Recommendation for tissue collection and handling ii. Tissue Transport d. Chain of custody 3. Specimen receipt by laboratory (date/time/name)	• Specimen receipt procedure must be available to all personnel in the pathology department. • All specimens must be signed off on the chain of custody form carried by the transporter and logged into the LIS system of the pathology department for accessioning. • The pathology lab must have a logging system that identifies the person receiving the specimen, the date and time received. • The pathology lab must have a process for documenting who handles the original specimen and all sub-specimens throughout the entire examination, testing and reporting process.	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements Laboratory General Checklist, GEN.40900 – Specimen Date Received	The Joint Commission. (2014). 2014 National Patient Safety Goals Hospital Program. US Dept of Health and Human Services. Summary of the HIPAA privacy rule. 2003. World Health Organization. Guidelines for the safe transport of infectious substances and diagnostic specimens. 1997. Association of Surgical Technologists (AST) Recommended Standards of Practice for Handling and Care of Surgical Specimens. www.ast.org
Collection and Handling E. Recommendation for tissue collection and handling ii. Tissue Transport e. Quality Assurance Monitors 1. Labeling discrepancies	• A policy and procedure must be made available that identify the process to follow for labeling discrepancies. • In some instances, the specimen can be considered to be a rejection specimen and only the originator should be making the appropriate labeling changes. • Label and requisition must be a match. Common mistakes are gender or site. • Records of all errors should be maintained.	All Common Checklist, COM.06100 – Primary Specimen Container Labeling All Common Checklist, COM.06200 - Secondary Specimen Container Labeling Laboratory General Checklist, GEN.40492 - Specimen Labeling Correction All Common Checklist, COM.06300 - Specimen Rejection Criteria	Association of Surgical Technologists (AST) Recommended Standards of Practice for Handling and Care of Surgical Specimens. www.ast.org

Collection and Handling E. Recommendation for tissue collection and handling ii. Tissue Transport e. Quality Assurance Monitors 2. Specimen rejection criteria	• The pathology department must have a policy and procedure that handles specimen acceptance and rejection • The information on the specimen container must match the information submitted on the requisition form. • Grounds for rejection may include: ○ Wrong name ○ Wrong site ○ Wrong identifiers ○ State of specimen	All Common Checklist, COM.06300 – Specimen Rejection Criteria	The Joint Commission. (2014). 2014 National Patient Safety Goals Hospital Program. US Dept of Health and Human Services. Summary of the HIPAA privacy rule. 2003. 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): [42CFR493.1283(a)(3)] World Health Organization. Guidelines for the safe transport of infectious substances and diagnostic specimens. 1997. Association of Surgical Technologists (AST) Recommended Standards of Practice for Handling and Care of Surgical Specimens. www.ast.org
Collection and Handling E. Recommendation for tissue collection and handling ii. Tissue Transport e. Quality Assurance Monitors 3. Tissue Acceptance	• The specimen collection and handling procedures should include the parameters for specimens deemed acceptable. ○ Identification of the patient sample (labeling) ○ Completion of the requisition to include all required demographic and clinical data ○ Specimen container to be used ○ Type and volume of fixation ○ Transport packing, temperature and method ○ Additional specialized instructions	All Common Checklist, COM.06300 – Specimen Rejection Criteria	International Standard ISO 20166-4:2020 - Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue for – Part 4: In situ detection techniques: section 6 – Inside the laboratory. The Joint Commission. (2014). 2014 National Patient Safety Goals Hospital Program.

			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): [42CFR493.1283(a)(3)] Association of Surgical Technologists (AST) Recommended Standards of Practice for Handling and Care of Surgical Specimens. www.ast.org Carson F, Hladik C. Histotechnology A Self-Instructional Text, 3rd ed. Chicago, IL: ASCP Press 2009.
Collection and Handling E. Recommendation for tissue collection and handling iii. Specimen specific recommendations 1. Specialized biopsies	• A policy and procedure should be made available that identify the process to follow for different types of specimens/biopsies: ○ Muscle - enzyme studies ○ Renal/Skin - Immunofluorescence ○ Nerve/CNS ○ Cardiac ○ Lymphatic tissue - mercuric fixative; thinner sections, etc. ○ Specimens that contain radioactive implants	Anatomic Pathology Checklist, ANP.11670 - Specimen- Gross Examination Anatomic Pathology Checklist, ANP.11275 - Radioactive Material Handling	Clinical Laboratory Standards Institute CLSI MM13-A: Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline; 2005:Vol 25 No31 Carson F, Hladik C. Histotechnology A Self-Instructional Text, 3rd ed. Chicago, IL: ASCP Press 2009 AFIP, Laboratory Methods in Histotechnology.
Collection and Handling E. Recommendation for tissue collection and handling iii. Specimen specific recommendations	• Health care facility policy and procedure should be followed for the proper collection and handling of general biopsies. Procedures to include: ○ Type of collection container ○ Type and volume of fixative ○ Transport and holding instructions • All fresh biopsies not needing special handling are to be submitted to the pathology department immediately for processing.	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements	Carson F, Hladik C. Histotechnology A Self-Instructional Text, 3rd ed. Chicago, IL: ASCP Press 2009. Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008.

2. General biopsies	• If this cannot be completed in a timely manner, the biopsy should be placed in a sterile container and kept moist with sterile saline or wrapped in saline-dampened sponges until the biopsy can be properly placed in fixative • Specimens must be placed in appropriate fixative as specified in collection/handling and submission procedure.		The Joint Commission. (2011). 2011 National Patient Safety Goals Hospital Program. Makary MA, Epstein J, Pronovost PJ, Millman EA, Hartmann EC, Freischlag JA. Surgical specimen identification errors: A new measure of quality in surgical care. Surgery. 2007.141:450-455.
Collection and Handling E. Recommendation for tissue collection and handling iii. Specimen specific recommendations 3. Bone marrows	• Health care facility policy and procedure should be followed for the proper collection and handling of bone marrow cores and aspirates. • Bone marrow cores/aspirates should be placed in fixative immediately after the procedure. • Bone marrow cores/aspirates should be stored at room temperature. • Cores/aspirates must be received in the laboratory, as soon as possible, for immediate handling according to written protocols.	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements	Carson F, Hladik C. Histotechnology A Self-Instructional Text, 3rd ed. Chicago, IL: ASCP Press 2009. Foucar, KM, Bone Marrow Pathology. 2nd ed. Chicago, IL, ASCP Press: 2001.
Collection and Handling E. Recommendation for tissue collection and handling iii. Specimen specific recommendations 4. Large specimen(s)	• Health care facility policy and procedure should be followed for the proper collection and handling of specimens. Procedures to include: ○ Type of collection container ○ Type and volume of fixative or no fixative ○ Transport and holding instructions • All fresh specimens are to be submitted to the pathology department immediately with instructions for special testing or processes. • Large specimens require a longer amount of time for tissue to be properly fixed (Ex. Uterus, spleen, lung, liver, etc.) • Breast tissue must follow the ASCO guidelines for strict fixation timing and processing. (please see section D, iv, e for specific fixation times)	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements. Anatomic Pathology Checklist, ANP.22983 – Fixation – HER2 and ER Predictive Marker Testing	American Society of Clinical Oncology. (2013). ASCO Guidelines. Retrieved December 18, 2013, from American Society of Clinical Oncology (ASCO): http://www.asco.org/Guidelines/ Lester, S. C. (2010). Manual of Surgical Pathology (3rd ed.). Saunders.

	• Placentas should be refrigerated until delivery to the pathology department.	Anatomic Pathology Checklist, ANP.11250 Adequate Storage	Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Path Lab Med*; Nov 2018, Vol. 142, No. 11. pp. 1364-1382
HANDLING PRIOR TO GROSS	**HANDLING PRIOR TO GROSS**		
Guideline Section	Statement	CAP Checklist	Reference
Collection and Handling F. Accessioning i. Specimen Identifiers and Labelling	• Specimen must be identified/labeled following parameters identified in section B. • Each specimen container received must be compared to the requisition to ensure correct match of at least 2 patient-specific identifiers: ○ Full patient name ○ Assigned identification number e.g. health record / master index number ○ Date of Birth • Additional requisition information to be checked: ○ Number of specimen containers ○ Type of specimens submitted ○ Complete clinical history ○ Name of requesting physician to return report to ○ Collection data related to fixation (section D)	All Common Checklist, COM.06100 – Primary Specimen Container Labeling All Common Checklist, COM.06200 - Secondary Specimen Container Labeling Laboratory General Checklist, GEN.40490 - Patient Identification	Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2011:Vol 30 No7. International Standard ISO 15189:2012 - Medical Laboratories; section 5.4 - Pre-examination Processes Zarbo RJ, Tuthill JM, D'Angelo R, et al. The Henry Ford Production System: reduction of surgical pathology in-process misidentification defects by bar code-specified work process standardization. Am J Clin Pathol. 2009; 131:469-477
Collection and Handling F. Accessioning ii. Accessioning order a. Avoiding Error	• It is good laboratory practice to avoid accessioning like-specimens back to back • If like specimens must be accessioned in sequence it is suggested to separate by size (e.g. skin punch biopsy followed by skin excision followed by skin punch	All Common Checklist, COM.06100 – Primary Specimen Container Labeling	

	biopsy) or to be identified by use of multi colored inks (punch one black ink, punch two is green ink, punch three blue ink etc.)	All Common Checklist, COM.06200 - Secondary Specimen Container Labeling Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements	

Collection and Handling
 	
G. Handling prior to Gross Examination
	• There should be sufficient space available in the surgical pathology suite to store surgical specimens in an orderly fashion after accessioning, and prior to gross examination:
	  ○ Space for the containers and accompanying paperwork/request slips.
	  ○ Storage area should be clean, free of clutter, and well ventilated.
		Laboratory General Checklist, GEN.60000 - Adequate Space
		
		Laboratory General Checklist, GEN.60100 - Adequate Space
		
		Anatomic Pathology Checklist, ANP.11250 Adequate Storage	

Collection and Handling
 	
G. Handling prior to Gross Examination
 	
i. Immediate Gross Examination and Handling
	• Site specific documentation on how to handle specimens requiring immediate gross examination (i.e., microbiological cultures, electron microscopy, cytogenetics, flow cytometry or other special studies) must be available to all staff handling the specimens and should include:
	  ○ Specialized grossing techniques i.e. sterile procedures
	  ○ Sample collection for submission into specialized media i.e. cytogenetic or EM
	  ○ Requisition completion for further testing i.e. microbiology or pathology referral lab
	  ○ Labeling procedure for sub - specimens
	  ○ Holding and transport instructions for specialized testing (i.e. refrigerate)
	  ○ Specimen cross contamination
	
	• Specimens submitted fresh for immediate gross examination (i.e., frozen sections, margin determination, etc.) should be kept in their labeled containers at room temperature
		Anatomic Pathology Checklist, ANP.11670 - Specimen Gross Examination
		
		Anatomic Pathology Checklist, ANP.11600 - Gross Examination – Pathologist
		
		Anatomic Pathology Checklist, ANP.11605 - Gross Examination – Non-Pathologist
		
		Anatomic Pathology - ANP.11680 – Cross Contamination - Grossing
		
		Anatomic Pathology Checklist, ANP.11810 - Frozen Section Preparation Quality
		
		Anatomic Pathology Checklist, ANP.11670 - Specimen Gross Examination
		
		All Common Checklist, COM.06100 – Primary Specimen Container Labeling
			Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 1992(Feb 28):7183 [42CFR493.1489(b)(6)]

	• If there is a delay, the fresh specimen should be kept in its labeled container and refrigerated until it can be examined. • Written procedure to prevent cross contamination	All Common Checklist, COM.06200 - Secondary Specimen Container Labeling Anatomic Pathology Checklist, ANP.11250 - Adequate Storage Anatomic Pathology - ANP.21397 – Cross Contamination - Histology	
Collection and Handling G. Handling prior to Gross Examination ii. Delayed time to Gross Examination	• Specimens in fixative requiring gross examination should be assembled/stored in an orderly fashion after accessioning, with appropriate paperwork/request slips and labeled cassettes available. • The containers should be sealed to avoid spillage, loss of fixative, loss of specimen, and to prevent drying of the specimen prior to gross examination.	Anatomic Pathology Checklist, ANP.11600 - Gross Examination - Pathologist Anatomic Pathology Checklist, ANP.11605 - Gross Examination – Non-Pathologist Laboratory General Checklist, GEN.40125 – Handling of Referred Specimens	
Collection and Handling G. Handling prior to Gross Examination ii. Delayed time to Gross Examination a. Monitoring of Environmental Parameters	• An appropriate room temperature should be maintained, so that specimens are neither frozen nor damaged by excessive heat. • Appropriate ventilation should be maintained so that there is adequate air movement around the specimen containers, without buildup of fixative or other noxious vapors.	Laboratory General Checklist, GEN.61300 - Climate Control Laboratory General Checklist, GEN.76720 - Formaldehyde and Xylene Safety	
Collection and Handling G. Handling prior to Gross Examination ii. Delayed time to Gross Examination	• Adequate fixative should be added to the specimen container as soon as possible. If insufficient fixative is present when the specimen is received in the laboratory additional fixative should be added.	Laboratory General Checklist, GEN.40125 – Handling of Referred Specimens	Carson F, Hladik-Cappellano C. Histotechnology A Self-Instructional Text, 4th ed. Chicago, IL: ASCP Press 2014.

b. Addition of fixative to specimen(s)	• Generally, this should be a volume such that there is a 15-20:1 ratio of fixative to tissue specimen. If a large specimen (i.e., uterus, colon, breast, etc.) is submitted, the specimen should be opened or regularly sliced and covered or wrapped in an absorptive material (i.e., paper towels, etc.) to maximize surface exposure to fixative reagents. • The specimen container should remain sealed so that drying or other specimen damage cannot occur.		Brown RW. et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008.
Collection and Handling H. Intra-Operative Consultation (I.e., Frozen Sections)	• Health care facility policy and procedure should be followed for the proper collection and handling of specimens for intra-operative consultation. Procedures to include: ○ Gross examination only. ○ Frozen sections ○ Touch preps, scrap preps • All intra-operative consultation results and tissue diagnoses are made and signed by a pathologist. • Reagents and slides used for intra-operative consultation are properly labeled. • Intra-operative consultation preparations are adequate for diagnosis.	Anatomic Pathology Checklist, ANP.11670 - Specimen – Gross Examination Anatomic Pathology Checklist, ANP.11850 - Intra-Operative Results Anatomic Pathology Checklist, ANP.11660 - Pathologist Diagnosis Anatomic Pathology Checklist, ANP.11756 - Reagents All Common Checklist, COM.06100 – Primary Specimen Container Labeling All Common Checklist, COM.06200 - Secondary Specimen Container Labeling Anatomic Pathology Checklist, ANP.11810 - Frozen Section Preparation Quality	Cibull ML. Q&A. Northfield, IL: College of American Pathologists CAP Today. 1997;11(7):112

	• Intra-operative slides are retained and made part of the permanent case. • Residual tissue(s) used for intra-operative examination are processed into paraffin for comparison with the frozen section interpretation.	Anatomic Pathology Checklist, ANP.12050 - Frozen Section Slides Anatomic Pathology Checklist, ANP.12075 - Residual Frozen Tissue Anatomic Pathology Checklist, ANP.12500 - Record Retention	Nakhleh RE, Fitzgibbons PL, editors. College of American Pathologists. Quality improvement manual in anatomic pathology, 2nd edition. Northfield, IL: CAP, 2002 Rickert RR. Quality assurance goals in surgical pathology. Arch Pathol Lab Med. 1990;114:1157-1162 Association of Directors of Anatomic and Surgical Pathology. Recommendations on quality control and quality assurance in anatomic pathology. Am J Surg Pathol. 1991;15:1007-1009 Gephardt GN, et al. Interinstitutional comparison of frozen section consultations. A College of American Pathologists Q-probes study of 90 538 cases in 461 institutions. Arch Pathol Lab Med. 1996;120:804-809 Novis DA, et al. Interinstitutional comparison of frozen section consultation in small hospitals. Arch Pathol Lab Med. 1996;120:10871093 Nakhleh RE, Fitzgibbons PL, editors. College of American Pathologists. Quality improvement manual in anatomic pathology, 2nd edition. Northfield, IL: CAP, 2002

Collection and Handling H. Intra-Operative Consultation i. Reporting	• When giving a verbal report, the pathologist must be able to speak directly with intra-operative medical/surgical personnel. • The patient's identification is checked and confirmed before delivery of any verbal report. • All intra-operative consultation reports are made a part of the final surgical pathology report.	Anatomic Pathology Checklist, ANP.11900 - Verbal Reports Anatomic Pathology Checklist, ANP.11950 - Verbal Report/Patient ID Anatomic Pathology Checklist, ANP.12000 - Final Report	
Collection and Handling H. Intra-Operative Consultation ii. Cryostat decontamination	• There is a documented procedure for the routine decontamination of the cryostat at defined intervals. • Decontamination of the cryostat is documented, and records are available for examination.	Anatomic Pathology Checklist, ANP.23410 - Cryostat Decontamination	Clinical Laboratory Standards Institute CLSI. Protection of Laboratory Workers from Occupational Acquired Infections, Approved Guideline M29-A4; 2014;Vol34 No8 http://www.epa.gov/oppad001/list_b_tuberculocide.pdf
Collection and Handling H. Intra-Operative Consultation iii. Hematoxylin and Eosin stain (H&E) Stain	• Establish operation procedures for H&E staining: ○ Reagents to be used – concentration and volumes ○ Staining schedule for each staining program ○ Rotation or change schedule for the reagents ○ Disposal and or recycle process for reagents • Establish quality assurance criteria for the staining and evaluation of H&E staining.	Laboratory General Checklist, GEN.77800 – Hazardous Chemical Waste Disposal Anatomic Pathology Checklist, Quality Control, ANP.11756 - Reagents All Common Checklist, COM.30400 – Reagent Expiration Date Anatomic Pathology Checklist, ANP.11734 – Slide Quality	Lott RL. HQIP: H&E Staining. HQIP - A Final Critique. Chicago, IL: College of American Pathologists; 2010. Brown RW. et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009 Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009

			Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008 Sheehan DC, Hrapchak BB., Theory and Practice of Histotechnology, 2nd ed. Columbus, OH: Battelle Press; 1980 Horobin RW. Troubleshooting Histology Stains, 1998, Churchill Livingstone

PART II	II. LABORATORY PROCESSES - Guidelines		
Guideline Section	Statement	CAP Checklist	Reference
Laboratory Processes A. Guidelines i. Facility Requirements	• The laboratory has sufficient space and utilities are adequate for gross examination and specimen storage. • Gross examination area has adequate lighting.	Anatomic Pathology Checklist, ANP.11250 - Adequate Storage. Laboratory General Checklist, GEN.60150 – Adequate Space Laboratory General Checklist, GEN.60250 – Working Environment	Clinical Laboratory Standards Institute CLSI: QMS01-A4: Quality Management System: A Model for Laboratory Services; Approved Guideline, 4th Edition 2011,Vol31 No15

	• Gross examination area has adequate ventilation system, with policy for monitoring exposure levels to formalin.
	• Formalin exposure level of grossing personnel should be examined annually to assure proper ventilation.
	• Grossing area should have readily available:
	  ○ Photographic equipment
	  ○ Dictation system (unless grossing personnel enters gross dictation directly into electronic laboratory information system)
	  ○ Access to anatomic pathology laboratory information system
	  ○ Access to diagnostic imaging PACS system if located in a clinical hospital setting
		Laboratory General Checklist, GEN.76720 - Formaldehyde and Xylene Safety	

Laboratory Processes A. Guidelines ii. Personnel	• All macroscopic tissue examinations are performed by a pathologist or pathology resident, or under the supervision of a qualified pathologist. • Activities and the nature of supervision is defined in a written protocol Qualification requirements for non-pathologist or pathology resident personnel who assist in gross examination of specimens: • *An earned associate degree in laboratory science or medical laboratory technology, obtained from an accredited institution, OR* • *Education/training equivalent to the above that includes at least 60 semester hours or equivalent from an accredited institution.*	Anatomic Pathology Checklist, ANP.11600 - Gross Examination - Pathologist. Anatomic Pathology Checklist, ANP.11605 - Gross Examination - Non-Pathologist.	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], 1071-1072. http://www.naacls.org/news/naacls-news/archives.asp?article_id=599.

	• *This education must include 24 semester hours of medical laboratory technology courses, OR 24 semester hours of science courses that includes 6 semester hours of chemistry, 6 semester hours of biology, and 12 semester hours of chemistry, biology or medical laboratory technology in any combination.*
	
	• *In addition, the individual must have laboratory training including either completion of a clinical laboratory* training program *approved or accredited by the NAACLS, ABHES, or other organization approved by HHS (note that this training may be included in the 60 semester hours listed above), OR at least 3 months documented laboratory training in each specialty in which the individual performs high complexity testing.*
	
	• *CLIA regulations include exceptions for grandfathered individuals; Refer to CLIA regulations 42CFR493.1489 and 1491 for details.*
	
	• *The laboratory director is responsible in determining whether an individual's education, training, and experience satisfy the requirements.*
	
	• Protocols should be in place to specify nature of pathologist supervision of non-pathologist for differing types of specimens.
	  ○ Protocol for small simple specimens that do not require knowledge of anatomy can specify indirect supervision.
	  ○ Protocol for more complex specimens can require direct or indirect supervision based on laboratory director's determination of each grossing personnel's ability to properly examine specimen.
	
	• Pathologist must define in writing the gross activities and the specimen types the individual is permitted to perform.
	
	• Performance of non-pathologist who performs gross examination should be evaluated by a pathologist on a regular basis.
	  ○ Annual review with documentation of errors in grossing, to include specimen mix-ups, improperly grossed specimens, and other parameters that are felt to be important by the laboratory director.
		Anatomic Pathology Checklist, ANP.11610 - Gross Examination Qualifications.
		
		Anatomic Pathology Checklist, ANP.11670 - Specimen – Gross Examination.
		
		Anatomic Pathology Checklist, ANP.11605 - Specimen – Gross Examination non-pathologist
		
		Anatomic Pathology Checklist, ANP.11640 - Competency Assessment of Non-Pathologists
			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], 1071-1072 [42CFR493.1491]
			
			Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical Laboratory Improvement Amendments of 1988; final rule. Fed Register. 1992(Feb 28):7183 [42CFR493.1489(b)(6)]
			
			Cibull ML. Q&A. Northfield, IL: College of American Pathologists CAP Today. 1997;11(7):112
			
			Grzybicki DM, et al. The usefulness of pathologists' assistants. Am J Clin Pathol. 1999;112:619-626
			
			Galvis CO, et al. Pathologists' assistants practice. A measurement of performance. Am J Clin Pathol. 2001;116:816-822
			
			The Joint Commission. Laboratory Services (CAMLAB) 2012
			
			The Joint Commission. Laboratory Services (CAMLAB) 2012

Laboratory Processes A. Guidelines iii. Specimen Gross Sectioning	• Identity of every specimen is maintained at all times during the gross examination steps.	All Common Checklist, COM.06100 – Primary Specimen Container Labeling	
	• There are documented instructions or guidelines available for the proper dissection, description, and histologic sampling of various specimen types (e.g., gastrointestinal biopsy, mastectomy, colectomy, hysterectomy, renal biopsy, nerve biopsy, muscle biopsy, etc).	All Common Checklist, COM.06200 - Secondary Specimen Container Labeling	
	○ Complex specimens should be dissected, described, and histologically sampled in a way that: ▪ Ensures proper microscopic evaluation and diagnosis can be performed by the pathologist by following established guidelines for specimen dissection and histologic sectioning. ▪ All required parameters of CAP Cancer Checklists can be assessed by pathologist.	Anatomic Pathology Checklist, ANP.11670 - Specimen – Gross Examination.	CAP Cancer Protocols and Checklists. http://www.cap.org/apps/cap.portal Barnes CA. False-negative frozen section results. *Am J Clin Pathol*. 2000;113:900; 6)
	• There are specific policies and procedures for the safe handling, storage, and disposal of tissues that may contain radioactive material. ▪ Procedures should be developed in conjunction with institutional radiation safety guidelines and must comply with state regulations for safe handling of radioactive materials. ▪ Procedures should distinguish policy regarding specimens with low radioactivity levels (such as sentinel lymph nodes) and high radioactivity level specimens such as implant devices. ▪ Procedure should specify specific handling details and laboratory should include specific storage area of higher radioactive material.	Anatomic Pathology Checklist, ANP.11275 - Radioactive Material Handling.	Glass EC, et al. Editorial: radiation safety considerations for sentinel node techniques. Ann Surg Oncol. 1999:6:10 Miner TJ, et al. Guideline for the safe use of radioactive materials during localization and resection of sentinel lymph nodes. Ann Surg Oncol. 1999;6:75-82 Cibull ML. Handling sentinel lymph node biopsy specimens. A work in progress. Arch Pathol Lab Med. 1999;123:620-621

	▪ Procedure should include institute specific directions for the disposal of potentially radioactive tissues.		Pfeifer JD. Sentinel lymph node biopsy. *Am J Clin Pathol*. 1999; 112:599-602. Fitzgibbons PL, et al. Recommendations for handling radioactive specimens obtained by sentinel lymphadenectomy. *Am J Surg Pathol*. 2000; 24:1549-1551.
	• There is a policy regarding what type of surgical specimens (if any) may be exempt from submission to the pathology department. ▪ Such a policy should be approved by the medical staff or appropriate health care committee. ▪ Examples of typical exempt specimens include prosthetic devices, tonsils and adenoids in children below a certain age, foreskin in children, varicose veins, cataracts, and pannus.	Anatomic Pathology Checklist, ANP.10016 - Surgical Pathology Exclusion. Anatomic Pathology Checklist, ANP.10032 - Surgical Pathology Microscopic Exemptions.	Zarbo RJ, Nakleh RE. Surgical pathology specimens for gross examination only and exempt from submission. A College of American Pathologists Q-Probes study of current policies in 413 institutions. *Arch Pathol Lab Med.* 1999;123:133-139 Nakhleh RE, Fitzgibbons PL, editors. College of American Pathologists. Quality improvement manual in anatomic pathology, 2nd ed.. Northfield, IL: CAP, 2002,113-114
	• There is a complete list of devices required for tracking under the Safe Medical Devices Act of 1990. • There is a policy for handling sup-optimal specimens (unlabeled specimens, specimens unaccompanied by adequate requisition information, left unfixed or unrefrigerated for extended period of time, received in a container/bag with a contaminated outside surface.	Laboratory General Checklist, GEN.20351 – Adverse Patient Event Reporting	Medical devices; device tracking. Fed Reg. May 29,119;57:22966-22981
	• There is written procedure for the storage and disposal of all specimens submitted for examination. The guideline should include: o Time of retention – minimum of two weeks after report issued and results reported to the referring physician o Approved disposal method of fixative as per local and state guidelines	All Common Checklist, COM.06300 – Specimen Rejection Criteria Anatomic Pathology Checklist, ANP.11550 - Specimen Retention.	College of American Pathologists. Policies and guidelines manual. Surgical specimens to be submitted to pathology for examination. Northfield, IL: CAP, 1999:Appendix M

	○ Approved disposal method of solid waste (tissue)		Nakhleh RE, Fitzgibbons PL, editors. College of American Pathologists. Quality improvement manual in anatomic pathology, second edition. Northfield, IL: CAP, 2002
Laboratory Processes A. Guidelines iv. Tissue Submission	Document physical parameters of sections submitted for histologic examination: • General information ○ Sample size must be thin (3-4 mm) enough to ensure adequate fixation and processing of the tissue. ○ Sample must small enough to fit in the cassette and allow space for processing fluids to enter the cassette on all sides. ○ Bloody or friable tissues should be wrapped so that the tissue sample is contained within the cassette to avoid cross contamination with other samples. ○ The number of biopsies or cores should be limited to enable proper embedding, all samples flat and within the same plane. ○ Number of cassettes per sample should be recorded. ○ Number of pieces per cassettes should be recorded ○ Specialized embedding directions should be documented. • Small biopsies ○ Multiple small pieces for most small biopsies (e.g.: stomach, colon, endometrium) can be submitted in one cassette. For needle core biopsies, one or at most a few (less than 5) pieces per cassette. • Larger tissue fragments or samples from whole organs ○ If more than one section is submitted in a block, the combined sections meet the above-mentioned parameters and that there is sufficient space between each piece to allow adequate fixation and embedding.	Anatomic Pathology Checklist, ANP.12200 – Gross Description Reporting	College of American Pathologists. Policies and guidelines manual. Surgical specimens to be submitted to pathology for examination. Northfield, IL: CAP, 1999:Appendix M Nakhleh RE, Fitzgibbons PL, editors. College of American Pathologists. Quality improvement manual in anatomic pathology, 2nd ed.. Northfield, IL: CAP, 2002

Laboratory Processes
 	
B. Tissue cassette identification
	• All tissue cassettes must be identified with a unique identifier.
	
	• The unique identifier must be indelible throughout all subsequent procedures.
	
	• The unique identifier can be applied manually or electronically through the use of automated printers.
	
	• Minimum requirements for a unique identifier include:
	  ○ Accession case identifier – to include year, subsection type (surgical, cytology etc.)
	  ○ Specimen identifier – alpha or numeric
	  ○ Block identifier – alpha or numeric
	
	• Additional identifiers: to be used but not required:
	  ○ Laboratory name or identifier
	  ○ Color coded cassette: tissue type, fixative used, pathologist etc.
	
	• Barcodes must not be the only identifying mark; a human readable identifier is also required.
	
	• If a barcode is applied to the cassette it should be readable by all tracking modalities used in the laboratory; LIS, Hospital Information system, associated testing equipment (slide writers) and third-party tracking software
		All Common Checklist, COM.06100 – Primary Specimen Container Labeling
		
		All Common Checklist, COM.06200 - Secondary Specimen Container Labeling
		
		Laboratory General Checklist, GEN.40825 - Specimen ID
		
		(see above)
			International Standard ISO 15189:2012 - Medical Laboratories; section 5.4- Pre-examination Processes
			
			Clinical Laboratory Standards Institute CLSI – LIS02A2 – Specifications for Transferring Information Between Clinical laboratory Instruments and Information Systems; 2004: Vol 24 No 33.
			
			Clinical Laboratory Standards Institute
			
			CLSI – Auto07A – Laboratory Automation; Data Content for Specimen Identification; 2004: Vol 24 No 20.

FIXATION	LABORATORY PROCESSES – FIXATION		
Guideline Section	Statement	CAP Checklist	Reference
Laboratory Processes C. Fixation Parameters i. Type of fixative a. Formalin, types	• Guidelines for the correct fixative to use for each specimen type should be documented and include: ○ Fixative to be used ○ Recommended duration of fixation ○ Required documentation of cold and warm ischemia times ○ References to mandatory fixation guidelines for breast tissues ○ Safety precautions and spill clean up	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements Anatomic Pathology Checklist, ANP.22983 – Fixation – HER2 and ER Predictive Marker Testing	Compton CC, Robb JA, Anderson MW, Berry AB, et.al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Path Lab Med.* Nov 2019, Vol. 143, No. 11. pp. 1346-1363. Clinical Laboratory Standards Institute CLSI – MM13, Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: 2020. International Standard ISO 20166-4:2020 - Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue for – Part 4: In situ detection techniques: section 6 – Inside the laboratory. Allison KH, Hammond EH, Dowsett M, McKernin SE et al. Estrogen and Progesterone Receptor Testing in Breast Cancer American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Path Lab Med*. Early Online Release. doi: 10.5858/arpa.2019-0904-SA

			Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Path Lab Med*; Nov 2018, Vol. 142, No. 11. pp. 1364-1382. Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Lott RL. HQIP: H&E Staining. HQIP - A Final Critique. Chicago, IL: College of American Pathologists; 2010. Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008
Laboratory Processes C. Fixation Parameters i. Type of fixative b. Recycling formalin fixatives	• A written policy and procedure for the use of recycled formalin should include: ○ Documentation of the initial verification of quality of recycled formalin. ○ Documentation of changes and reverification of quality of recycled formalin after any procedural changes or repairs to equipment used. ○ What formalin can be recycled: from tissue samples or tissue processor ○ Recycled formalin be used with new tissue samples, samples to be stored, and on tissue processors ○ Procedure for recycling formalin ○ Procedure for testing quality of recycled formalin		Section 19 of Occupational Safety and Health Act (OSHA) 1970 - Public Law 91-596. 29 CFR 1910.1000 (OSHA) Toxic and Hazardous Substances 29 CFR 1910.1048 (OSHA) Formaldehyde 29 CFR 1910.1200 (OSHA) Hazard Communication 29 CFR 1910.1048 (OSHA) Formaldehyde, Irritant and Potential Cancer Hazard

	○ Procedure for disposal of non-reusable waste ○ Procedure for cleaning and maintenance of recycling equipment ○ Validation studies comparing the filtered/tested solution to new solution are required. ○ Documentation to show licensing agencies is required.		29 CFR 1910.1450 (OSHA) Occupational Exposure to Hazardous Chemicals in Laboratories 40 CFR 262 (EPA) Standards Applicable to Generators of Hazardous Wastes 49 CFR 172.101 (DOT) Table of Hazardous Materials and Special Provisions http://www.osha.gov/dsg/hazcom/index.html
Laboratory Processes C. Fixation Parameters i. Type of fixative c. Non-Formalin, types	• Guidelines for the use of specialized fixatives for each specimen type must be documented and include: ○ Fixative to be used ○ Recommended duration of fixation ○ Specialized handling requirements i.e. refrigeration or flammable storage ○ Specialized preparation or usage i.e. mix before use ○ Safety precautions and spill clean up	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements	Carson F. Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Dapson RW: Glyoxal fixation: How it works and why it only occasionally needs antigen retrieval. *Biotech Histochem* 82:161; 2007 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008 Michel B, et al., Preservation of tissue fixed immunoglobulins in skin biopsies of patients with lupus erythematous and bullous diseases: preliminary report. *J Invest Dermato* 59:449, 1972. Elias JM, et al, New method for shipment of renal biopsies. *J Histotechnol* 1:15. 1977

Laboratory Processes C. Fixation Parameters ii. Fixation Times/Factors a. Fixative type	• Using 10% neutral buffered formalin (10%NBF), complete fixation of a 4 mm thick section of tissue is achieved in approximately 24 hours. • As a general recommendation, when using 10% NBF, ALL clinical tissue specimens should be fixed for a minimum of 6 hours and a maximum of 72 hours. • The general recommendations above are fixative dependent and relate specifically to the use of 10% NBF. Other fixatives, such as alcoholic formalin or Bouin, may have different guidelines.	Anatomic Pathology Checklist, Immunohistochemistry, ANP.22300 - Specimen Modification	Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Path Lab Med*; Nov 2018, Vol. 142, No. 11. pp. 1364-1382 Goldstein NS, Ferkowicz M, Odish E, et al: Minimum formalin fixation time for consistent estrogen receptor immunohistochemical staining of invasive breast carcinoma. Am J Clin Pathol 120:86–92, 2003
Laboratory Processes C. Fixation Parameters ii. Fixation Times/Factors b. Tissue type	• Guidelines for the fixation and handling of specific tissue types must be documented based on: ○ Accepted standards – CAP/ASCO guidelines for breast tissues ○ Tissue anatomy: ▪ Brain ▪ Fatty tissue – requires extended fixation ▪ Dense tissue such as uterus or cervix- requires extended fixation ▪ Lung - requires inflation	Laboratory General Checklist, GEN.40100 - Specimen Collection Manual Elements Anatomic Pathology Checklist, ANP.11670 – Specimen - Gross Examination	Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Path Lab Med*; Nov 2018, Vol. 142, No. 11. pp. 1364-1382

	▪ Whole organs • Dense tissues, such as uterus or cervix, and those that are especially fatty or bloody, like breast, colon and spleen, usually require extended times in most routine fixatives.		Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008
Laboratory Processes C. Fixation Parameters ii. Fixation Times/Factors c. Tissue Size	• Gross dissection manual should include information about the size and thickness of the tissue sample – see section A iv • A gross dissection manual should include specific instructions related to the fixation of the specimen to include: o Total fixation time required prior to processing o Preparation of large specimen to improve fixation: ▪ Opening / slicing of whole organs ▪ Exchange fixative • Thickness of tissue specimens is especially important because of its effect on reagent penetration. Large specimens should be opened or regularly sliced to maximize surface exposure to fixative reagents. Gross tissue sections should be no thicker than 3-4 mm. and easily fit between the top and bottom of the processing cassette.	Anatomic Pathology Checklist, ANP.11670 – Specimen - Gross Examination	Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008 Compton CC, Robb JA, Anderson MW, Berry AB, et.al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Path Lab Med.* Nov 2019, Vol. 143, No. 11. pp. 1346-1363.

			Clinical Laboratory Standards Institute CLSI – MM13, Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: 2020. International Standard ISO 20166-4:2020 - Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue for – Part 4: In situ detection techniques: section 6 – Inside the laboratory.
Laboratory Processes C. Fixation Parameters ii. Fixation Times/Factors d. Total Fixation time	• Guidelines for the total fixation of the specimens should be documented. • Total fixation time required prior to processing to include: ○ Time from placement in fixative to lab ○ Time large specimen is held prior to final dissection ○ Time in cassettes prior to processing – hold time and time on processor • Tissues for clinical assessment should be placed into an appropriate fixative immediately after surgical removal. Duration of fixation is an important variable in achieving excellent processing, microtomy, staining, and special staining. • Total fixation time should be recorded for each specimen and may be dictated into the body of the surgical report.	Anatomic Pathology Checklist, ANP.22983 – Fixation – HER2 and ER Predictive Marker Testing	Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009. Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Path Lab Med; Nov 2018, Vol. 142, No. 11. pp. 1364-1382. Compton CC, Robb JA, Anderson MW, Berry AB, et.al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Path Lab Med.* Nov 2019, Vol. 143, No. 11. pp. 1346-1363.

			Clinical Laboratory Standards Institute CLSI – MM13, Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: 2020. International Standard ISO 20166-4:2020 - Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue for – Part 4: In situ detection techniques: section 6 – Inside the laboratory.
Laboratory Processes C. Fixation Parameters ii. Fixation Times/Factors e. Environmental Parameters 1. Temperature	• Guidelines for the temperature at which the fixative must be used should be documented. ○ Storage temperature of fixative prior to use ○ Temperature the specimen in fixative to be stored at after collection ○ Temperature the specimen in fixative to be stored at during transport to testing laboratory. • Almost all fixatives are effectively used at room temperature (22-25°C). • Some fixatives such as acetone are more effective when used cold (4°).		Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009. Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008.
Laboratory Processes C. Fixation Parameters ii. Fixation Times/Factors e. Environmental Parameters 2. Use of	• Guidelines for use and operation of specialized microwave equipment used to assist with fixation should include: ○ Safety instructions to include radiation testing process ○ What solutions can be used in microwave ○ Type of tissues that can be microwave fixed	Anatomic Pathology Checklist, ANP.27170 - Microwave usage Anatomic Pathology Checklist, ANP.28290 - Microwave Monitoring	Clinical Laboratory Standards Institute CLSI – GP28-A, Microwave Device Use in the Histology Laboratory; Approved Guideline; 2005;Vol25 No10 Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009.

Microwaves	○ Size of tissue that can be microwave fixed ○ Protocols to be applied	Anatomic Pathology Checklist, ANP.28860 - Microwave Container Venting Anatomic Pathology Checklist, ANP.29430 - Microwave Venting	Login GR, Giammara B. Rapid microwave fixation, staining and embedding for light and electron microscopy. Microscopy Society of America Workshop; Cincinnati, OH. 1993.
PROCESSING	**LABORATORY PROCESSES – PROCESSING**		
Guideline Section	Statement	CAP Checklist	Reference
Laboratory Processes D. Processing i. Time	• Procedures must be written and validated for each processing schedule used. • Documented processing schedules must include: ○ Unique title that can be related to program on the tissue processor ○ Identify what tissue types the schedule can be used for ▪ Rush/urgent, biopsies, breast tissue ○ Indicate any pretreatment of the tissues ▪ i.e. Tissue must be fully fixed prior to processing as program starts in alcohol ○ Total processing time ○ Schedule: ▪ Name of reagent ▪ Expiration date ▪ Concentration ▪ Location on processor ▪ Order of application of reagents	Anatomic Pathology Checklist, ANP.23120 – Tissue Processing Programs. Anatomic Pathology Checklist, ANP.23130- Tissue Processing Programs. All Common Checklist, COM.30400 – Reagent Expiration Date	Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. New York, NY: Churchill Livingstone, 6th ed. 2008: 53-92. Brown RW, et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009: 4-8. Carson F. Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009: 31-42. Sheehan D, Hrapchak B. Theory and Practice of Histotechnology. Columbus, OH: Battelle Press, 2nd ed., 1980:59-78.

	▪ Ensure reagents are compatible with each other- i.e. alcohol following neutral buffered formalin must be 70% or less to stop precipitation of phosphate salts. ▪ Duration of application ▪ Specialized functions: ▪ Heat – actual temperature ▪ Pressure /vacuum – actual levels ▪ Mixing/stirring/agitation – Yes / No • Maintenance programs for the processor must be established: ○ Preventative maintenance and service contracts ▪ Completed by lab staff ▪ Completed by vendor service ○ Operational maintenance:	All Common Checklist, COM.30675 - Instrument /Equipment Records	Llewellyn, B.D., StainsFile, http://stainsfile.info/StainsFile/prepare/process/auto.htm Clinical Laboratory Standards Institute CLSI –GP28-A, Microwave Device Use in the Histology Laboratory; Approved Guideline; 2005. Willis, D., Minshew, J., Microwave Technology in the Histology Laboratory. Histologic. 2002; 35:1-4. Login GR, Dvorak AM. The Microwave Toolbook. A Practical Guide for Microscopists. Boston, MA: Beth Israel Hospital; 1994. Kok, L.P., Boon, M.E., Microwave Cookbook of Microscopists. 3rd Edition, Coulomb Press, Leyden, 1992. Kok LP, Boon ME. Ultrarapid vacuum-microwave histoprocessing. Histochem J. 1995;27(5):411-419 Clinical Laboratory Standards Institute CLSI GP31-A Laboratory Instrumentation, Implementation, Validation and Maintenance; Vol.29 No. 11.

	▪ Reagent top up / exchange / rotation schedule based on: • Number of cassettes processed • Number of time program run • Monitored and established by processor software ▪ Establish if re-cycled reagents can be used on processor ▪ Cleaning of reagent reservoir containers		
Laboratory Processes D. Processing ii. Tissue Processor Reagents a. Fixative	• Establish and document for fixative to be used on the tissue processor: o Type of fixative to be used ▪ 10% neural buffered formalin (NBF) ▪ Zinc formalin ▪ Alcoholic formalin ▪ Formalin substitute or proprietary fixative o Number of reservoirs of fixative to be used o Duration of time in fixative o Temperature / vacuum/ agitation o Rotation or change schedule • Verify and document that the fixative used is compatible with the tissues to be processed. • Establish if recycled fixative can be used on processor. • Establish and document procedures for fixative handling that include: o Storage o Safety to include:	Anatomic Pathology Checklist, ANP.23120 – Tissue Processing Programs. Anatomic Pathology Checklist, ANP.23130-Tissue Processing Programs.	Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. New York, NY: Churchill Livingstone, 6th ed. 2008: 53-92. Brown RW, et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009: 4-8. Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009: 31-42. Sheehan D, Hrapchak B. Theory and Practice of Histotechnology. Columbus, OH: Battelle Press, 2nd ed., 1980:59-78.

	▪ Use of personal protective equipment ▪ Spill control and clean up ▪ Monitoring of exposure levels ○ Disposal methods that follow regulatory guidelines		
Laboratory Processes D. Processing ii. Tissue Processor b. Reagents for dehydration	• Develop documentation that establishes the parameters of the dehydrant used on the tissue processor: ○ Type – alcohol or proprietary product ○ Type of alcohol – ethanol or isopropanol ○ Concentration – grades alcohols i.e. 70%, 80%, 95%, 100% ○ Number of reservoirs of each alcohol concentration ○ Duration of time for each alcohol reservoir and total time ○ Temperature / vacuum/ agitation ○ Rotation or change schedule • Verify and document that the dehydrant is compatible with the tissues to be processed and changed at intervals appropriate for workload. • Ensure that dehydrant following fixative is compatible with fixative: ○ 10% NBF- the first alcohol in the dehydrating series should be 70% or less to prevent the precipitation of phosphates from the 10% NBF ○ Alcoholic formalin – the first alcohol in the dehydrating series can be 95% as the tissue has already been in 70% alcohol. ○ Formalin substitute or proprietary fixatives – must follow guidelines provided by the manufacturer	Anatomic Pathology Checklist, ANP.23100 – Tissue Processor Solutions Anatomic Pathology Checklist, ANP.23120 – Tissue Processing Programs. Anatomic Pathology Checklist, ANP.23130- Tissue Processing Programs.	Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. New York, NY: Churchill Livingstone, 6th ed. 2008:53-92. Brown RW, et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009:4-8. Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009:31-42. Sheehan D, Hrapchak B. Theory and Practice of Histotechnology. Columbus, OH: Battelle Press, 2nd ed., 1980: 59-78.

	• Validate that the dehydrant is compatible with the reagent that follows in the processing cycle; this could be xylene or xylene substitute or paraffin. • Develop a documentation process for recording the purchase, use and disposal of ethanol. Ethanol is strictly controlled by the federal government. • Develop procedures for alcohol: o Storage o Safety to include: ▪ Use of personal protective equipment ▪ Spill control and clean up ▪ Monitoring of exposure levels o Disposal methods that follow regulatory guidelines o Recycling procedures: ▪ Testing method to prove quality ▪ What alcohol can be recycled ▪ When recycled alcohol can be used	Laboratory General Checklist – GEN.76000 – Chemical Hygiene Plan Laboratory General Checklist - GEN.76500 – Flammable Storage Laboratory General Checklist, GEN.77800 – Hazardous Chemical Waste Disposal	
Laboratory Processes D. Processing ii. Tissue Processor c. Reagents for clearing	• Develop documentation that establishes the parameters of the clearant used on the tissue processor: o Type – xylene, xylene substitute or proprietary product ▪ Verification that clearant is compatible with dehydrants and paraffin o Number of reservoirs of clearant o Duration of time for each reservoir of clearant and total time o Temperature / vacuum/ agitation	Anatomic Pathology Checklist, ANP.23100 – Tissue Processor Solutions Anatomic Pathology Checklist, ANP.23350 – Paraffin Baths, Flotation Baths, and Embedding Stations	Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. New York, NY: Churchill Livingstone, 6th ed. 2008: 53-92. Brown RW, et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009 4-8.

	○ Rotation or change schedule • Verification that the clearant to be used is compatible with the tissues to be processed and changed at intervals appropriate for workload. • Develop procedures for clearant: ○ Storage ○ Safety to include: ▪ Use of personal protective equipment ▪ Spill control and clean up ▪ Monitoring of exposure levels ○ Disposal methods that follow regulatory guidelines ○ Recycling procedures: ▪ Testing method to prove quality ▪ When recycled clearant can be used	Laboratory General Checklist – GEN.76000 – Chemical Hygiene Plan Laboratory General Checklist, GEN.77800 – Hazardous Chemical Waste Disposal	Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009:31-42. Sheehan D, Hrapchak B. Theory and Practice of Histotechnology. Columbus, OH: Battelle Press, 2nd ed., 1980: 59-78.
Laboratory Processes D. Processing ii. Tissue Processor d. Reagents for infiltration 1. Paraffin(s)	• Develop documentation that establishes the parameters of the paraffin to be used on the tissue processor: ○ Type – with or without additives ▪ Verification that paraffin is compatible with the dehydrant or clearant used ○ Melting point of paraffin ○ Number of reservoirs of paraffin ○ Duration of time for each reservoir of paraffin and total time ○ Temperature / vacuum/ agitation	Anatomic Pathology Checklist, ANP.23350 – Paraffin Baths, Flotation Baths, and Embedding Stations	Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. New York, NY: Churchill Livingstone, 6th ed. 2008: 53-92. Brown RW. et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009: 4-8.

	○ Rotation or change schedule ▪ Format of wax to be used; melted wax, pellets, solid block		Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009: 31-42. Sheehan D, Hrapchak B. Theory and Practice of Histotechnology. Columbus, OH: Battelle Press, 2nd ed., 1980:59-78.
EMBEDDING	**LABORATORY PROCESSES - EMBEDDING**		
Guideline Section	Statement	CAP Checklist	Reference
Laboratory Processes E. Embedding i. General Recommendations	• Develop standardized guidelines for routine embedding and handling of special biopsies: ○ Opening of cassettes – one cassette at time ○ Mold size ○ Storage and temperature of molds ○ Placement of tissue in mold ▪ Similar surfaces in same direction ▪ Direction of surface in orientation to block placement on the microtome ○ Orientation of the tissue types ○ Method for cooling embedded blocks ○ Method for release of blocks from molds and removal of excess paraffin ○ Method for cleaning and reuse of molds • Develop quality assurance procedures: ○ Manual or electronic workload log used to compare recorded number of cassettes with the actual number of cassettes. ○ Documentation and follow up of discrepancies	Anatomic Pathology Checklist, ANP.23350 – Paraffin Baths, Flotation Baths, and Embedding Stations	Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008 Luna L. Histopathologic Methods and Color Atlas of Special Stains and tissue Artifacts; American Histolabs Inc;1992 (embedding table)

	• Establish guidelines for the order of embedding cassettes:
	  ○ Urgency
	  ○ Tissue type; biopsy, routine tissues
	• Establish guidelines for the use and operation of the embedding center:
	  ○ Temperature of embedding paraffin – monitored daily
	  ○ Set temperature of other heated elements: holding paraffin, work surface and forceps
	  ○ Cleaning of forceps and work surfaces
	  ○ Addition of paraffin to reservoir: liquid, pellets solid block
	  ○ Cleaning of the paraffin reservoir and filter
		Anatomic Pathology Checklist, ANP.21350 – Specimen Preparation Records
		
		Anatomic Pathology Checklist, ANP.21397 – Cross Contamination - Histology	

Laboratory Processes
E. Embedding
ii. Paraffin Wax
	• Establish type of paraffin wax to be used for embedding:
	  ○ Specialized paraffin or the same as processing paraffin
	  ○ Additives - beeswax, plastic polymers, diethylene glycol distearate, ceresin
	  ○ Melting point
		Anatomic Pathology Checklist, ANP.23350 – Paraffin Baths, Flotation Baths, and Embedding Stations	Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008

MICROTOMY	**LABORATORY PROCESSES - MICROTOMY**		
Guideline Section	Statement	CAP Checklist	Reference

Laboratory Processes
F. Microtomy
i. Microtome Maintenance
	• Written instructions for the operation of all makes/models of microtomes:
	  ○ Manual vs. automated
	  ○ Cleaning and maintenance
	  ○ Acceptable cleaning products
	  ○ Lubrication schedule and reagent
		Anatomic Pathology Checklist, ANP.23400 - Microtome Maintenance	Clinical Laboratory Standards Institute CLSI GP31-A Laboratory Instrumentation, Implementation, Validation and Maintenance 2009:Vol. 29, No. 11..

	• Schedule and document annual preventative maintenance, service, or repair	All Common Checklist, COM.30675 - Instrument /Equipment Records	Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008
Laboratory Processes F. Microtomy ii. Section preparation a. Block trimming	• Develop technique to standardized position of microtome chuck (block holder) on all microtomes to ensure blocks can be recut on any microtome. • Establish guidelines for the orientation of block placement in microtome chuck: o Block identifier to face to the right, left, up or down. • Establish cutting guidelines: o Placement of the slide label o Limiting one patient tissue to a slide o Thickness of section ▪ Routine tissues ▪ Specialized tissues i.e. brain, lymph nodes ▪ Specialized techniques i.e. amyloid, immunohistochemistry	All Common Checklist, COM.06100 – Primary Specimen Container Labeling All Common Checklist, COM.06200 - Secondary Specimen Container Labeling see above	Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2010:Vol 30 No7. Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008

	<table><tr><td>Tissue</td><td>Thickness</td></tr><tr><td>Routine Paraffin</td><td>4 to 5 microns</td></tr><tr><td>Renal Sections</td><td>1 to 3 microns</td></tr><tr><td>Bone Marrow</td><td>2 to 3 microns</td></tr><tr><td>Nerve histochemical staining</td><td>6 to 15 microns</td></tr><tr><td>Amyloid demonstration</td><td>6 to 12 microns</td></tr></table> ○ Number of sections / ribbons per slide ▪ Sections/ ribbons are same depth ▪ Each section / ribbon is a different depth ▪ Amount of trim between each section/ribbon ○ Placement of sections on the slide ○ Number of slides per tissue type i.e. 2 slides for biopsy blocks ○ Use of specialized slides: ▪ Adhesive or no adhesive ▪ Control slides – specialized markings ○ Addition of additives to water bath ▪ Adhesives – i.e. gelatin, agar, Elmer's glue or proprietary products ▪ Surfactants – i.e. tween	Anatomic Pathology Checklist, ANP.11716 – Paraffin Microtomy	

Laboratory Processes F. Microtomy iii. Flotation Bath a. Temperature	• Establish guidelines for the use and maintenance of flotation/water bath: ○ Temperature of flotation/water bath – documentation of temperature ○ Type of water to be used – tap versus distilled ○ Use of additives – gelatin, agar, Elmer's glue, proprietary product(s) ○ Cleaning method ▪ Frequency ▪ Cleaning products to be used	All Common Checklist, COM.30675 - Instrument /Equipment Records Anatomic Pathology Checklist, ANP.23350 – Paraffin Baths, Flotation Baths, and Embedding Stations	Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008
Laboratory Processes F. Microtomy iv. Slides a. Labelling	• All slides must be clearly labeled to identify the following: ○ Specimen accession number ○ Block identifier ○ Slide level number ○ Patient name ○ Stain identifier • Establish a labeling procedure to be used; It is good laboratory practice to label slides only as required and to avoid the practice of pre-labeling large numbers of slides in advance. • Establish a quality assurance process of matching slides against the block before delivery out of the laboratory.	All Common Checklist, COM.06100 – Primary Specimen Container Labeling All Common Checklist, COM.06200 - Secondary Specimen Container Labeling see above	Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2010: Vol 30 No7. Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Brown RW, Della Speranza V, Alvarez JO, et al. Uniform labeling of blocks and slides in surgical pathology: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center and the National Society for Histotechnology. *Arch Pathol Lab Med*. 2015;139(12):1515-24.
Laboratory Processes F. Microtomy iv. Slides b. Slide Drying	• Drying times for slides with paraffin sections should be established and made available to all technical staff. The following recommendations should be considered: ○ Air drying of cut sections before placing into the drying oven		Clinical Laboratory Standards Institute CLSI - GP33A, Accuracy in Patient and Sample Identification; 2010: Vol 30 No7.

	○ Use of a forced air dryer maintained at a temperature just above the melting point of the paraffin. ○ Drying time and temperature, commonly slides are dried at 58-60°C for 15-30 minutes. • Special techniques, such as immunohistochemistry or in-situ hybridization may require longer drying times. The required drying time should be included in the written procedure. • Dry slides in an oven for a minimum of 60 minutes at a temperature between 50-60°C. Optimal results are achieved at room temperature for 24 hours; however, this is impractical in a clinical laboratory setting. (Note: Some molecular testing protocols require that slides not be oven dried.)		Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Clinical Laboratory Standards Institute CLSI – I/L28-A2, Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays,2011: Vol. 31 No.4.
Laboratory Processes F. Microtomy iv. Slides c. Disposal of Blocks/Slides	• Guidelines to be established for the retention and disposal of all glass paraffin blocks and slides.	Anatomic Pathology Checklist, ANP.12500 - Record Retention Anatomic Pathology Checklist, ANP.27150 – Glass Slide/Block Disposal	Clinical Laboratory Standards Institute CLSI – GP05-A3 Clinical Laboratory Waste Management; 2011: Vol. 31, No. 3.

STAINING	LABORATORY PROCESSES – STAINING		
Guideline Section	Statement	CAP Checklist	Reference
Laboratory Processes G. Staining i. Hematoxylin & Eosin (H&E)	• Establish operation procedure for manual or automated staining: o Reagents to be used – concentration and volumes o Staining schedule for each specific staining program o Rotation or change schedule for the reagents o Disposal and or recycle process for reagents • Establish quality assurance criteria for the staining and evaluation of hematoxylin and Eosin stain. • **HEMATOXYLIN:** When applied correctly, in well-fixed, well processed tissues, epithelial cells will demonstrate: o A well-defined nuclear membrane o Clear, open (vesicular) karyoplasm (cytoplasm of the nucleus) o Crisp, fine-spiculed chromatin patterns ▪ Also, in most tissue sections, there are some dense closed (hyperchromatic) nuclear patterns present in lymphoid tissue. o Prominent “eosinophilic” nucleoli. (if present) o Cartilage and calcium deposits stain dark blue o The hematoxylin should appear blue to blue-black	All Common Checklist, COM.10000 – Procedure Manual Laboratory General Checklist, GEN.77800 – Hazardous Chemical Waste Disposal Laboratory General Checklist, GEN.30000 – Monitoring Analytic Performance Anatomic Pathology Checklist, ANP.21395 – Special Stains/Studies Anatomic Pathology Checklist, ANP.11734 – Slide Quality All Common Checklist, COM.30675 - Instrument /Equipment Records	Clinical Laboratory Standards Institute CLSI GP31-A Laboratory Instrumentation, Implementation, Validation and Maintenance: 2009: Vol. 29, No. 11. Lott RL. HQIP: H&E Staining. HQIP - A Final Critique. Chicago, IL: College of American Pathologists; 2010 Brown RW. et. al., Histologic Preparations Common Problems and Their Solutions. College of American Pathologists, 2009 Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009 Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008

	• **EOSIN:** When applied correctly, in well-fixed, well processed tissue, eosin produces, at least, a "tri-tonal" (three-color) effect.
	  ○ Muscle cells (smooth, skeletal, cardiac) and epithelial cell cytoplasm will stain deep red-pink.
	  ○ Collagen will stain a distinct lighter pink.
	  ○ Red blood cells (RBC) will stain a bright orange-red.
	  ○ Nucleoli (if present) should exhibit a reddish-purple color due to their high protein and RNA content.
	▪ It is essential, when applying eosin, that the smooth muscle/cell cytoplasm and collagen be differentially stained. (different shades of red/pink).
	• Complete and document results of a H&E control prior to staining routine workload.
	  ○ Documentation to include changes or actions taken to correct substandard staining of the control.
	• Establish a preventative maintenance program that includes annual service and emergency service.
		Anatomic Pathology Checklist, ANP.21360 Automated Stainer.	Prophet EB, Mills B, Arrington JB, Sobin LH. AFIP Laboratory Methods in Histotechnology, AFIP;1992
			
			Sheehan DC, Hrapchak BB., Theory and Practice of Histotechnology, 2nd ed. Columbus, OH: Battelle Press; 1980
			
			Horobin RW. Troubleshooting Histology Stains, Churchill Livingstone; 1998

Laboratory Processes
G. Staining
ii. Histochemical and enzymatic stains (special stains)
	• Establish written procedures for manual or automated staining procedures to include:
	  ○ Special cutting or preparation of tissue section
	  ○ Reagents used
	  ▪ Access to material data sheets
	  ▪ Concentration
	  ▪ Storage
	  ▪ Disposal
		All Common Checklist, COM.10000 – Procedure Manual
		
		Laboratory General Checklist, GEN.76411 – Chemical Safety Document Access
		
		Anatomic Pathology Checklist, ANP.21395 - Special Stains/Studies	

	○ Specific steps of staining procedure ○ Quality assurance process ▪ Define positive control tissue ▪ Define expected stain results ▪ Records of acceptability		
	• Establish operation procedures for automated staining equipment: ○ Validation process ○ Cleaning and maintenance procedures	Laboratory General Checklist, GEN.77800 – Hazardous Chemical Waste Disposal	
	• Establish a preventative maintenance program that includes annual service and emergency service.	Anatomic Pathology Checklist, ANP.23100 - Tissue Processing Programs	
	• Histochemical stains, or special stains, refer to a group of secondary stains used in conjunction with H&E staining. They were developed to provide differential coloration and contrast to cell and tissue constituents with the goal of understanding cell structure and function.	All Common Checklist, COM.30550 – Instrument/Equipment Performance Verification All Common Checklist, COM.30600 – Instrument/Equipment Function Checks	
	• Many are used to identify morphological entities such as bacteria, fungi, nerve fibers, and for connective tissues including collagen and reticular fibers. • Other special histochemical stains are used for specific tissue components and include stains for iron, mucins, glycogen, amyloid, and nucleic acids.		Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008 Carson F, Hladik C., Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009
	• Enzyme histochemical staining refers to a subclass of histochemistry that identifies enzymes by employing substrates containing one of a number of various naphthol compounds.	All Common Checklist, COM.30675 - Instrument /Equipment Records	

			Sheehan DC, Hrapchak BB., Theory and Practice of Histotechnology, 2nd ed. Columbus, OH: Battelle Press; 1980 Kiernan J. Histological and Histochemical Methods: Theory and Practice 4th ed. Oxfordshire, England; 2008 Pearse AGE, Stoward PJ. Histochemistry, Theoretical and Applied, 4th ed. Vol. 2. Analytical Technique. Edinburgh: Churchill-Livingstone, 1985 Lillie RD, Fullmer HM. Histopathologic Technic and Practical Histochemistry. 4th ed. New York: McGraw-Hill;1976
Laboratory Processes G. Staining iii. Immunohistochemical stains	• Establish a procedure for selection and development of antibodies and clones to be added to menu: ○ Fixation of tissue ○ cutting of tissue section ▪ Paraffin ▪ Frozen ○ Selection and validation of antibody and clone ○ Selection, validation and monitoring of reagents ○ Validation of application method ▪ Pretreatment ▪ Antibody dilution ▪ Retrieval method – if required	Anatomic Pathology Checklist, ANP.22983 – Fixation - HER2 and ER– Predictive Marker Testing Anatomic Pathology Checklist, ANP.22300 – Specimen Modification Anatomic Pathology Checklist, ANP.22500 - Buffer pH Anatomic Pathology Checklist, ANP.22750 - Antibody Validation	Clinical Laboratory Standards Institute CLSI: ILA28-A2: Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline –2011;Vol31 No4 Fitzgibbons PL, Bradley LA, Fatheree LA, Alsabeh R, et.al. Principles of Analytic Validation of Immunohistochemical Assays: Guideline From the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Path Lab Med*; Nov 2014, Vol. 138, No. 11. pp. 1432-1443.

	▪ Detection method • DAB • Alkaline phosphatase • Fluorescent ○ Documentation of scoring methodology ▪ Manual or automated ○ Documentation of validation; record test tissue, expected results actual results and changes to method ○ Storage of antibody and reagents • Establish re- validation procedures after change of: ○ Methodology ○ Reagent ○ Antibody	Anatomic Pathology Checklist, ANP.22978 – Predictive Marker Testing – Validation/Verification Anatomic Pathology Checklist, ANP.22969 – Report Elements All Common Checklist, COM.30350 – Reagent Storage and Handling Anatomic Pathology Checklist, ANP.22615 – Endogenous Biotin Anatomic Pathology Checklist, ANP.22900 – Slide Quality Anatomic Pathology Checklist, ANP.22760 - New Reagent Lot Confirmation of Acceptability Anatomic Pathology Checklist, ANP.22780 – IHC Assay Performance All Common Checklist, COM.30550 – Instrument/Equipment Performance Verification	Troxell ML, Fulton RS, Swanson PE, Bellizzi AM, Fitzgibbons PL, et.al. Predictive Markers Require Thorough Analytic Validation. *Arch Path Lab Med*; Aug 2019, Vol. 143, No. 8. pp. 907-909. Torlakovic EE. How Validate Predictive Immunohistochemistry Testing in Pathology? *Arch Path Lab Med*; Aug 2019, Vol. 143, No. 8. pp. 907-907.Validation doc Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008 Dabbs D. Diagnostic Immunohistochemistry: Theranostic and Genomic Applications, Expert Consult: Online and Print , 3rd Edition Fitzgibbons PL, Bradley LA, Fatheree LA, Alsabeh R, et.al. Principles of Analytic Validation of Immunohistochemical Assays: Guideline From the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Path Lab Med*; Nov 2014, Vol. 138, No. 11. pp. 1432-1443.

	▪ Clone ▪ Lot number ▪ Dilution ○ Equipment ▪ New model ▪ major service repair ▪ move or relocation • Establish procedures for cleaning and maintenance of equipment ○ Calibration of pipettes ○ Monitoring of refrigerator and freezer temperature ▪ NIST calibration procedure ○ Ancillary equipment ▪ Microwave oven ▪ Steamer ○ Stainer • Establish a preventative maintenance program that includes annual service and emergency service.	All Common Checklist, COM.30820 – Quantitative Pipette Accuracy and Reproducibility All Common Checklist, COM.30750 – Temperature Checks All Common Checklists, COM.30600 – Maintenance and Function Checks All Common Checklist, COM.30675 - Instrument /Equipment Records Laboratory General Checklist, GEN.77800 – Hazardous Chemical Waste Disposal	Taylor, Cote; Immunomicroscopy Volume 19 in Major Problems in Pathology Series, 3rd ed. Hayat MA.Microscopy, Immunohistochemistry and Antigen Retrieval Methods: For Light and Electron Microscopy, Springer Press; 2002. Elias JM. Immunohistopathology: A Practical Approach to Diagnosis; 2nd ed. Chicago, IL: ASCP Press, 2003 Hayat MA. Immunogold-Silver Staining: Principles, Methods, and Applications, CRC;1995 Javois LC. Immunocytochemical Methods and Protocols, 3rd ed.:BIOS Scientific; 2003 Polack JM. Introduction to Immunocytochemistry, 3rd ed.:BIOS Scientific; 2003 Hayat MA. Microscopy, Immunohistochemistry and Antigen Retrieval Methods: For Light and Electron Microscopy, Springer Press; 2002 Javois LC. Immunocytochemical Methods and Protocols, 3rd ed.:BIOS Scientific; 2003 Shi S, Taylor CR. Antigen Retrieval Techniques: Immunohistochemistry and Molecular Morphology, Eaton Publications;2000

	• Establish procedure for the disposal of reagents as per local, state and national requirements • Immunohistochemistry (IHC) staining refers to the method of localizing specific antigens (e.g., proteins) in cells of a tissue by the principle of an antibody / antigen recognition. This reaction is labelled by a detection technique and visualized by a chromagen.		Immunochemical Staining Methods Handbook, 3rd ed., Dako Corp, Carpinteria, CA Clinical Laboratory Standards Institute CLSI – I/L28-A2, Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays,2011.
Laboratory Processes G. Staining iv. Immunohistochemical Stains a. Quality Control	• Establish Quality Control and Quality Assurance procedures to include: ○ Selection of appropriate control material ○ Validation of control material ▪ Documentation of test of control at accredited lab ○ Use and application of controls ▪ Patient and antibody reagent control ▪ Positive and negative • Establish procedures for the review of controls and release of patient slides for interpretation • Records of review need to be retained. ▪ IHC quality control measures are essential to provide and ensure consistency of performance and reproducibility of the intended target.	Anatomic Pathology Checklist, ANP.21395 – Special Stains/Studies Anatomic Pathology, ANP.21850 - QC - Immunofluorescence Anatomic Pathology ChecklistANP.22550 – QC - Antibodies Anatomic Pathology Checklist, ANP.22570 – QC - Antibodies Anatomic Pathology Checklist, ANP.22660 - Control Slide Review Anatomic Pathology Checklist, ANP.22780 – IHC Assay Performance	Fitzgibbons PL, Bradley LA, Fatheree LA, Alsabeh R, et.al. Principles of Analytic Validation of Immunohistochemical Assays: Guideline From the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Path Lab Med*; Nov 2014, Vol. 138, No. 11. pp. 1432-1443. Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008 Dabbs D. Diagnostic Immunohistochemistry: Theranostic and Genomic Applications, Expert Consult: Online and Print , 3rd Edition Taylor C, Cote RJ; Immunomicroscopy Volume 19 in Major Problems in Pathology Series, 3rd ed.

		Laboratory General Checklist, GEN.30000 – Monitoring Analytic Performance	Hayat MA.Microscopy, Immunohistochemistry and Antigen Retrieval Methods: For Light and Electron Microscopy, Springer Press; 2002 Elias JM. Immunohistopathology: A Practical Approach to Diagnosis; 2nd ed. Chicago, IL: ASCP Press; 2003 Taylor C, Cote RJ. Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist, 3rd ed., WB Saunders; 2005 Immunochemical Staining Methods Handbook, 3rd ed., Dako Corp, Carpinteria, CA
Laboratory Processes G. Staining iv. Immunohistochemical stains b. Intended Use of the Antibody	• Establish procedure for clinical validation of each antibody: ○ Number of tissue sections to be tested per antibody ○ Comparison of results to previous stained slides or duplicate slides stained by accredited lab • Each antibody MUST be clinically validated to be relevant to its intended target antigen.	Anatomic Pathology Checklist, ANP.22750 - Antibody Validation Anatomic Pathology Checklist, ANP.22760 - New Reagent Lot Confirmation of Acceptability Anatomic Pathology Checklist, ANP.22550 - QC- Antibodies Anatomic Pathology Checklist, ANP.22570 - QC – Antibodies Anatomic Pathology Checklist, ANP.22978 – Predictive Marker Testing – Validation/Verification	Clinical Laboratory Standards Institute CLSI – I/L28-A2, Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays,2011: Vol. 31 No.4 Fitzgibbons PL, Bradley LA, Fatheree LA, Alsabeh R, et.al. Principles of Analytic Validation of Immunohistochemical Assays: Guideline From the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Path Lab Med*; Nov 2014, Vol. 138, No. 11. pp. 1432-1443. Troxell ML, Fulton RS, Swanson PE, Bellizzi AM, Fitzgibbons PL, et.al. Predictive Markers Require Thorough Analytic Validation. *Arch Path Lab*

			Med; Aug 2019, Vol. 143, No. 8. pp. 907-909. Torlakovic EE. How to Validate Predictive Immunohistochemistry Testing in Pathology? *Arch Path Lab Med*; Aug 2019, Vol. 143, No. 8. pp. 907-907.

Laboratory Processes
 	
G. Staining
 	
v. In Situ Hybridization
	• Establish a procedure for selection and development of probes to be added to menu:
	  ○ Preparation and cutting of tissue section
	  ○ Selection of probe
	  ○ Validation of application method
	  ▪ Pretreatment
	  ▪ Antibody dilution
	  ▪ Retrieval method – if required
	  ▪ Detection method
	  • DAB
	  • Alkaline phosphatase
	  • Fluorescent
	  ○ Selection and validation of control material
	  ○ Instructions on how to score slide and expected results
	  ○ Documentation of validation; record test tissue, expected results, actual results, and changes to method
	  ○ Storage of probe and reagents
	  ○ Retention and storage of slides and or images
		Anatomic Pathology Checklist, ANP.22956 - ISH Probe Validation/Verification
		
		Anatomic Pathology Checklist, ANP.22978 – Predictive Marker Testing – Validation/Verification
		
		Anatomic Pathology Checklist, ANP.22964 – ISH Controls
		
		Anatomic Pathology Checklist, ANP.22959 – ISH Assay Performance
		
		Anatomic Pathology Checklist, ANP.22963 – ISH scoring
		
		Anatomic Pathology Checklist, ANP.22965 - Retention - Images and Slides
			Clinical Laboratory Standards Institute CLSI – MM13, Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: 2020.
			
			International Standard ISO 20166-4:2020 - Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue for – Part 4: In situ detection techniques: section 6 – Inside the laboratory.
			
			Clinical Laboratory Standards Institute CLSI.- MM7-A2 Fluorescence In Situ Hybridization (FISH) Methods for Clinical Labs, Approved Guideline,2nd Ed. 2013:Vol.33,No.10
			
			Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008.
			
			David J. Dabbs.Diagnostic Immunohistochemistry: Theranostic and Genomic Applications, 3rd ed. Philadelphia, PA: Saunders Elsevier; 2010.

	• Establish procedures for change of:
	  ○ Methodology
	  ○ Reagent
	  ○ Antibody
	  ▪ Clone
	  ▪ Lot number
	  ▪ Dilution
	  ○ Equipment
	  ▪ New model
	  ▪ major service repair
	  ▪ move or relocation
	
	• Establish procedure for clinical validation of each probe:
	  ○ Number of tissue sections to be tested per probe
	  ○ Comparison of results to previous stained slides or duplicate slides stained by accredited lab
	
	• In Situ Hybridization (ISH) staining refers to a method using probes made up of complementary strands used to target sequences of mRNA, viral DNA or chromosomal DNA located in tissue cells.
	
	• Retention of Images and permanent slides
		Anatomic Pathology Checklist, ANP.22956 - ISH Probe Validation/Verification
		
		Anatomic Pathology Checklist, ANP.22963 – ISH Scoring
		
		Anatomic Pathology Checklist, ANP.22964 – ISH Controls
		
		Anatomic Pathology Checklist, ANP.22978 – New Reagent Lot – ISH probes
		
		Anatomic Pathology Checklist, ANP.22966 - ISH Interpretation
			Awatif I. AL-Nafussi, 2nd ed. Tumor Diagnosis, Practical Approach and Pattern Analysis. London, Hodde Arnold; 2005
			
			American College of Medical Genetics Laboratory. Standards and guidelines for clinical genetics laboratories, 2nd ed. Bethesda, MD: ACMG; 1999.
			
			Clinical Laboratory Standards Institute CLSI.- MM7-A2 Fluorescence In Situ Hybridization (FISH) Methods for Clinical Labs, Approved Guideline,2nd Ed. 2013:Vol.33,No.10
			
			Jennings L, Van Deerlin VM, Gulley ML (2009) Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests. Archives of Pathology & Laboratory Medicine: Vol. 133, No. 5: 743-755.
			
			Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Path Lab Med; Nov 2018, Vol. 142, No. 11. pp. 1364-1382.

			Tanner M, Gancberg D, Di Leo A, Larsimont D, Rouas G, Piccart MJ, et al. Chromogenic in situ hybridization: a practical alternative for fluorescence in situ hybridization to detect HER-2/neu oncogene amplification in archival breast cancer samples. Am J Pathol 2000;157(5):1467-72. Di Palma S, Collins N, Faulkes C, Ping B, Ferns G, Haagsma B, et al. Chromogenic in situ hybridisation (CISH) should be an accepted method in the routine diagnostic evaluation of HER2 status in breast cancer. J Clin Pathol 2007;60(9):1067-8. Gong Y, Gilcrease M, Sneige N. Reliability of chromogenic in situ hybridization for detecting HER-2 gene status in breast cancer: comparison with fluorescence in situ hybridization and assessment of interobserver reproducibility. Mod Pathol 2005;18(8):1015-21. Hauser-Kronberger C, Dandachi N. Comparison of chromogenic in situ hybridization with other methodologies for HER2 status assessment in breast cancer. J Mol Histol 2004;35(6):647-53. Saez A, Andreu FJ, Segui MA, Bare ML, Fernandez S, Dinares C, et al. HER-2 gene amplification by chromogenic in situ hybridisation (CISH) compared with fluorescence in situ hybridisation (FISH) in breast cancer-A study of two hundred cases. Breast 2006;15(4):519-27.

			Bhargava R, Lal P, Chen B. Chromogenic in situ hybridization for the detection of HER-2/neu gene amplification in breast cancer with an emphasis on tumors with borderline and low-level amplification: does it measure up to fluorescence in situ hybridization? Am J Clin Pathol 2005;123(2):237-43. Dietel M, Ellis IO, Hofler H, Kreipe H, Moch H, Dankof A, et al. Comparison of automated silver enhanced in situ hybridisation (SISH) and fluorescence ISH (FISH) for the validation of HER2 gene status in breast carcinoma according to the guidelines of the American Society of Clinical Oncology and the College of American Pathologists. Virchows Arch 2007;451(1):19-25. van de Vijver M, Bilous M, Hanna W, Hofmann M, Kristel P, Penault- Llorca F, et al. Chromogenic in situ hybridisation for the assessment of HER2 status in breast cancer: an international validation ring study. Breast Cancer Res 2007;9(5):R68. Bilous M, Morey A, Armes J, Cummings M, Francis G. Chromogenic in situ hybridisation testing for HER2 gene amplification in breast cancer produces highly reproducible results concordant with fluorescence in situ hybridisation and immunohistochemistry. Pathology 2006;38(2):120-4.

			Di Palma S, Collins N, Bilous M, Sapino A, Mottolese M, Kapranos N, et al. A quality assurance exercise to evaluate the accuracy and reproducibility of chromogenic in situ hybridisation for HER2 analysis in breast cancer. J Clin Pathol 2008;61(6):757-60
Laboratory Processes G. Staining v.Immunohistochemistry and In Situ Hybridization a. Quality assurance	• Establish Quality Assurance procedures for IHC and ISH procedures to include: o Compilation of predictive marker results ▪ Total cases ▪ % positive, % negative ▪ Comparison to benchmarks ▪ Corrective action taken • Documented participation in external proficiency testing for HER2 and ER	Anatomic Pathology Checklist, ANP.22970 - Annual Result Comparison – Breast Carcinoma All Common Checklist, COM.01520 – PT and Alternative Performance Assessment for IHC and ISH Predictive Marker Interpretation	Allison KH, Hammond EH, Dowsett M, McKernin SE et al. Estrogen and Progesterone Receptor Testing in Breast Cancer American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Path Lab Med*. Early Online Release. doi: 10.5858/arpa.2019-0904-SA Wolff AC, Hammond ME, Allison KH, Harvey BE, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Path Lab Med*; Nov 2018, Vol. 142, No. 11. pp. 1364-1382. Fitzgibbons PL, Bradley LA, Fatheree LA, Alsabeh R, et.al. Principles of Analytic Validation of Immunohistochemical Assays: Guideline From the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Path Lab Med*; Nov 2014, Vol. 138, No. 11. pp. 1432-1443.

COVERSLIPPING	LABORATORY PROCESSES - COVERSLIPPING		
Guideline Section	Statement	CAP Checklist	Reference

Laboratory Processes
 	
H. Coverslipping
 	
i. Manual/Automated
	• Establish manual coverslipping procedures that:
	  ○ Include ergonomic techniques
	  ○ Reduce chemical exposure
	• Use mounting media with an appropriate refractive index for proper resolution:
	  ○ Aqueous vs. non aqueous
	  ○ Non fluorescent
	• Identify size and weight of coverslip to be used
	• Identify drying method of coverslip and slide
	• Establish validation and operation procedures for an automated coverslipper:
	  ○ Speed of operation
	  ○ Type of mounting media
	  ○ Size and type of coverslip
	  ○ Type and volume of transfer fluid (xylene or xylene substitute)
	  ○ Cleaning and maintenance
	  ○ Reagent filling or change
	  ○ Filter change
	  ○ Drying time
	• Establish a preventative maintenance program that includes annual service and emergency service.
		Laboratory General Checklist, GEN.77200 - Ergonomics
		
		All Common Checklist, COM.30575 – Instrument Operation
		
		All Common Checklist, COM.30675 - Instrument /Equipment Records
			Bancroft J, Gamble M. Theory and Practice of Histological Techniques, 6th ed. New York, NY: Churchill Livingston; 2008.
			
			Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009

END			