Eastern Health

FORMALIN FIXATION	Laboratory Medicine LAB-020

Issuing Authority	**Kenneth Baird, Vice President Signed by Kenneth Baird Dated May 28, 2021**
Office of Administrative Responsibility	Laboratory Medicine
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Overview

Correct fixation is one of the most important steps for preservation of human tissues for pathology diagnosis. The quality of fixation will affect every subsequent step in histotechnology. Adequate fixation will facilitate histological assessment and diagnosis. In addition, standardized pre-analytical procedures, such as fixation, will lead to the most consistent results.

Standardized fixation is defined as: "Immersion of all specimen types, including large specimens and biopsies, within 1 hour at room temperature, in a volume of 10% neutral buffered formalin ten times that of the specimen, for no less than 24 hours and ideally for no greater than 72 hours".

The approximate fixation duration of specimens will be duly noted in the grossing report, and will be taken into consideration when interpreting test results, particularly for immunohistochemistry and molecular studies.

The cold ischemic time is defined as the period of time between removal of the specimen from the body to when it is placed in fixative and should be no longer than 1 hour. If it is anticipated to be greater than 1 hour, the specimen should be refrigerated at 4 degrees Celsius and fixed as soon as possible thereafter.

Tissue procured for flow cytometry analysis, frozen section analysis, immunofluorescence, electron microscopic examination, tumor banking, cytogenetics studies and some molecular procedures must not be fixed in formalin.

Specimens of extreme clinical urgency, as defined by the physician, in collaboration with, and at the discretion of the staff pathologist on call, may be processed within less than 24 hours fixation. For such cases, in the absence of analytical protocols validated for fixation less than 24 hours, the specific number of hours of fixation must be noted in the grossing report, and taken into consideration when interpreting test results, particularly for immunohistochemistry and molecular studies.

POLICY

Laboratory staff adhere to a standardized fixation protocol to achieve consistent results.

Scope

Pathology laboratory staff, pathologists, residents, clinicians, nursing staff and all other users of pathology laboratory services.

Purpose

To ensure that tissues for all Pathology procedures that require formalin fixation are appropriately and adequately fixed. Fixation preserves cells and tissues in as reproducible and lifelike state as possible.

Procedure

Formalin must be handled in a safe manner in a well-ventilated area by all staff. If it is necessary to decant formalin, the employee must wear appropriate personal protective equipment, including gloves and face shield or goggles in an approved fume hood. If an approved fumehood is not available, full face respirator masks must be worn while decanting formalin.

1. 10% neutral buffered Formalin (4% Formaldehyde) is the fixative of choice for most tissues.

2. Place all specimens in appropriately sized containers surrounded by a volume of formalin at least 10 times that of the tissue so that there is adequate tissue penetration of the fixative.

3. Place all tissues requiring formalin fixation in formalin as soon as possible and no later than 1 hour after removal from the body. Every effort should be made to place the specimen in formalin immediately after acquisition; however, whenever a delay in placing the specimen in formalin is anticipated, the specimen should be refrigerated at 4 (+/-2) degrees Celsius.

4. Submitting staff must document the time of tissue acquisition ("Cold Ischemia Start Time column") and time of placement of the specimen in formalin on the pathology requisition. When there has been a delay in placing the specimen in formalin for greater than 1 hour, it should be noted in the "Comment" section of the pathology requisition by the submitting staff. These comments must be dictated by the grossing staff in the gross description.

5. Grossing personnel will open large hollow organ specimens and immerse in formalin to allow uniform contact of fixative with all areas of the specimen.

6. Grossing personnel will slice solid specimens at 5 millimeters (mm) intervals and immerse in fixative as soon as possible, preferably before 1 hour from acquisition time (excision or resection), to expose all surfaces of the specimen, for adequate tissue penetration of the fixative.

Supporting Documents *(References, Industry Best Practice, Legislation, etc.)*

- American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. Hammond ME, Hayes DF, Dowsett M, et al. J Clin Oncol. 2010 Jun 1;28(16):2784-95
- Minimum formalin fixation time for consistent estrogen receptor immunohistochemical staining of invasive carcinoma. Goldstein NS, Ferkowicz M, Odish E et al. Am J Clin Pathol 2003 Jul;120(1):86-92
- Rosai and Ackerman's Surgical Pathology (10th edition)
- Lester's Manual of Surgical Pathology (3nd edition)
- Material Safety Data Sheet - Formalin Solution.
- Applied Immunohistochemistry & Molecular Morphology. 16(6):513-520, December 2008.
- Delay to formalin fixation effect on breast biomarkers. Thaer Khoury, Sheila Sait, Helena Hwang, Rameela Changrasekhar, Gregory Wilding, Dongfeng Tan and Swati Kulkarni. Modern Pathology (2009) 22, 1457-1467
- Effects of Preanalytical Variables on Detection of Proteins by Immunohistochemistry in Formalin-Fixed Paraffin –Embedded Tissue. Kelly B. Engel, PhD; Helen M. Moore PhD Arch Pathol Lab Med. 2011;135;537-543
- Fitzgibbons et al., (2014) Principles of analytic validation of immunohistochemical assays: Guideline from the College of American Pathologists pathology and laboratory quality center. Archiv., Pathol Lab Med 138, 1432-1442.

Linkages

- Tissue Handling-Suboptimal Pathology Specimens #11751
- Specimen Labeling #4579
- Tissue Handling – Breast Specimen, Needle Localization #4608

- Tissue Handling – Kidney Biopsy #4610
- Tissue Handling – Lymph Nodes and Tissue for Lymphoma #4613
- Tissue Handling – Frozen Section Specimens #4609
- Tissue Handling – Limbs #4611
- Tissue Handling – Lung Resection #4612
- Tissue Handling – Muscle and/or Nerve Biopsy #4614
- Tissue Handling – Skin Biopsy for Immunofluorescence #4615
- Tissue Handling – Formalin Fixed Specimens on Weekends #4582
- Handling of Alcohol, Formalin, and Xylene #4786

Key Words

Fixation, formalin

Definitions & Acronyms

Fixation	A chemical process by which biological tissues are preserved from decay, either through autolysis or putrefaction. Fixation terminates any ongoing biochemical reactions, and may also increase the mechanical strength or stability of the treated tissues.