

Tissue Processing and Paraffin Embedding: Procedural Manual

I. Principle

Tissue processing is the preparation of fixed tissue for microscopic examination by removing water and replacing it with a medium that provides sufficient support for sectioning.

In routine paraffin histology, tissue is **dehydrated through graded alcohols, cleared with a suitable clearing agent, and infiltrated with molten paraffin wax**. The processed tissue is then oriented and embedded in paraffin to form a firm block suitable for microtomy and subsequent staining.

Proper processing and embedding preserve tissue architecture, provide adequate support, and allow thin, continuous sections to be produced for microscopic examination.

II. Scope

This procedure applies to routine histologic tissue specimens that have been adequately fixed and are intended for paraffin embedding.

It covers:

- Specimen preparation
- Dehydration
- Clearing
- Paraffin infiltration
- Paraffin embedding
- Quality control
- Safety
- Storage of completed paraffin blocks

The procedure is intended for use by authorized and appropriately trained laboratory personnel in a histopathology laboratory.

III. Processing Parameters

Processing Step	Purpose	Typical Reagent
Fixation	Preserves tissue morphology and prevents autolysis	10% neutral buffered formalin
Dehydration	Removes water from tissue	Graded ethanol
Clearing	Replaces alcohol and prepares tissue for paraffin	Xylene or approved substitute
Paraffin infiltration	Replaces clearing agent with paraffin	Molten paraffin wax

Embedding	Provides support and establishes tissue orientation	Paraffin wax
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IV. Specimen Requirements

The specimen must:

- Be properly identified.
- Be adequately fixed before routine processing.
- Be appropriately trimmed to permit penetration of processing reagents.
- Be placed securely in a labeled tissue cassette.
- Be identified if it requires special processing.

V. Materials and Equipment

A. Reagents

- 10% neutral buffered formalin, when applicable
- Graded ethanol solutions
- Absolute ethanol
- Xylene or approved clearing substitute
- Paraffin embedding medium
- Cleaning and disinfecting materials

B. Equipment

- Tissue cassettes
- Automated tissue processor
- Paraffin embedding station
- Embedding molds
- Cold plate or cooling surface
- Forceps
- Labeling materials
- Laboratory timer
- Appropriate PPE

VI. Quality Control

Quality control should be performed according to the laboratory's established histopathology procedures.

1. Verify reagent identity, concentration, condition, and expiration.
2. Check that the correct processing program is selected.
3. Monitor reagent quality and replace reagents according to the laboratory schedule.
4. Ensure paraffin is maintained at the appropriate operating temperature.
5. Check that tissue is adequately processed before embedding.
6. Verify specimen identification and cassette-to-block traceability.
7. Inspect completed blocks for proper orientation and adequate tissue support.
8. Investigate and correct processing or embedding problems before sectioning.

VII. Operating Conditions

Processing times, reagent concentrations, paraffin temperature, and equipment settings should follow the laboratory's validated tissue-processing schedule and the manufacturer's instructions.

Tissue size, thickness, density, and composition may affect reagent penetration and processing time.

Excessive heat should be avoided because it may adversely affect tissue morphology.

VIII. Procedure

A. Preparation

1. Verify the specimen identification and corresponding laboratory record.
2. Perform hand hygiene and put on appropriate PPE.
3. Confirm that the specimen has been adequately fixed.
4. Place the specimen in a properly labeled tissue cassette.
5. Ensure that the cassette is securely closed.
6. Prepare the tissue processor and required reagents.
7. Check reagent levels, expiration dates, and equipment readiness.
8. Select the appropriate tissue-processing program.

B. Tissue Processing

1. Place the labeled tissue cassettes into the tissue processor.
2. **Dehydrate** the tissue by sequential exposure to graded concentrations of ethanol.
3. Continue dehydration through higher concentrations of ethanol until adequate dehydration is achieved.
4. **Clear** the tissue using xylene or an approved clearing agent.
5. Clearing replaces the alcohol and prepares the tissue for paraffin infiltration.
6. **Infiltrate** the tissue with molten paraffin wax.
7. Allow paraffin to penetrate the tissue completely and provide internal support.
8. Complete the validated processing cycle.
9. Remove the cassettes after the processing cycle has finished.
10. Inspect the processed tissue before embedding.
11. If the tissue is inadequately processed, follow the laboratory's reprocessing procedure.

C. Paraffin Embedding

1. Prepare the embedding station, embedding mold, molten paraffin, forceps, and labeled cassette.
2. Select an appropriately sized embedding mold.
3. Fill the mold with sufficient molten paraffin.
4. Using clean forceps, place the processed tissue into the paraffin.
5. **Orient the tissue correctly** according to the desired plane of sectioning.
6. Position the labeled cassette on top of the mold.
7. Add sufficient molten paraffin to completely surround the tissue and attach the cassette.
8. Transfer the mold to the cooling surface.
9. Allow the paraffin to completely solidify.
10. Remove the hardened paraffin block from the mold.
11. Inspect the block for correct identification, orientation, and adequate tissue support.
12. Store the completed paraffin block in the designated area until sectioning.

D. Block Verification

1. Confirm that the block label corresponds to the original specimen.

2. Confirm that the tissue is completely surrounded by paraffin.
3. Check that the tissue is firmly supported.
4. Verify that the tissue orientation is appropriate for microtomy.
5. Document any processing or embedding abnormalities.
6. Proceed to sectioning only when the block is adequately hardened and suitable for microtomy.

IX. Troubleshooting

Problem	Possible Cause	Corrective Action
Tissue remains soft	Incomplete dehydration, clearing, or infiltration	Review processing schedule and reprocess if necessary
Tissue is excessively hard or brittle	Over-processing	Review processing time and reagent condition
Tissue does not section properly	Poor processing, incorrect orientation, or inadequate cooling	Check processing quality and block hardness
Tissue separates from paraffin	Poor infiltration or embedding	Review infiltration and embedding technique
Incorrect tissue orientation	Improper placement during embedding	Correct orientation or prepare a replacement block

X. Result / Acceptance Criteria

A properly processed and embedded specimen should be:

- Adequately dehydrated
- Properly cleared
- Completely infiltrated with paraffin
- Firm and well-supported
- Correctly labeled
- Properly oriented
- Suitable for microtomy and subsequent staining

XI. Biosafety

Tissue specimens and materials that have contacted human tissue or body fluids must be handled as potentially infectious.

- Perform hand hygiene before and after specimen handling.
- Wear appropriate PPE.
- Handle formalin, alcohols, clearing agents, and molten paraffin carefully.
- Avoid direct contact with specimens and contaminated materials.
- Use appropriate ventilation when handling volatile clearing agents.

- Handle heated equipment and molten paraffin carefully to prevent burns.
- Dispose of contaminated materials according to laboratory waste procedures.
- Clean and disinfect work surfaces after use and following spills.
- Follow established procedures for spills and exposure incidents.
- Do not eat, drink, or apply cosmetics in laboratory work areas.

XII. Documentation and Records

The following should be documented according to laboratory policy:

- Specimen identification
- Processing information
- Reagent information and lot numbers, when required
- Processing schedule
- Equipment checks
- Processing or embedding errors
- Corrective actions
- Paraffin block identification and traceability

XIII. References

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