Clinical Histology
Procedure
Histo27.04

Paraffin Block Microtomy and Case Preparation for Microscopic Interpretation

Final Approval: May 2010 Effective: May 2010

List all stakeholder(s) and dates of approval:

<table>
<thead>
<tr>
<th>Stakeholder Name(s):</th>
<th>Shelly M. Siegel HT Date: 7/12/12 Reviewed</th>
<th>Revised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stakeholder Name(s):</td>
<td>Shelly M. Siegel HT Date: 8/17/12 Reviewed</td>
<td>Revised</td>
</tr>
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<td>Stakeholder Name(s):</td>
<td>Shelly M. Siegel HT Date: 2/17/13 Reviewed</td>
<td>Revised</td>
</tr>
<tr>
<td>Stakeholder Name(s):</td>
<td>Shelly M. Siegel HT Date: 7/30/13 Reviewed</td>
<td>Revised</td>
</tr>
<tr>
<td>Stakeholder Name(s):</td>
<td>Shelly M. Siegel HT Date: 6/17/14 Reviewed</td>
<td>Revised</td>
</tr>
</tbody>
</table>

Describe briefly the most recent revision made to this policy, procedure or protocol & why:
1/15/14 Cutting guide has been revised with the prostate needle cores to only make 1 unstained instead of 2. The negative was deleted from the pin-4 protocol.
7/30/13 Cutting guide has been revised with visuals to help the Histotech perform the correct amount of cuts based on part type. Two identifiers; accession number and patient last name will be added to all slides throughout the histology process added from February 2013.
The pathologists request that large skin excisions show 1 full representation on a slide instead of 4 levels.
The CoPath upgrade allows the cassette printer to be interfaced. The number of color cassettes is limited to 6 colors eliminating the brown and lavender.
Effective August 1, 2011, the Pathology Department will no longer perform routine cytokeratin immunohistochemistry on sentinel lymph nodes removed for breast cancer, except in cases of invasive lobular carcinoma.
This decision was made after review of literature and discussion by members of the Salem Cancer Institute Breast Cancer Committee on June 9, 2011. This literature suggests that the detection of individual tumor cells (ITCs) in Sentinel nodes has little or no prognostic or therapeutic significance. (Weaver DL, et al. N Engl J Med. 2011;364:412–421).

Purpose/Policy Statement:
Using specifically designed, precision instrumentation, commonly practiced histologic techniques will enable the technician to routinely cut 4-micron thick sections of paraffin blocks which are mounted on glass microslides. Lymph node or kidney specimens will be cut at 3-micron thick sections. Central nervous system tissue will be cut at 6-8 micron thick sections. Hematoxylin & Eosin stained slides will be organized by accession number into presentation trays.

Definitions:
- n/a

STEPS / KEY POINTS

PROCEDURE:
1. Fill the tissue flotation bath with DH2O water and bring the temperature to 42-46°C.
2. Following paraffin embedding, blocks are aligned on the Histology Lab counter by color-coded priority and in somewhat numerical order. NOTE: Color-coding is used as a helpful tool to expedite work processes; current colors are used as follows, but are subject to change at any time:
   - Red - Early Read Out (M or L)
   - Yellow - Bone marrow (LB/MB)
   - Purple – cytology cell block (CN)
   - Pink – physician office or out of town hospital (L) (Santiam Community Hospital, Silverton Hospital, West Valley Hospital, Willamette Valley Medical Center)
3. Use **Histology Data Entry/Edit** in Copath to validate on each case the accession number, the patients last name, part type, number of specimens and/or blocks.
   - Reference the Procedure Notes and CUTTING GUIDE contained within this procedure to determine number of slides and sample representation required for each paraffin block.

4. Place microtome knife or blade system into microtome knife holder, set predetermined cutting angle, tighten securely, and position knife guards or similar safety precautions.

5. Using microtome controls, bring block face to knife-edge. Make certain the upper and lower edges of the paraffin block are parallel to the knife-edge (essential for ribboning).

6. Depending upon the size and orientation of the tissue sample, shave conservatively into the block surface taking appropriate cuts that may measure between 3-60 micrometers:
   a. Samples of small biopsy tissue may be trimmed only to the depth of the first representation of several levels that will be collected.
   b. Samples of large dissected tissue may be trimmed to the desired depth of the earliest full representation of tissue.

7. Place blocks on an ice tray to cool and absorb water to achieve quality sections.

8. Using microtome controls, begin tissue block ribboning. As 3-4 micron thick sections are cut, grasp the beginning of ribbon sections; gently lift up and away from knife-edge.

9. When the paraffin ribbon contains desired number of tissue sections, using forceps, brush, dissecting probe, or fingers, lift the end of the ribbon from knife-edge.

10. Using a delicate and gradual pulling motion carefully lay the ribboned tissue sections onto the clean water surface of the tissue flotation bath.

11. If wrinkles or bubbles occur, use same implements to gently and cautiously ease these artifacts from tissue sections, taking care not to create stretch-artifact that may affect pathologic interpretation.

12. Print and/or label the microslide with minimum of two patient identifiers to the individual cut block. (Accession # and patients last name with a minimum of 3 letters.)

   **For quality assurance purposes: as the microtomy for each unique and individual block is completed, correctly and accurately label all corresponding slides prior to beginning microtomy on another block.**

13. Perpendicularly insert a CLEAN glass microslide into the water and orient the tissue sections(s) on the slide, either centrally or according to positioning of first representative level, and lift the sections from the surface of the water.

14. Drain excess water from slides.

15. Following the preparation of paraffin sections from each block, use Kleenex or Kim-wipe to thoroughly wipe clean the surface of the water and the edges of the flotation bath to prevent floaters or contamination.

16. Do not expose your tissue flotation bath to consecutive like specimens or more than one block.

17. Load slides into staining rack and or drying trays.

18. Dry slides in a 75-80 degree oven for minimum of 20 minutes or until water droplets are no longer visible on the slides.

19. Stain slides routinely with Hematoxylin and Eosin on the designated **Leica Autostainer** program.


**CALCULATIONS:** N/A

**CALIBRATION:** N/A

**QUALITY CONTROL:**

1. **Waterbath temperature log** is recorded by the histotech.

2. Use only CLEAN microslides; those contaminated with fingerprints, dust, dirt, or any other product must be discarded in a designated glass sharps container.
3. As the slide is labeled (Step 12), the accession number and patient last name on the slide must be verified with the cut block.

4. At any time during microtomy when extraneous tissue or paraffin “floater” contaminants are visualized in the flotation bath water, discard the water, thoroughly clean the reservoir, and refill with fresh deionized water.

5. At the end of each day’s microtomy, discard the water from the tissue flotation bath, thoroughly clean the reservoir with detergent soap to remove tissue, and refill with fresh deionized water.

6. A secondary container for deionized water needs to be replaced every 2 weeks to prevent bacteria or fungus growth contamination in the tissue flotation bath.

7. At the end of each day’s microtomy, perform daily microtome maintenance and document on the form HISTOLOGY LABORATORY MICROTOME MAINTENANCE.

8. Following daily stainer maintenance, an H&E test slide will be stained and the satisfactory results documented on the form HISTOLOGY LAB H&E STAIN PERFORMANCE IMPROVEMENT.

9. Pathologists will utilize the form PATHOLOGY FEEDBACK to record and report exceptions found in the pathology laboratory.

10. 1 form per day, HISTOLOGY QUALITY CONTROL, will be filled out by a reading pathologist.

**PROFICIENCY TESTING:** College of American Pathologists HistoQIP.

**PROCEDURE NOTES:**

1. Cut paraffin sections routinely at 4 microns (except for lymph node or kidney tissue cut routinely at 3 microns and central nervous system tissue cut routinely at 6-8 microns).

2. On small diagnostic biopsy samples, the original H&E shall be multiple levels that represent approximately 75% of the original specimen depth. Leave 25% of the tissue sample in the paraffin block.
   - Depending on the sample size, trim redundant paraffin from the block edges to maximize the number of levels obtained on each slide. The microtomist must utilize his/her training and expertise to insure that the prepared slides represent the blocked tissue specimen and that satisfactory representation of that sample has been achieved on the original H&E slide.

3. Concurrently, some samples require technologist discretion to ensure the integrity of the specimen; the sample must not be compromised by excessive cutting-through the block and tissue must remain for possible future additional testing unless otherwise excluded in the cutting guide.

4. On Cytology Cell Blocks, stop cutting at the first diminishment in size of the sample.

5. The microtomist is responsible in determination of malorientation and initiating re-embedding of the sample. When re-embedding is not desirable, the microtomist is responsible for preparing H&E level and saving additional UnStained (US) slides at representative levels through the block until satisfactory sample representation has been achieved.

**TECHNIQUE NOTES:**

1. Do not trim too much paraffin from section this will lose the support from the block and lead to cutting “thick then thin” sections.

2. Paraffin that loops around edge of slide will remove or tear sections away after immediately picking up from waterbath and loading in a rack. Wipe edge of extra paraffin or allow slide to dry before loading.

3. To prevent “chasing” attach ribbon(s) to edge of waterbath.

4. Use forceps/slide/brush to detach section. Use forceps/brush/pick to gently guide orient section(s) onto slide.

5. If you are having trouble ribboning, gently scrape your thumb nail across the bottom edge of the block.

6. Each section/ribbon needs to be orientated the same way on slide(s).

7. If you are having trouble ribboning try trimming the top and bottom of the block.
8. Minimize your sections with “floaters”. Do not use your fingers in the waterbath to orient sections on slide. This will contaminate your waterbath/sections with epithelial cells.

9. For single sections on unstains, place on the lower half of slide but not low enough where IHC staining does not stain tissue. See example.

**LIMITATIONS OF PROCEDURE:**

**Equipment/Supplies (If Applicable):**

**SPECIMEN:** Tissue-containing paraffin blocks.

**MATERIALS, REAGENTS:**
- Microtome, accompanying knife or blade system
- Thermometer
- Forceps, Brush, Probe
- Permanent marking pen
- Glass microslides plus (+) charged
- Rapid Freezing Spray
- Ice Tray
- Tissue Flotation Bath (water bath)
- Deionize Water

**Histology Tissue Processing Batch Log**
- Light Microscope
- SAKURA coverslipping instrument

**Form Name & Number or Attachment Name (If Applicable):**
- Histo27, Cutting Guide found H:\Dragon\Procedures\POLICY AND PROCEDURES\Anatomic Pathology-Histology-RL\approved procedure\Cutting Guide

**Author Position:**
- Lead Histologist

**Review/Revision Authority (Position Not Individual Name):**
- Lead Histologist

**Expert Consultant Position/s (Not Individual Name/s):**
- N/A

**References (Required for Clinical Documents):**

**MANUFACTURER’S PACKAGING BROCHURE/INSERT:**
- Operator’s Manual for each microtome instrument.
Is there a Regulatory Requirement?  Yes ☐ No ☐
If yes, insert requirement information here:

Review History (No Changes):
Shelly M. Siegel HT Date: 8/26/2010 Reviewed ☒ Revised ☐
Mark Magilner, MD Date: 8/27/10 Reviewed ☒ Revised ☐

Revision History (Note changes in area under header):
Shelly M. Siegel HT Date: 7/27/2011 Reviewed ☐ Revised ☒

Computer Search Words:
N/A

Policy, Procedure or Protocol Cross Reference Information:
N/A
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<th>PART TYPE</th>
<th>LEVELS/RIBBONS FOR H&amp;E</th>
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<tbody>
<tr>
<td>BREAT: needle core biopsy</td>
<td>3</td>
<td>NO</td>
</tr>
<tr>
<td>BREAT: STR stereotactic/microcalcifications</td>
<td>6</td>
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</tr>
<tr>
<td>BONE MARROW: aspiration (clot)</td>
<td>3</td>
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</tr>
<tr>
<td>BONEMARROW: biopsy (decal)</td>
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</tr>
<tr>
<td>CELL BLOCK:</td>
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<tr>
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<td>3</td>
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<tr>
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<tr>
<td>EN TOTO Small biopsies submitted</td>
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</tr>
<tr>
<td>LIVER: Hep C biopsy</td>
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</tr>
<tr>
<td>LUNG: biopsy (multiple fragments)</td>
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<tr>
<td>LUNG: conization</td>
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<tr>
<td>LUNG: H&amp;E</td>
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<tr>
<td>LUNG: 3 OR MORE UNSTAINED, UP TO 3 RIBBONS PER SLIDE</td>
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<tr>
<td>LIVER: LYMPH NODE: LUNG: SOFT TISSUE MASS:</td>
<td>4</td>
<td>YES, (1-XX), 1 SECTION PER SLIDE,</td>
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<tr>
<td>SKIN: other than cyst/tag/debridement</td>
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<tr>
<td>PROSTATE: H&amp;E</td>
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<td>TEMPORAL ARTERY: biopsy</td>
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<tr>
<td>TEMPORAL ARTERY: UNSTAINED FOR ELASTIC STAIN</td>
<td>US= LEVEL 3, 6, &amp; 9</td>
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