Clinical Surgical Pathology - Gross Room Procedure
surgpath09.01

H&E Stain On Frozen Sections

Final Approval: May 2010 Effective: May 2010
Next Review Date: April 2015

List all stakeholder(s) and dates of approval:

<table>
<thead>
<tr>
<th>Stakeholder Name(s)</th>
<th>Date</th>
<th>Reviewed</th>
<th>Revised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geoffrey Werner</td>
<td>5/3/10</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Mark Magilner, MD</td>
<td>5/2010</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Geoffrey Werner</td>
<td>4/12/13</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Geoffrey Werner</td>
<td>3/26/14</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

Describe briefly the most recent revision made to this policy, procedure or protocol & why:
New Procedure

Purpose/Policy Statement:
For diagnostic purposes, a RAPID nuclear & cytoplasmic staining technique is required to demonstrate relationships among cells and tissue components.

Definitions:
- N/A

STEPS / KEY POINTS

SPECIMEN: Tissue embedded in OCT medium is cut at 5 micron-thickness using a cryostat microtome and mounted on a glass microslide.

MATERIALS, REAGENTS:

Hematoxylin II- Richard-Allan #7231

Methyl-Formalin Fixative:
Methyl alcohol . . . . . 720 ml
37% Formaldehyde . . . 80 ml

CAUTION – FLAMMABLE, IRRITANT, SERIOUS HEALTH HAZARD, SUSPECTED CARCINOGEN.
Wear appropriate protective equipment.
CONTAINS FORMALDEHYDE.
Toxic by inhalation and if swallowed.
Irritating to the eyes, respiratory system, and skin.
May cause sensitization by inhalation or skin contact.
Risk of serious damage to eyes.
May cause cancer.
Repeated or prolonged exposure increases the risk.

CAUTION – FLAMMABLE, IRRITANT.
Wear appropriate protective equipment.

100% Ethyl Alcohol (EtOH)

CAUTION – FLAMMABLE, IRRITANT.
Wear appropriate protective equipment.

70% Ethyl Alcohol (EtOH)

CAUTION – FLAMMABLE, IRRITANT.
Wear appropriate protective equipment.

WORKING Phloxine Eosin
95% EtOH . . . . . . . 3120 ml
1% Eosin . . . . . . 400 ml
1% Phloxine B . . . . 40 ml
Acetic Acid . . . . . . 16 ml

H&E Stain on Frozen Section
Page 1 of 3
INSTRUMENTATION OR EQUIPMENT:
Frozen section H&E stain set.

PROCEDURE:
1. Immediately immerse frozen-section slides in Methyl-Formalin for at least 30 sec.
2. Rinse in clean tap H2O for 10 dips.
4. Rinse in clean tap H2O for 10 dips.
5. Blue in Scott's Tap Water for 5 sec.
6. Rinse in clean tap H2O for 10 dips.
7. 70% EtOH for 5 dips
8. Stain in Eosin for 20 sec.
9. 95% EtOH for 5 dips.
10. Absolute EtOH for 15 dips.
11. Absolute EtOH for 15 dips.
14. Mount with synthetic medium.

CALCULATIONS: N/A
CALIBRATION: N/A

QUALITY CONTROL:
1. Stains and reagents are filtered or changed daily and documented on a QA recordsheet.
2. Mounting medium consistency is validated on a daily basis. Reagent consistency must be balanced to avoid evaporation extremes following coverslipping. Either replace or add fresh mounting medium to the container. The mounting medium may also be “thinned”: transfer the container to the Histology Lab., add small incremental amounts of Cleartile III or xylene (reagents prohibited from the Pathology Gross Room), and mix thoroughly.

RESULTS: Nuclei - blue.
Cytoplasm - pink.

PROCEDURE NOTES: NONE.

LIMITATIONS OF PROCEDURE: N/A


MANUFACTURER PROCURE/INSERT: NONE.

DISTRIBUTION OF PROCEDURE: Histology Policy & Procedure.
Equipment/Supplies (If Applicable):
Disposable gloves
Laboratory coat
Eye protection
Surgical mask or face shield if there is a possibility of specimen or chemical release into the air

Form Name & Number or Attachment Name (If Applicable):
surgpath09

Author Position:
Histology Supervisor

Review/Revision Authority (Position Not Individual Name):
Medical Director, Operation Manager, Histology Supervisor, Pathologist Assistant

Expert Consultant Position/s (Not Individual Name/s):
Pathology Physician Assistant

References (Required for Clinical Documents):
n/a

Is there a Regulatory Requirement? Yes ☐ No ☐
If yes, insert requirement information here:

Review History (No Changes):
n/a

Revision History (Note changes in area under header):
n/a

Computer Search Words:
n/a

Policy, Procedure or Protocol Cross Reference Information:
n/a