Clinical Histology
Procedure
Histo26.03

Oil Red O Stain

Final Approval: May 2010 Effective: May 2010

List all stakeholder(s) and dates of approval:

Stakeholder Name(s): Shelly Siegel Date: 6/17/14 Reviewed ☑ Revised ☐
Stakeholder Name(s): Shelly Siegel Date: 5/3/11 Reviewed ☑ Revised ☐
Stakeholder Name(s): Shelly Siegel HT Date: 6/15/12 Reviewed ☑ Revised ☐
Stakeholder Name(s): Shelly Siegel HT Date: 6/28/13 Reviewed ☑ Revised ☑

Next Review Date: June 2015

Describe briefly the most recent revision made to this policy, procedure or protocol & why:
6/28/13 Cytospins need to be air-dried only, not fixed in 95% alcohol. All tissue needs to be fresh and unfixed to do this stain.

Purpose/Policy Statement:
The procedure will demonstrate the presence of cellular fat in tissue sections for diagnostic purposes.

Definitions:
• n/a

STEPS / KEY POINTS

PROCEDURE:
1. Fix Frozen Sections in absolute Propylene Glycol for 2 min.
2. Collect a Fat CONTROL slide from the designated storage jar to be stained simultaneously with the patient slide.
3. Stain slides in 57-62°C Oil Red O for 6 min. (Oil Red O must be at temperature before staining.)
4. Differentiate in 85% Propylene Glycol for 1 min.
5. Two DH₂O rinses.
6. Counterstain in fresh filtered Harris Hematoxylin for 1 min. (Dispose Hematoxylin down sink.)
7. Two DH₂O rinses.
8. Two tap H₂O rinses.
9. DO NOT COVERSLIP WITH FILM. Mount with Vectashield (Found in refrigerator) aqueous medium using 22 x 40 cover glass.
10. DO NOT COVERSLIP WITH FILM. Seal edges of cover glass with LA colors base/top coat nail polish.

CALCULATIONS: N/A
CALIBRATION: N/A

QUALITY CONTROL: Each time the procedure is performed, a known fat-positive slide labeled with the current date and "FAT CONTROL" shall be stained simultaneously with the patient slide(s). Patient slide(s) shall be labeled with the current date, to correspond with the matching date on the CONTROL slide.

PROFICIENCY TESTING: College of American Pathologists HistoQIP.
RESULTS:
Fat - red.
Nuclei - blue.

PROCEDURE NOTES:
1. Prepare a fat-positive CONTROL by making a direct smear from a “fresh” unfixed fatty tissue specimen onto the fully frosted slide.
2. Fat-positive controls are prepared in advance and stored in a separate container of 100% Propylene Glycol.
3. Oil Red O stain needs to be at 57-62°C at time of staining.

LIMITATIONS OF PROCEDURE: False positive results may occur due to grease marks on the microslides. Pre-cleaning in 95% alcohol and avoiding fingerprints on the slides reduces this possibility.

Equipment/Supplies (If Applicable):
SPECIMEN: Fresh, frozen tissue sections cut at 6-8 microns, fresh smears or touch preparations mounted on plus (+) charged microslides.
MATERIALS, REAGENTS:

**CAUTION: IRRITANT**
Wear appropriate protective equipment.

<table>
<thead>
<tr>
<th>100% Propylene Glycol</th>
<th>Harris Hematoxylin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Am. Hist. Reagent Co. - #KTORO</td>
<td>Surgipath - #01562</td>
</tr>
<tr>
<td>Expires 3 months</td>
<td>Filter before use, dispose after use</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oil Red O Stain</th>
<th>Aqueous Mounting Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Mastertech. - #STORO100</td>
<td>Vectashield - #H1000</td>
</tr>
<tr>
<td>Filter before each use.</td>
<td>Keep refrigerated.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>85% Propylene Glycol</th>
<th>Fat Control Slides</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Propylene Glycol . . . 42.5 ml</td>
<td>S/P Dakin Micro Slides Cat # M6150-1</td>
</tr>
<tr>
<td>Distilled H2O . . . . . . . . 7.5 ml</td>
<td>Fully Frosted</td>
</tr>
<tr>
<td>Expires 3 months</td>
<td></td>
</tr>
</tbody>
</table>

**CAUTION: FLAMMABLE, IRRITANT:**
Wear appropriate protective equipment.

| L.A. Colors base/top coat | Nail polish |

INSTRUMENTATION OR EQUIPMENT:

Form Name & Number or Attachment Name (If Applicable):
Histo26

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):

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

Review History (No Changes):
Mark Magilner, MD  Date: 8/27/10  Reviewed  ◐  Revised  □

Revision History (Note changes in area under header):
Shelly M. Siegel HT Date: 8/26/2010 Reviewed  ◐  Revised  ◐

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N/A

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N/A