Clinical Histology
Procedure
Histo26.01

Oil Red O Stain

<table>
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<tr>
<th>Final Approval: May 2010</th>
<th>Effective: May 2010</th>
<th>Next Review Date: May 2012</th>
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List all stakeholder(s) and dates of approval:

| Stakeholder Name(s): Shelly M. Siegel HT Date: 8/26/2010 | Reviewed ☒ | Revised ☒ |
| Stakeholder Name(s): Mark Magilner, MD Date: 8/27/10 | Reviewed ☒ | Revised ☐ |
| Stakeholder Name(s): Shelly Siegel Date: 5/3/11 | Reviewed ☒ | Revised ☐ |
| Stakeholder Name(s): | Date: | Reviewed ☐ | Revised ☐ |

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

Purpose/Policy Statement:
For diagnostic purposes, to demonstrate the presence of cellular fat in tissue sections.

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.
4. Differentiate in 85% Propylene Glycol for 1 min.
5. Two DH2O rinses.
6. Counterstain in Harris Hematoxylin for 1 min.
7. Two DH2O rinses.
8. Two tap H2O rinses.
9. Mount with Vectashield (Found in refrigerator) aqueous medium using 22 x 40 cover glass.
10. Seal edges of cover glass with fast-dry nail enamel.

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 stored 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:

<table>
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<th>CAUTION: IRRITANT</th>
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<td>Wear appropriate protective equipment.</td>
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- **100% Propylene Glycol**
  Am. Hist. Reagent Co. - #KTOPR
  Replace QUARTERLY

- **Harris Hematoxylin**
  Surgipath - #01562
  Filter before each use, replace MONTHLY

- **Oil Red O Stain**
  American Mastertech. - #STORO100
  Filter before each use, replace QUARTERLY

- **Aqueous Mounting Medium**
  Vectashield - #H1000

- **85% Propylene Glycol**
  100% Propylene Glycol . . . 42.5 ml
  Distilled H2O . . . . . . . . . . . 7.5 ml
  Replace QUARTERLY

- **Fat Control Slides**
  S/P Dakin Micro Slides Cat # M6150-1
  Fully Frosted

- **Coverslip**
  22 x 40 cover glass

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

- **Fast-dry Nail Enamel**
  Maybelline Express Finish

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):
N/A

Revision History (Note changes in area under header):
N/A

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N/A

Policy, Procedure or Protocol Cross Reference Information:
N/A