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|  **Oil Red O Stain** |
| **Purpose** | To demonstrate fat in an abnormal place, such as kidneys or brain. Lipid stains may identify cell structure degenerative change and detect changes in lipid metabolism. |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | This technique involves primarily a physical process which involve chemical mechanisms. The dye is dissolved in a lipid solvent and sections are treated with the dye-solvent solution. Since the dye is comparatively more soluble than in the original solvent, the dye will move out of the solvent and color the lipid. |
| **Materials** | **Supplies** | **Reagents** |
|  | • Coplin jars with lids• Graduated cylinders• PPE | • Propylene Glycol• Oil Red O Stain• Hematoxylin• Bluing |
| **Sample** | Cryostat sections: cut at 4 microns mounted on charged slides, air-driedCytology preparations: Air-dried, no fixation required. |
| **Quality Control** | Important - Do not expose tissue to any lipid solvents. Always use frozen sections for staining to demonstrate simple fats in tissue.Use known positive material as a control, such as frozen section of adrenal gland or tissue demonstrated to have stores of fat Known positive Bronchial washing cytospin preparations for cytology specimens. Control material for both are prepped, air-dried and stored in the -70 freezer. |
| **Special Safety Precautions** | Oil Red O is a suspected carcinogen and should be handled according to established Safety precautions. Waste stain and Propylene Glycol containing any ORO are collected in labeled Hazardous Waste satellite containers for disposal. |
| **Stock Solutions** | **Oil Red O Solution****Hematoxylin 1****Bluing** |
| **Working Solutions** |  **Propylene Glycol, 85%**Propylene Glycol……….85.0 mLDistilled water……….….15.0 mL |
| **Procedure** | **Step** | **Action** |
|  | 1 | Frozen sections are air-dried **5-10** minutes minimum prior to stainingCytology preparations are air-dried |
|  | 2 | Place slides in Oil Red O Solution with lid on……….**15-20** minutes (The slides may be left in the stain up to 30 minutes) |
|  | 3 | Remove slides and drain/ blot to remove excess stain. |
|  | 4 | Place slides in Propylene Glycol, 85%……….**1** minute. Gently agitate |
|  | 5 | Rinse slides well in Distilled water….**1** minute |
|  | 6 | Counterstain with Hematoxylin…….**2** minutes |
|  | 7 | Rinse slides gently in tap water |
|  | 8 | Place slides in Bluing solution……….**1** minute |
|  | 9 | Rinse slides gently in running tap water ……..**3** minutes |
|  | 10 | Coverslip gently using aqueous mounting media. Do not push out air bubbles. |
| **Results** | Lipids/Fat……….….RedNuclei……..........….Blue |
| **Result Reporting** | By Pathologists |
| **References** | Sheehan, D.C., Hrapchak, B.B., Theory and Practice of Histotechnology; C.V, 1980, 2nd editionHistotechnology A Self-Instructional Text, F.Carson 1990 |

**Historical Record**

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| Version | Revised by | Effective Date | Summary of Revisions |
| 1 |  |  | Initial version. |
| 2 | A. Dubbelde | 6/27/19 | Update format, add version, and update to match current staining procedure used. |
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