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| **Alcian Blue 1%, pH 2.5 Stain** |
| **Purpose** | The demonstration of sulfated mucosubstances.  |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | The use of various pH values will aid in separating and identifying the different acid mucins. Strongly sulfated mucins react most consistently at low pH levels. Above pH 1.0, their staining reactions tend to be uncertain and will vary from moderate positive to negative. This procedure is used to differentiate between neutral and acidic mucosubstances. |
| **Materials** | **Supplies** | **Reag Reagents** |
|  | • Coplin jars with lids• PPE  | • Alcian Blue,1%• Nuclear Fast Red• Acetic Acid, 3% |
| **Sample** | * FFPE tissue
* Fresh frozen/ Cryostat tissue: air-dry.
* Imprints and cytological preparations: Fixed in 95% alcohol and air-dried.
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| **Quality Control** | Use a section of small intestine, appendix or colon as a control. |
| **Special Safety Precautions** | Glacial Acetic Acid has strong fumes and should be used under a fume hood. |
| **Stock Solutions** | Acetic Acid, 3%Alcian Blue, pH 2.5Nuclear Fast Red |
| **Working Solutions** | **All Reagents are ordered from Newcomer Supply ready to use.** |
| **Procedure** | **Step** | **Action** |
|  | 1 | Deparaffinize slides and hydrate to water |
|  | 2 | Place slides in Solution A: Acetic Acid, 3%……….**3** minutes Do not rinse slides with water. |
|  | 3 | Stain sections in Solution B: Alcian Blue, 1% solution……….**30** minutes |
|  | 4 | Wash in running tap water, then rinse in Distilled water |
|  | 5 | Place slides in Solution C: Nuclear Fast Red……….**5** minutes |
|  | 6 | Wash slides in running tap water |
|  | 7 | Dehydrate, clear and coverslip |
| **Results** | Acid Epithelial Mucin....................………....BlueStromal(mesenchymal mucin)....................BlueNuclei............………………………………...Pink/RedCytoplasm.......................................……….Pale Pink |
| **Result Reporting** | By Pathologist |
| **References** | Sheehan, D.C. and Hrapchak, B.B.: Theory and Practices of Histotechnology, 1980Carson, F.L.: Histotechnology, A Self-Instructional Text, ASCP Press,1990 |

**Historical Record**

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| Version | Revised by | Effective Date | Summary of Revisions |
| 1 | A. Dubbelde | 6/27/19 | Initial version. |
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