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| **Alcian Blue-PAS 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 | | • Acetic Acid, 3%  • Alcian Blue,1%  • Nuclear Fast Red  • Periodic Acid, 0.5%  • Schiff Reagent  • Hematoxylin |
| **Sample** | * FFPE tissue * Fresh frozen/ Cryostat tissue: air-dry. * Imprints and cytological preparations: Fixed in 95% alcohol and air-dried. | | |
| **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 with a fume hood. | | |
| **Stock Solutions** | **Acetic Acid**  **Periodic Acid, 0.5% Storage: All stored at room temp**  **Alcian Blue, 1%**  **Schiff Reagent**  **Hematoxylin** | | |
| **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:** 3% Acetic Acid……….**3** minutes  Do not rinse slides with water. | |
|  | 3 | Stain sections in **Solution B:** Alcian Blue solution……….**15** minutes | |
|  | 4 | Wash in running tap water, then rinse in Distilled water | |
|  | 5 | Place slides in **Solution C:** 0.5% Periodic Acid……….**5** minutes | |
|  | 6 | Wash in running tap water for 1-2 minutes; rinse in Distilled water | |
|  | 7 | Place slides in a glass coplin jar of **Solution D:** Schiff reagent……. **10** minutes | |
|  | 8 | Wash slides in running luke warm tap water……….**5-10** minutes | |
|  | 9 | Stain lightly in **Solution E:** Mayer Modified Hematoxylin...............**1** minute | |
|  | 10 | Rinse in running tap water for 1-2 minutes | |
|  | 11 | Dehydrate, clear and coverslip | |
| **Results** | Exclusively acid mucosubstances……………………………………..Blue  Neutral polysaccharides………………………………………………..Magenta/red  Certain substances stain with both Schiff’s and Alcian Blue……….Purple | | |
| **Result Reporting** | **By Pathologist** | | |
| **References** | Sheehan, D.C. and Hrapchak, B.B.: Theory and Practices of Histotechnology, 1980  Carson, 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 |  |  | Initial version. |
| 2 | A. Dubbelde | 6/27/19 | Update format, add version, update to match current staining procedure used. |
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