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| **PAS with and without Digestion Stain** |
| **Purpose** | To determine glycogen by **digesting** out and **staining** with **PAS stain,** demonstration of fungus and basement membrane. |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | The diastase (or a-amylase) act on glycogen to de polymerize it into smaller sugar units, maltose and glucose, that are washed out of the section. |
| **Materials** | **Supplies** | **Reagents** |
|  | • Coplin jars with lids• PPE• Graduated cylinder• Waterbath | • Periodic Acid, 0.5%• Schiff Reagent• a-Amylase• Hematoxylin • Acid Alcohol, 1%• Lithium Carbonate |
| **Sample** | Tissue: FFPEBone Marrow/ Heme Smears: Formaldehyde fumes, then Methanol fixation, air-dry.Cytology preparations: 95% Alcohol fix, then air-dry. |
| **Quality Control** | A section of normal liver, a normal appendix or containing fungusCytological preparations: tissue containing fungusHematological smears: Peripheral smear with large number of neutrophilsCoplin jars should be extremely clean. Rinse with Distilled water before use |
| Stock Solutions | a-AmylaseSchiff ReagentPeriodic Acid, 0.5%Hematoxylin, HarrisLithium CarbonateAcid Alcohol, 1% |
| **Working Solutions** | **Working Amylase Digestion Solution** Make fresh each use a-Amylase….....……..…...........0.5 gm Phosphate buffer, pH 6.0…..…50.0 mLMix using magnetic stirrer  |
| **Procedure** | **Step** | **Action** |
|  | 1 | Deparaffinize slides and hydrate to water. (Skip to step #4 if not running digestion)  |
|  | 2 | Label 2 slides; 1 "with" and 1 "without" |
|  | 3 | Preheat a coplin jar of each; Working Amylase Digestion Solution and Phosphate Buffer, pH 6.0 to 37° C |
|  | 4 | Place slides labeled "with" in preheated Amylase Digestion Solution. Place slides labeled "without" in preheated Phosphate Buffer Solution. Incubate slides for 60 minutes at 37° C |
|  | 5 | Wash all slides in running tap water..........**5** minutesRinse slides in Distilled water and combine slides for remaining steps |
|  | 6 | Oxidize sections in Solution A: Periodic Acid, 0.5%……............**10** minutes |
|  | 7 | Wash in 3 changes of tap water and rinse in Distilled water |
|  | 8 | Place slides into Solution B: Schiff Reagent ………..**20** minutes  |
|  | 9 | Wash slides in lukewarm tap water………..........**5-10** minutes |
|  | 10 | Stain in Solution C: Hematoxylin, Harris……….**1-5** minutes |
|  | 11 | Wash in tap water................**2-3** minutes |
|  | 12 | Differentiate in Solution D: Acid Alcohol, 1%.............**1-2** quick dips |
|  | 13 | Wash in tap water………**1** minute |
|  | 14 | Blue slides in Solution E: Lithium Carbonate..........**3-4** dips |
|  | 15 | Rinse in several changes of tap water; rinse in Distilled water |
|  | 16 | Dehydrate, clear and coverslip |
| **Procedure Notes** | Schiff’s reagent may produce a strong odor. Disposal in appropriate satellite Hazardous Waste container under a fume hood is suggested. |
| **Results** | Glycogen............................................MagentaGlycogen digestion.............................Abscence of magentaAcid and nuetral epithelial mucin........MagentaFungal cell walls.................................Red to purpleBasement membranes……………..…Red to purpleNuclei…………………………………...Blue |
| **Result Reporting** | By Pathologist |
| **References** | Sheehan, D.C. and Hrapchak, B.B.: Theory and Practice of Histotechnology, 2nd edition, p. 166; C.V. Mosby Co., St. Louis, Mo., 1980.Histotechnology 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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