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| **Grocott’s Methenamine Silver Stain** | | | |
| **Purpose** | For the demonstration of Pneumocystis, yeast, and fungal elements. | | |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. | | |
| **Principle** | Chromic Acid is used to oxidize the tissue and fungal polysaccharides to aldehyde groups. After the oxidation treatment, slides are placed in a solution of Sodium Bisulfite, which removes traces of Chromic Acid left in the tissue. A water wash follows and slides are then exposed to the alkaline Silver reagent. This reagent produces a selective blackening of the polysaccharides after the Chromic Acid oxidation. (The aldehyde oxidation renders them visible). Methenamine is added to the Silver reagent to give alkaline properties necessary for proper reaction; Borax is added to the working solution as a buffer. Gold Chloride is used to tone the tissue after the silver treatment and eliminate yellow tones from the section. Sodium Thiosulfate fixes the silver reaction in the tissue by stopping all previous reactions and removing unreduced Silver Nitrate. | | |
| **Materials** | **Supplies** | | **Reagents** |
|  | • Coplin jars and lids  • Gloves, PPE  • Graduated cylinders  • water bath | | • Chromic Acid, 5%  • Silver Nitrate  • Sodium Bisulfite, 1%  • Methanamine Borate  • Gold Chloride, 0.1%  • Sodium Thiosulfate, 2%  • Light Green SF Yellowish Stain, 0.02% |
| **Sample** | **Tissue:** FFPE  **Smear preps:**  95% Ethyl Alcohol………………………..…..2-10 minutes.  **Cryostat sections:**  Fix in 95% alcohol or FS fixative………2-10 minutes,rinse in  Distilled water.  **Imprints/ touch preps**: Fix in 95% alcohol, then airdry ……2-10 minutes. | | |
| **Quality Control** | Use control tissue positive for fungus and Pneumocystis.  All plastic and glassware must be extremely clean. Rinse in distilled water before use. Do not use metal forceps.  The more slides there are in a Coplin jar; the faster the silver will heat and turn a metallic color and become ineffective. | | |
| **Special Safety Precautions** | Silver Nitrate may cause severe burns. Avoid contact with skin and eyes. Poison: avoid breathing in, corrosive. Disopse of in a satellite waste container labeled with hazardous waste label.  Sodium Metabisulfite – avoid inhalation | | |
| **Stock Solutions** | Chromic Acid, 5%  Silver Nitrate  Sodium Bisulfite, 1%  Methanamine Borate  Gold Chloride, 0.1%  Sodium Thiosulfate, 2%  Light Green SF Yellowish Stain, 0.02% | | |
| **Working Solutions** | **Working Silver- Methenamine Solution**  Solution C: Silver Nitrate ……………………20 ml  Solution D: Methenamine Borate ………..…20 ml  **All other reagents are purchased from Newcomer Supply ready to use** | | |
| **Procedure** | **Step** | **Action** | |
|  | 1 | Deparaffinize slides and hydrate to Distilled water. | |
|  | 2 | Oxidize in Solution A: Chromic Acid, 5%................1 hour  Preheat Working Silver- Methenamine Solution to 45-60°C at this time | |
|  | 3 | Rinse well in tap water follow by Distilled water | |
|  | 4 | Rinse in Solution B: Sodium Bisulfite. 1%……….**1** minute | |
|  | 5 | Rinse slides in running water.............5 minutes. Then in Distilled water | |
|  | 6 | Place slides in preheated Working Silver- Methenamine Solution in water bath……**12-18** minutes (or at room temp) until sections appear paperbag brown. Periodically remove control, rinse in warm Distilled water, check microscopically for adequate silver impregnation. Fungi should be dark brown. If organisms are not sufficiently dark, return slides to warm silver solution and recheck every 2-3 minutes | |
|  | 7 | Rinse slides in several changes of Distilled water- Do NOT use tap at this step | |
|  | 8 | Tone slides in Solution E: Gold Chloride, 0.1% until sections turn gray……….20 seconds to **1** minute | |
|  | 9 | Rinse in Distilled water | |
|  | 10 | Reduce slides in Solution F: Sodium Thiosulfate, 2%……….**2** minutes | |
|  | 11 | Wash in running water......5 minutes. Rinse in Distilled water | |
|  | 12 | Counter stain in Solution G: Light Green SF Yellowish Stain, 0.02%........**2** minutes | |
|  | 13 | Dehydrate, clear and coverslip | |
|  | \*NOTE: If overstaining in the working Silver-Methenamine solutions occurs, the slides may be placed briefly in a solution of 0.5% Potassium Ferrocyanide. | | |
| **Interpretation/**  **Results/Alert Values** | Fungi………………………………………Crisp black walls with visible internal structures  Mucin………………………………………Taupe to dark gray  Background……………………………….Green | | |
| **Result Reporting** | By Pathologist | | |
| **References** | Sheehan/Hrapckak/Mosby: Theory and practice of Histotechnology, G.V. Mosby company, 1973. St. Louis, NO., pp170-189.  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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