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| **Gram Stain** | | | |
| **Purpose** | This technique differentiates the bacteria present into Gram-positive and Gram-negative categories. | | |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. | | |
| **Principle** | This procedure involves the application of a Crystal Violet solution, followed by an Iodine mordant to form a dye lake. Both Gram-positive and Gram-negative cells are colored blue-black after these first two steps. Decolorization is the third step, rendering the Gram-negative cells colorless while leaving the blue-black dye lake in the Gram-positive cells. The decolorization step extracts lipid from the cells wall of Gram-negative bacteria, thereby increasing the porosity of the cell wall and allowing the crystal violet-iodine complex to diffuse from the cell. | | |
| **Materials** | **Supplies** | | **Reagents** |
|  | • PPE  • Coplin jars with lids  • Slide staining rack | | •Gram Crystal Violet  •Gram Iodine  •Gram Saffranin  • 95% Alcohol  • Acetone |
| **Sample** | Tissue: FFPE  Cytology preparations: fixation in 95% alcohol, then air dry. (Air dried slides acceptable but not preferred).  Imprints or touch preps: air dried and post fixed in 95% alcohol  Hematology/ blood smears: fixation in Formaldehyde fumes, 10 minutes, then air dry. Secondary fixation in Methanol, then air dry before staining. | | |
| **Quality Controls** | The control slide should contain one tissue sample with Gram-positive organisms and one tissue sample with Gram-negative organisms. | | |
| **Special Safety Precautions** | Acetone should be used under a hood and disposed of in a separate satellite Hazardous Waste container. | | |
| **Stock Solutions** | Gram Crystal Violet  Gram Iodine  Gram Safranin  Acetone  95% Alcohol | | |
| **Working Solutions** | Decolorizing Solution.......................1:1 ratio of Acetone and 95% Alcohol  **All other Reagents are ordered from BD BBL ready to use.** | | |
| **Procedure** | **Step** | **Action** | |
|  | 1 | Deparaffinize tissue sections and hydrate to water  **Note:** For 2-3 slides, use a slide rack. For more, use Coplin jars | |
|  | 2 | Place slides in Gram Crystal Violet……….....….**1** minute | |
|  | 3 | Rinse slides in running tap water and drain | |
|  | 4 | Place slides in Gram Iodine ……………………...**1** minute | |
|  | 5 | Rinse slides in running water | |
|  | 6 | Decolorize sections by rinsing slides with Acetone/ 95% alcohol, until slides run clear | |
|  | 7 | Rinse slides immediately in running water | |
|  | 8 | Stain slides with Gram Safranin…………………**1** minute | |
|  | 9 | Rinse in Distilled water | |
|  | 10 | Air dry | |
|  | 11 | Dehydrate rapidly with 2 quick dips in 100% Alcohol, clear and coverslip | |
| **Results** | Gram-positive organisms………….Blue  Nuclei………………………………..Light Red  Gram-negative……………….…..…Pink/Red  Other tissue elements………….….Yellow  If sections are exposed too long to the action of the decolorizing process, even Gram-positive cells will lose the dye lake and become colorless and the stain will need to be repeated. | | |
| **Result Reporting** | By Pathologist | | |
| **References** | Bancroft, J.D. and Stevens, A.: Theory and Practice of Histology Techniques. Churchill Livingston, Edinburgh, London and New York, pp. 210-212, 1977.  American Journal of clinical Pathology, August 1973, Vol. 59:237-238, Brown, J.H. and Brenn, | | |

**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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