|  |
| --- |
| **Wright-Giemsa Stain** |
| **Purpose** | Used to perform **differential white blood cell counts and to study red blood cell morphology.** |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | This stain is a modification of the original Romanovsky procedure and is designated as a polychrome stain because it imparts metachromatic qualities to cell constituents and possesses the ability to color leukocyte granules differentially. This stain is insoluble in water but readily dissolved in methyl alcohol. |
| **Materials** | **Supplies** | **Reagents** |
|  | * PPE
* Coplin jars with lids
* Graduated cylinder
* Syringe, 10 mL
* Slide forceps
* Drying rack
 | * Methanol
* Wright-Giemsa Stain
* Phosphate Buffer, pH 6.4
 |
| **Sample** | Hematological preparations: Air dryCytological preparations: Air dry |
| **Quality Control** | Blood / Bone marrow smears, imprints and cytology preparations have internal control indicators; additional or separate controls are not necessary.Keep staining containers covered |
| **Special Safety Precautions** | Methanol and methanol-based stain waste is collected in a labeled satellite Hazardous Waste container. |
| **Stock Solutions**  | **Wright – Giemsa Stain****Phosphate Buffer, pH 6.4**   |
| **Working Solutions** | **Wright’s-Buffer Solution**Wright’s/Giemsa Stain……………...5.0 mL or 10.0 mLPhosphate Buffer, pH 6.4………...45.0 mL or 90.0 mL |
| **Procedure** | **Step** | **Action** |
|  | 1 | Fix slides in fresh Methanol …………..……**2** minutesSlides may remain in Methanol for up to 1 hour |
|  | 2 | Stain slides with Wright Stain (agitate a few times)….......….**10** minutes**Make fresh each use.**  |
|  | 3 | Place slides in Working Wright- Buffer Solution (agitate a few times)..............**30** minutes**Make fresh each use.**  |
|  | 4 | Quickly rinse slides in Distilled water to avoid stain precipitate |
|  | 5 | Place the slides in a vertical position to dry |
|  | 6 | Dip in Xylene and coverslip |
| **Trouble-Shooting Notes** | * If the stain is too light, restain the slides through the Wright’s and the Wright buffer solution. If the slides have already been cover slipped, place the slides in a xylene to remove the cover slip and the mounting media, then clear in several changes of Methanol and re-stain.
* Wright’s stain tends to be somewhat hygroscopic and can absorb water after a short period of time; even a small amount can cause marked red cell and other artifacts.
* Occasionally, it may be necessary to stain old unstained slides, restore poorly stained old slides, remove blue background, or decrease staining if cells are too blue. The following method can be helpful:
	+ Stain unstained or already stained slides with routine Wright’s stain and let air-dry.
	+ Rinse stained slides with 2 quick dips in acetic water (approximately 50 ml distilled water in a Coplin jar plus 1 drop of Glacial Acetic Acid)
	+ Wash immediately with several changes of distilled water and air dry.
 |
| **Results** | Red cells...................................Yellowish –redNeutrophils................................Dark purple chromatin, pale blue cytoplasm, lilac granulesEosinophils................................Dark purple chromatin, pale blue cytoplasm, bright red granulesBasophils...................................Dark purple chromatin, dark red granulesLymphocytes..............................Dark purple chromatin, light blue cytoplasmMonocytes..................................Medium blue chromatin, gray- blue cytoplasm, fine lilac granulesPlatelets.....................................Violet to purple |
| **Result Reporting** | By Pathologist |
| **References** | Sheehan, D.C. and Hrapchak, B.B.: Theory and Practice of Histotechnology, c 1980, C.V. Mosby Company, pp 154-155Williams, William J.; Beutler, Ernest; Erslu, Allen J.; and Rundles R. Wayne: Hematology, c 1972, McGraw-Hill, Inc., pp. 1356-1357.Clinical Hematology, 1993, Ninth edition, Lea & Febiger, pp23-24. |

**Historical Record**

|  |  |  |  |
| --- | --- | --- | --- |
| 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. |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |