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| **Myeloperoxidase Stain** |
| **Purpose** | To demonstrate peroxidase present in primary granules of neutrophils, granules of eosinophils and monocytes in the cytoplasm of leukemic cells. |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | The peroxidase activity of leukocytes transfers hydrogen from dye substrates to hydrogen peroxidase, yielding a colored derivative of the dye, which is localized at the site of the enzyme activity. |
| **Materials** | **Supplies** | **Reagents** |
|  | • PPE• Coplin jars with lids• Erlenmeyer Flasks• Plastic slide forceps• Disposable pipettes• Graduated cylinders• Filter paper• Disposable funnel• Parafilm | **•** Benzidine Dihydrochloride**•** Ethyl Alcohol, 100%**•** Formaldehyde,37%• Hydrogen Peroxide,3%• Safranin O• Sodium Acetate• Sodium Hydroxide• Zinc Sulfate |
| **Sample** | Use freshly prepared smears of whole blood or Bone Marrow (aspirate or concentrate) smears; tissue imprints or cytospin preparations. Heparin and EDTA are not inhibitory. Exposure to light should be minimized, as leukocyte peroxidase is photolabile. Allow slides to air dry at least 10 minutes if slides are freshly prepared. |
| **Quality Control** | Use a normal peripheral blood smear with a large number of neutrophils |
| **Special Safety Precautions** | Benzidine Dihydrochloride is a **carcinogen**. Always wear personal protective equipment and work under a hood. All materials contaminated with Benzidine, such as gloves, gauze or pipettes, must be placed in a red biohazard bag for incineration separately from routine red-trash collection bin material.  |
| **Stock Solutions** |  Benzidine Dihydrochloride Ethyl Alcohol, 100%Formaldehyde,37% Hydrogen Peroxide,3% Safranin O Sodium Acetate Sodium Hydroxide, 1N Zinc Sulfate |
| **Working Solutions** | **Formol-Ethanol, 10% \*FIXATIVE\*** Formaldehyde, 37% …………….5.0 mL Ethyl Alcohol, 100%…………….45.0 mL Make fresh each use **Ethyl Alcohol, 30% Make fresh**  Ethyl Alcohol,100%…….......30.0 mL Distilled water……….....……70.0.0 mL  **Zinc Sulfate Make fresh** Zinc Sulfate...............……….0.38 gm Distilled water…….……..….10.0 mL **INCUBATION REAGENT** Ethyl Alcohol, 30%………………….…....100.0mLBenzidine Dihydrochloride ……………..0.3 gmZinc Sulfate, 0.132 M…………………….1.0 mLSodium Acetate ………………….……….1.0 gmHydrogen Peroxide, 3% …………………0.7 mLSodium Hydroxide, 1N ……..………........1.5 mLSafranin O ………………………..………..0.2 gmPrepare all reagents separately and prior to starting Incubation preparation. The reagents should be added in the order listed, mixing well with each addition. 1. Carefully weigh out the Benzidine Dihydrochloride and place into a flask for the mixing process.
2. Add the 100 mL of Ethyl Alcohol, 30% slowly. Cover the flask with Parafilm before mixing.

 The benzidine salt may contain a small amount of inert residue, which will not go into solution. 1. Add 1.0 mL of Zinc Sulfate. Precipitate forms upon addition of the zinc sulfate but will dissolve upon the addition of the remaining reagents.
2. Add 1.0 gm of Sodium Acetate.
3. Add 0.7 mL of Hydrogen Peroxide, 3%.
4. Check the pH of the reagent. Start to add Sodium Hydroxide drop by drop until the pH is 5.5-6.5. The full 1.5 mL is usually not needed.
5. After pH-ing the solution with a test strip, add 0.2 gm of Safranin O and filter the solution into a storage container. This incubation reagent is stable up to 6 months.

When staining slides, use staining rack and disposable pipettes. Collect ALL stain waste in labeled satellite Hazardous Waste Container. |
| **Procedure** |  |
|  | **Step** | **Action** |
|  | 1 | Fix air dried smears in freshly prepared Formal-Ethanol, 10%…….………**45-60** seconds |
|  | 2 | Wash in Distilled water……….**15** to **30** seconds Shake off excess water and wipe the back of slides |
|  | 3 | Place well-drained slides on staining rack and flood with Incubation Reagent………..……….**1** minute. When the granules start to fade in intensity, the staining time may be adjusted in 30 second increments |
|  | 4 | Rinse well in Distilled water and check microscopically for fading of granules |
|  | 5 | Wash briefly in running tap water |
|  | 6 | Air dry and coverslip |
| **Procedure Notes** | * If better nuclear staining is desired at the expense of slightly less contrast, Safranin O may be eliminated from the incubation reagent and the slides stained with Richard Allan Hematoxylin 1 for 6-8 minutes. Wright’s stain is unsatisfactory as a counterstain.
* The amount of hydrogen peroxide added must be carefully measured. If too much hydrogen peroxide is used, the enzyme is destroyed before oxygen can be liberated. If not enough hydrogen peroxide is added or if the solution is old, the reaction will be weak or negative.
* No staining or weak staining may occur if:
	+ Slides are not completely well rinsed after the fixation step.
	+ The pH of the incubation reagent is not 5.5-6.5
	+ The incubation reagent is old or has become contaminated
* Permount coverslip mounting media causes fading of the color and should not be used for mounting of the coverslip.
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| **Limitations of Procedure** | * Prolonged fixation will destroy the peroxidase activity.
* Myeloperoxidase is sensitive to light, heat, methanol and bleach fumes.
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| **Results** | Nuclei...........................................RedCytoplasm....................................RedNeutrophil series..........................Blue granules Eosinophil granules......................Blue Basophils......................................No stainMonocytes...................................Either tiny blue granules or faint, diffuse cytoplasmic stainingLymphocytes & Erythrocytes.......No stain |
| **Result Reporting** | By Pathologist |
| References | Maile, J.B.: Laboratory Medicine Hematology, c. 1982 by C.V. Mosby Co., pg 206.Sheehan, D.C. and Hrapchak, B.B.: Theory and Practice of Histotechnology, 2nd edition, p. 309, 322; C.V. Mosby Co., St. Louis, MO., 1980Spangers, Ella: The University of Minnesota, Special Hematology Department, Peroxidase Staining Procedure, 1985. |

**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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