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| **Alpha-Naphthyl Acetate Esterase (NSE)** |
| **Purpose** | For the demonstration of Non-specific Leukocyte Esterase. This enzyme is detected primarily in monocytes, macrophages and histiocytes, and is absent in granulocytes. Lymphocytes may occasionally exhibit enzyme activity.  |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | When alpha-naphthyl acetate, in the presence of freshly formed diazonium salt, causes hydrolysis of ester linkages, free naphthol compounds are released. Free naphthol compounds couple with the diazonium salt to form highly colored deposits at the sites of alpha-naphthyl acetate esterase enzyme activity. |
| **Materials** | **Supplies** | **Reagents** |
|  | • Coplin jars with lids• PPE• Disposable pipettes• Plastic slide forceps• Erlenmeyer flask• Waterbath  | • Formaldehyde, 37%• Acetone |
| **Sample** | Appropriate specimens include:* Peripheral blood smears (blood may be anticoagulated with either heparin or EDTA)
* Bone marrow aspirate and/or concentrate smears
* tissue touch imprint slides
* Body fluids: CSF, Pleural fluid, Peritoneal fluid, etc.

\*\*Allow all slides to air-dry 1 hour before placing in the NSE’s CAF Fixative. Slides may be stored unfixed at room temperature for several days prior to staining. |
| **Quality Control** | Blood or Bone Marrow aspirate smears containing monocytes/ monoblasts. Peripheral blood smears for controls may be made fresh or smears prepared and saved at -70°C. |
| **Stock Solutions** | Alpha Naphthol Acetate SolutionFast Blue BB Base SolutionSodium Nitrite SolutionCitrate SolutionTrizmal 7.6 Buffer Concentrate Solution Gill HematoxylinAll purchased from Sigma Aldrich ready to use. Stored in refridgerator. |
| **Working Solutions** | **C.A.F. Fixative**Citrate Solution ………..6 mL Acetone ………………..16 mL Formaldehyde, 37%…...2 mL  |
| **Procedure** | **Step** | **Action** |
|  | 1 | Preheat at least 40.0 ml of distilled water to 37°C. |
| **Immediately prior to fixing slides** | 2 | Working Incubation Solution:In Erlenmeyer flask; add 1.0 ml Sodium Nitrite to 1.0 ml Fast Blue BB Base solution. Swirl and let stand for 2 minutes. The color will change from dirty brown to deep yellow |
|  | 3 | Add 40.0 ml of preheated distilled water to the Sodium Nitrite/Fast Blue Solution |
|  | 4 | Add 5.0 ml Trizmal 7.6 Buffer Concentrate Solution |
|  | 5 | Add 1.0 ml Alpha-Naphthyl Acetate solution. The solution should turn greenish.  Mix well,pour Working Incubation Solution into a glass coplin jar and set aside |
|  | 6 | Fix slides in a coplin jar of CAF Fixative solution for 30 seconds. Agitate slides vigorously for the last 5 seconds. |
|  | 7 | Rinse slides in running tap water for **5** seconds. |
|  | 8 | Do not allow slides to dry. Incubate slides in a coplin jar of the Working Incubation Solution at 37°C for 30 minutes protected from light. |
|  | 9 | After 30 minutes, remove slides and rinse in running tap water for **5** seconds.Examine microscopically for desired staining intensity. Slides may be returned to solution and stained an additional 10-15 minutes if necessary. Rinse in running tap water. |
|  | 10 | Counterstain in Gill Hematoxylin ……… **5 – 8** seconds. |
|  | 11 | Rinse in running tap water and air dry. |
|  | 12 | Coverslip with aqueous mounting media |
| **Results** | The following cells with show staining:* Monocytes show black granulation.
* Megakaryocytes
* Histiocytes
* Limited staining in Erythroblasts
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| **Result Reporting** | By Pathologists |
| **References** | Histotechnology A Self-Instructional Text, F.Carson 1990Sheehan & Hrapchak:Theory and Practice of Histotechnology, 2nd edition 1980 Battelle Press |

**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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