Papanicolaou Stain for Cytological Preparations - Technical Memo

SOLUTIONS:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Quantity</th>
<th>Part Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoxylin Stain, Gill I</td>
<td>500 ml</td>
<td>Part 1180A</td>
</tr>
<tr>
<td>Hematoxylin Stain, Gill II</td>
<td>500 ml</td>
<td>Part 1180D</td>
</tr>
<tr>
<td>Papanicolaou Stain, OG-6</td>
<td>500 ml</td>
<td>Part 1330A</td>
</tr>
<tr>
<td>Papanicolaou Stain, EA-36, Fast Green</td>
<td>500 ml</td>
<td>Part 1300A</td>
</tr>
<tr>
<td>Papanicolaou Stain, EA-50, Fast Green</td>
<td>500 ml</td>
<td>Part 1310A</td>
</tr>
<tr>
<td>Papanicolaou Stain, EA-65, Fast Green</td>
<td>500 ml</td>
<td>Part 1320A</td>
</tr>
<tr>
<td>Papanicolaou Stain, EA-36, Light Green</td>
<td>500 ml</td>
<td>Part 1302A</td>
</tr>
<tr>
<td>Papanicolaou Stain, EA-50, Light Green</td>
<td>500 ml</td>
<td>Part 1312A</td>
</tr>
<tr>
<td>Papanicolaou Stain, EA-65, Light Green</td>
<td>500 ml</td>
<td>Part 1322A</td>
</tr>
</tbody>
</table>

Additionally Needed:

- Alcohol, Ethyl Denatured, 95% Part 10842
- Lithium Carbonate, Saturated Aqueous Part 12215
- Alcohol, Ethyl Denatured, 70% Part 10844
- Alcohol, Ethyl Denatured, 100% Part 10841
- Xylene, ACS Part 1445
- Scott Tap Water Substitute Part 1380

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Papanicolaou (Pap) Stain provides three classic staining solutions for cytology preparations, allowing for crisp and distinct nuclear detail and differentially stained cytoplasm. Gill Hematoxylin is the optimal nuclear stain and the use of two counterstains, Orange G (OG) and Eosin Azure (EA) provide the subtle range of green, blue, and pink hues to the cellular cytoplasm.

Papanicolaou Stain, EA, is comprised of a combination of two dyes; Eosin Y and Light Green SF or Fast Green. EA-36, EA-50 and EA-65 denote the varying proportions of dyes in each solution and the formula of choice will depend upon a laboratories staining preference. The Pap Stain, EA also varies with each EA formulation made with either Light Green SF or modified with Fast Green. The choice of which green dye to use will depend upon staining intensity preference.

METHOD:

Fixation/Technique: Choice of fixative and technique is dependent on specimen types analyzed in the laboratory. The stains offered are suitable for both Gyn (EA-36, EA-50) and Non-Gyn (EA-50, EA-65) specimens as determined by the user.

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

STAINING PROCEDURE:

1. Place slides in 95% Ethyl Alcohol for 10 minutes.
2. Rinse in running tap water for 1 minute.
3. Stain in Hematoxylin Stain, Gill I or Gill II for 1 to 6 minutes, depending on preference of nuclear stain intensity.
4. Wash in tap water until clear.
5. Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 30 seconds.
6. Wash in tap water for 30 seconds.
7. Dehydrate in 70% Ethyl Alcohol; 10 dips.
8. Dehydrate in 95% Ethyl Alcohol, 10 dips.
9. Stain in Papanicolaou Stain, OG-6 for 30 seconds to 2 minutes, depending on specimen type and preference of stain intensity.
10. Rinse in two changes of 95% Ethyl Alcohol; 10 dips each.
11. Stain in Papanicolaou Stain, EA-36, EA-50 or EA-65 of choice for 1-3 minutes, depending on specimen type and preference of stain intensity.
12. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

- Chromatin: Blue
- Keratin: Orange
- Squamous cells: Shades of pink
- Cytoplasm: Shades of blue-green
- RBCs, nucleoli, cilia: Shades of pink

PROCEDURE NOTES:

1. The Papanicolaou stain solutions can be used for either manual or automated staining. Timings may vary depending on the staining platform used.
2. Solutions should be filtered or replaced on a regular basis to prevent cross-contamination.
3. Clean and fresh alcohol rinses (Step #10 and Step #12) after the counterstain steps are essential for optimal cytoplasmic staining.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for the clearing step.

REFERENCES:

4. Modifications developed by Newcomer Supply Laboratory.