Papanicolau-EA-36

Papanicolau stains are used for vaginal smear to detect vaginal, cervical and uterine cancer.

**Composition**

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Light green</td>
<td>45.0 gm</td>
</tr>
<tr>
<td>2) Bismark brown</td>
<td>10.0 gm</td>
</tr>
<tr>
<td>3) Eosin Y</td>
<td>45.0 gm</td>
</tr>
<tr>
<td>4) Phosphotungstic acid</td>
<td>0.20 gm</td>
</tr>
<tr>
<td>5) Lithium carbonate, saturated aqueous solution</td>
<td>1 drop</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

**Directions**

Fixation: Do not allow smears to dry and fix immediately in 95% alcohol for 5-15 min. The smears may be left in the fixative for 3 days if necessary, but prolonged fixation affects the staining reaction.

1) Rinse in 70% alcohol, 50% alcohol and distilled water.
2) Stain in Harris haematoxylin (without acetic acid) for 5-10 minutes.
3) Rinse in distilled water.
4) Rinse 3 or 4 times in 0.5% aqueous solution of hydrochloric acid.
5) Rinse thoroughly in water.
6) Leave for 1 minute in a weak solution of lithium carbonate (3 drops saturated aqueous solution / 100 ml water). Rinse thoroughly in water.
7) Rinse in distilled water, 50% alcohol, 70% alcohol, 80% alcohol and 95% alcohol.
8) Stain for 1 minute in the Papanicolau Orange G-6 (S035) solution.
9) Rinse 5-10 times in each of two jars containing 95% alcohol.
10) Stain in Papanicolau EA36 (S036) for 2 minutes.
11) Rinse 5-10 times in each of three jars containing 95% alcohol (not the same alcohol that was used after orange G-6 solution).
12) Rinse in absolute alcohol, then in a mixture of equal parts of absolute alcohol and xylene and then in xylene.
13) Mount in any satisfactory neutral medium and observe under microscope.

**Principle And Interpretation**

Papanicolau- EA-36 is Eosin Azure, comprising three dyes; the number denotes the proportion of the dyes, e.g. EA-36, EA-50, EA-65. This group of reagents provides excellent cytoplasmic staining of gynecological and non-gynecological samples. The wide range of formulations available allows the end user to select from various color intensities and hues. Papanicolau Stains are used in conjunction with Hematoxylin nuclear stains in the diagnosis of malignant cytological disease. EA-36 and EA-50 are used in conjunction with OG-6 for gynecological staining. EA-65 is used with OG-6 for non-gynecological staining. When performed properly, the stained specimen should display hues from the entire spectrum: red, orange, yellow, green, blue, and violet. The chromatin patterns are well visible, the cells from borderline lesions are easier to interpret and the photomicrographs are better. The staining results in very transparent cells, so even thicker specimens with overlapping cells can be interpreted.
On a well prepared specimen, the cell nuclei are crisp blue to black. Cells with high content of keratin are yellow, glycogen stains yellow as well. Superficial cells are orange to pink, and intermediate and parabasal cells are turquoise green to blue. Metaplastic cells often stain both green and pink at once (2).

**Quality Control**

**Appearance**
Dirty green coloured solution.

**Clarity**
Clear, without any precipitate

**Microscopic examination**
Staining is carried out and staining characteristics of the organisms is observed under microscope using oil immersion lens.

**Results**
- Nuclei: blue
- Cytoplasm: pink to pale pink

**Storage and Shelf Life**
Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

**Reference**
1) Microlab-inc.com/reagents-stains