Papanicolaou Stains

Intended use

Papanicolaou stains are used for staining the vaginal smear to detect vaginal, cervical, and uterine cancer. Papanicolaou EA – 36 / EA – 65 is used as counterstain with Hematoxylin Stains in Papanicolaou Staining method. Papanicolaou OG – 6 is a multichromatic cytological stain.

Summary

PAP stain is a polychromatic staining method containing multiple dyes to differentially stain various components of the cells which is used to differentiate cells in smear preparations of various bodily secretions. PAP stain is a very reliable technique. Papanicolaou Stains are used in conjunction with Hematoxylin nuclear stains in the diagnosis of malignant cytological disease. As such it is used for cervical cancer screening. Cancer cells may be found by the smear technique in imprints of puncture biopsy material and in smears of cervical cells, vaginal secretion, prostatic secretion, urine, gastric contents, bronchial aspirations, cavity fluids and sputum. A diagnosis of malignancy made from stained smears should be considered tentative and should be checked by tissue sections.

Principle

The classic form of PAP staining method involves five dyes in three solutions: A nuclear stain, hematoxylin, is used to stain cell nuclei. First OG-6 counterstain. The Orange G is used to stain keratin. Its original role was to stain the small cells of keratinizing squamous cell carcinoma present in sputum. Second EA (Eosin Azure) counterstain, comprising of three dyes; the number denotes the proportion of the dyes, eg. EA-36, EA-50, EA-65. This group of reagents provides excellent cytoplasmic staining of gynecological and non-gynecological samples. 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. The wide range of formulations available allows the end user to select from various color intensities and hues. Eosin Y stains the superficial epithelial squamous cells, nucleoli, cilia, and red blood cells.
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.

Reagents / Contents

Papanicolaou EA – 36

Light green	45.0 g
Bismark brown	10.0 g
Eosin Y	45.0 g
Phosphotungstic acid	0.20 g
Lithium carbonate, saturated aqueous solution	1 drop

Appearance: Green with pinkish/red tinge solution.

OR

Papanicolaou EA – 65

Eosin Y,	0.23%,
Bismarck brown,	0.05%,
Fast green FCF,	0.01%,
Phosphotungstic acid,	0.2%, in denatured alcohol

Appearance: Green with pinkish-orange tinge solution.

OR

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Papanicolaou OG – 6

Orange G-6 Certified	0.3 g
Phosphotungstic acid	0.015 g
Denatured alcohol	100.0 mL

Appearance: Orange coloured solution.

Storage and stability

Store at 15°C-25°C away from bright light. Use before expiry date on label.

Materials required but not provided

Clinical specimen on clean grease-free glass slide, smear collection brush, staining rack, blotting paper, immersion oil, Hematoxylin Harris , DPX and microscope.

Type of Specimen

Smear preparations of various bodily secretions; gynecological smears (PAP smears), sputum, brushings, washings, urine, cerebrospinal fluid, abdominal fluid, pleural fluid, synovial fluid, seminal fluid, fine needle aspiration material, tumor touch samples, or other materials containing cells.

Procedure

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 Hematoxylin Harris (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) or in Scott's tap 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 Papanicolaou Orange G-6 solution.
9. Rinse 5-10 times in each of two jars containing 95% alcohol.
10. Stain in Papanicolaou EA-36 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 allow to dry.
13. Dip in xylene and allow to dry.
14. Mount in DPX and observe under microscope.

Interpretation of results

Nuclei:	Blue
Cytoplasm:	Pink to pale pink
Acidophilic cells:	Red
Basophilic cells:	Blue Green
Erythrocytes:	Orange-red
Keratin:	Orange-red
Superficial cells:	Pink
Intermediate & Parabasal Cells:	Blue Green
Eosinophil:	Orange Red
Candida:	Red
Trichomonas:	Grey green

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Warranty

This product is designed to perform as described on the label and pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.

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