

## **M-800 PAPANICOLAOU STAIN**

**Fixation:** Acetone-Alcohol (1:1) solution immediately smears must not be allowed to dry as the exfoliated cells are liable to rapid degeneration.

**Staining:**

1. Dehydrate through several alcohol rinses, i.e. from 80%, 70%, 50% to distilled water. Use approximately 30 seconds each.
2. Stain in non-acetic **Harris Hematoxylin**, (M-800-1) 5 minutes.
3. Rinse in distilled water (6 dips, 1-5 each). The dipping process should insure smooth, even staining.
4. Differentiate in **Hydrochloric Acid, 0.25%** (M-800-2) 8 dips, 1-5 seconds each.
5. Rinse in running water, 6 minutes.
6. Transfer through distilled water to 50%, 70%, 80% and 95% alcohol, approximately 30 seconds each.
7. Counterstain in **Orange G Staining Solution** (M-800-3), 1-2 minutes.
8. Rinse in 95% alcohol, two changes, two slow dips each.
9. Counterstain further in the **EA 50 Counterstaining Solution** (M-800-4) (or other variant) for 1-2 minutes.
10. Rinse in 95% alcohol, three changes, two slow dips each. Then change to absolute alcohol, 30 seconds.
11. Clear through Xylene-Alcohol (1:1) for 4 minutes, Xylene for 2 minutes, change, and Xylene for 3 minutes.
12. Mount.

**Stain Results:**

Blue, sharp detail	Nuclei
Pink, green, yellow, blue, gray	Cytoplasm

**References:**

Lillie, R.D.: "Papanicolaou Stain" in Staining Procedures, 3<sup>rd</sup> ed., ed. G. Clark; Baltimore: Williams & Wilkins, Co., c. 1973, p. 165.

Papanicolaou, G.N.: "Papanicolaou Smear" in Atlas of Exfoliative Cytology Cambridge, MA: Harvard University Press, G 1954, p. 6.

