

## **PAPANICOLAOU'S STAIN - PROTOCOL**

### **Principle:**

Papanicolaou's stain and Pap stain is a multichromatic staining histological technique developed by George Papanikolaou. Pap staining is used to differentiate cells in smear preparations of various bodily secretions; the specimens can be 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. Pap staining is a very reliable technique. As such, it is used for cervical cancer screening in gynecology. The entire procedure is known as Pap smear.

### **Reagents:**

- i) PAPANICOLAOU'S EA-50 (PPAN-S50-500) - 500 ml
- ii) PAPANICOLAOU'S-OG-6 (PPAN-S06-500) - 500 ml
- iii) HAEMATOXYLIN (Harris) (HEMA-HPS-500) - 500 ml

### **Procedure: Rapid Economic & Papanicolaou Stain Method:**

1. Take a clean glass slide. Fix smears in 95% ethanol for 15 minutes.
2. Dip smears in 80% and 50% ethanol for 30 seconds each.
3. Rinse gently with water.
4. Add stain Harris's Haematoxylin, 3-5 minutes (preheated 60°C).
5. Rinse with tap water for 1-2 minutes.
6. Differentiate in 0.5 - 1% hydrochloric acid ethanol for several seconds.
7. Rinse with running tap water for 5-10 minutes or with lithium carbonate solution, and then rinse with water for 1-2 minutes.
8. Dip smears in 50% and 80% ethanol for 30 seconds each. And then dehydrate them in 95% ethanol for 2 minutes.
9. Stain for 1-3 minutes with Papanicolaou's Orange (OG-6).
10. Wash the smears in 95% ethanol twice for 10-20 seconds each.
11. Stain for 2-5 minutes with EA 50.
12. Wash the smears in 95% ethanol twice for 10-20 seconds each.
13. Dehydrate in 100% ethanol and then clear in Xylene or D.P.X. Mount with neutral resin and examine microscopically.

**Note:** Blotting was done after each step of washing.

**Results:**

1. **Epithelia:** Nucleus is indigo and Nucleolus is red; keratinized cells in cytoplasm appear pink, full-keratinized cells appear orange; cells prior to keratinized are sky-blue or light green.
2. **Erythrocyte:** vermil or salmon pink
3. **Leukocyte:** cytoplasm is sky- blue or light green, nucleolus is indigo.
4. **Mucus:** sky-blue or pink.

• **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.