

# PAPANICOLAOU STAIN

---

## Principle

Papanicolaou staining is applied to vaginal exudates for the detection of uterine or vaginal cancer. The technique uses a high number of dyes in its procedure.

- Hematoxylin: is the chosen nuclear staining, basically allows to reveal the nuclei of the cells present in the sample. Harris Hematoxylin is usually used.
- Orange G: is a synthetic acid dye that reveals basic compounds such as prekeratin (that stains pink) or keratin (that stains bright orange).
- Yellowish Eosin: stains cytoplasm of mature squamous cells, hair cells and erythrocytes into pink-orange.
- Green SF Yellowish light: stains squamous non-superficial cells (immature or partially mature) into greenish-blue.
- Bismark Brown R: does not stain the cellular cytoplasm but does mucin.
- Phosphotungstic acid: has a mordant function, especially important for Green Light SF.

When presenting its composition several dyes, it can reveal different types of cells. These characteristics are what make it optimal for cytological type studies.

The technique involves the use of three different solutions, on the one side, one corresponding to hematoxylin, on the other hand, the one containing Orange G (Papanicolaou OG solution) and the last with the remaining dyes (Papanicolaou solution EA).

## Material

Vaginal exudates.

## Reagents

<u>Code</u>	<u>Description</u>
253892	Papanicolaou's Solution OG 6 for clinical diagnosis (*)
253594	Papanicolaou's Solution EA 50 for clinical diagnosis (*)
256700	Histofix <sup>®</sup> Spray fixative for clinical diagnosis (*)
253949	Harris Hematoxylin solution for clinical diagnosis (*)
256991	Harris Hematoxylin modified solution for clinical diagnosis (*)
251086	Ethanol absolute for clinical diagnosis (*)
251085	Ethanol 96% v/v for clinical diagnosis (*)
192695	Ethanol 70% v/v (BP) pharma grade
251769	Xylene, mixture of isomers for clinical diagnosis (*)
253681	Eukitt <sup>®</sup> , mounting medium for clinical diagnosis

### **Procedure**

1. Fix the sample with spray.
2. Submerge successively in alcohol 80%, alcohol 70%, alcohol 50% and water, 1 minute in each liquid.
3. Stain with Hematoxylin Harris solution for approximately 5 minutes.
4. Immerse in water 6 times for 1 second.
5. Submerge in 0.5% Hydrochloric Acid, 8 times for 1 second.
6. Rinse with tap water for 5 minutes, and pass the sample through successive grade alcohols, 50%, 70%, 80% and 96% for 30 seconds in each of them.
7. Stain with Pap Smear or OG 6 for 1 to 1.5 minutes.
8. Wash the excess dye in two 96% Ethanol baths by immersing the preparation 2 times in each of 3 to 4 seconds.
9. Stain with Pap Smear or EA 50 for 1.5 to 2 minutes.
10. Wash in 3 different containers of Ethanol 96% v / v by immersing the preparation 2 times of 3 to 4 seconds in each of them.
11. Wash in absolute ethanol for 30 seconds.
12. Immerse the preparation for 4 minutes in a 1: 1 bath of Xylene, mixture of isomers and absolute ethanol.
13. Rinse with Xylene, mixture of isomers by immersing the preparation for 3 minutes in a bath.
14. Mount with Mounting medium
15. Observe under a microscope.

### **Technical note**

The microscope used should correspond to the requirements of a clinical diagnostic laboratory. If an automatic staining device is used, the operating instructions of the appliance manufacturer and the software must be observed.

### **Sample preparation**

All samples should be treated according to the state of the technology. All samples must be unambiguously labeled.

### **Diagnostics**

Diagnosis should be established only by authorized and qualified persons. Each application should involve appropriate controls to rule out erroneous results.

### **Storage**

The staining solution should be stored at room temperature.

### **Expiration**

The product stored at the indicated temperature and in a tightly closed container is usable until the expiration date indicated on the package.

### **Notes on use**

To avoid errors, the staining must be carried out by specialized personnel. For professional use only. The national directives on safety at work and quality assurance must be complied with.

### **Advise on disposal of waste**

Solutions used and expired solutions should be disposed of as hazardous waste and local waste disposal regulations must be observed. If further questions are asked about disposal, they may be processed through E-Mail: [info.es@itwreagents.com](mailto:info.es@itwreagents.com). Inside the EU are valid the requirements based on Council Directive 67/548/EEC on the approximation of the laws, regulations and laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances in the relevant version.

### **Classification of hazardous substances**

Observe the classification of dangerous substances on the label and the information on the safety data sheet.

### **Manufacturer**

Panreac Química S.L.U.  
an ITW Company  
C/Garraf, 2 – Polígono Pla de la Bruguera  
E-08211 Castellar del Vallès  
(Barcelona) España  
Tel. (+34) 937 489 400  
Fax (+34) 937 489 401

---

(\*) Sanitary product for In Vitro Diagnostics

