

# GRAM-NICOLLE STAIN

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## Principle

As a variant of Gram staining, Gram Nicolle staining is a differential staining in which the Carbol-Fuchsin Basic is used as an alternative contrast dye to Safranin to reveal certain Gram-negative microorganisms which, although colored, do so very faintly. The dyes are fixed to the bacterial walls differently depending on whether they are Gram-positive bacteria or Gram-negative bacteria.

## Material

Blood

## Reagents

Code	Description
251333	Ziehl-Neelsen Carbol-Fuchsin Basic solution for clinical diagnosis (*)
251766	Gentian Violet Phenique for clinical diagnosis (*)
251774	Lugol's Liquor with 0,4% of Iodine (diluted) for clinical diagnosis (*)
251803	Alcohol-Acetone 7:3 for clinical diagnosis (*)
251002	Immersion Oil for clinical diagnosis (*)

## Preparation of solutions

- Ziehl Carbol-Fuchsin Basic diluted: Dilute 1 ml Ziehl-Neelsen Carbol-Fuchsin Basic solution according to Ziehl to 10 ml with distilled water.

## Procedure

1. Fix the smear by heat.
2. Cover the preparation with the Gentian Violet Phenique for 1 to 5 minutes.
3. Wash excess dye gently with running water.
4. Cover the preparation with Lugol Liquid for 1 minute.
5. Decolor by dropping the Alcohol-Acetone 7: 3 dropwise onto the inclined slide until color is not released. Do not exceed 1 minute with discoloration.
6. Wash with water.
7. Coat with Carbol-Fuchsin Basic solution according to Ziehl diluted for 30 seconds.
8. Wash with water gently to remove excess dye.
9. Dry.
10. Observe the preparation under a microscope with immersion objective.

### **Results**

<b>Gram-positive bacteria</b>	Blue violet
<b>Bacterias Gram-negativas</b>	Red-pinkish

### **Technical note**

The color of the dropper can change over time. Does not affect staining. The microscope used should correspond to the requirements of a clinical diagnostic laboratory. If an automatic staining device is used, the manufacturer's instructions 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**

In order 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**

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(\*) Sanitary product for In Vitro Diagnostics

