

# BACTERIAL STAIN

## Gram's Stain

200 Tests

### PRINCIPLE :

In 1884 Gram described this method, which is the most important stain in routine bacteriology. It divides bacteria into two categories, depending on whether they can be decolorized with acetone, alcohol or aniline oil after staining with one of the rosaniline dyes such as crystal violet, methyl violet or gentian violet, and treating with iodine.

Those that resist decolorization remain blue or violet in color and are designated Gram-Positive, those that are decolorized and take up the red counterstain, such as neutral red, safranin or dilute carbol – fuchsin, are termed Gram-negative.

Although many investigators have tried to uncover the mechanism of the Gram reaction no universal answer has yet been found and it is possible that more than one mechanism exists.

### REAGENTS :

Reagent 1	Methyl violet 6B Distilled water
Reagent 2	Lugol's iodine Potassium iodide Iodine
Reagent 3	Decolorizer, Absolute Ethyl alcohol or acetone
Reagent 4	Counterstain, Neutral red

### PROCEDURE :

1. Prepare a smear, allow to dry and fix with gentle heat.
2. Apply Reagent 1 for 30 s. Rinse in water.
3. Apply Reagent 2 and allow to act for 30 sec. to 1 min.
4. Rinse with Reagent 3 and continue application until no more color appears to flow from the preparation.
5. Wash with water.
6. Apply Reagent 4 for 3 min.
7. Rinse with water, blot carefully and dry with gentle heat.

### RESULTS:

Gram-Positive organisms: Blue-black  
Gram-Negative organisms: Red

### N.B

This procedure is only a guide and it is preferable if each laboratory established his own conditions.

## BACTERIAL STAIN

**Gram's Stain**  
+15 to +25°C                      200 Tests  
For In Vitro Diagnostic Use

CAT. No.                                      GR 27 17

## REAGENTS

<b>R1</b>	Methyl violet	<b>100</b>	<b>ml</b>
<b>R2</b>	Lugol's iodine	<b>100</b>	<b>ml</b>
<b>R3</b>	Decolorizer	<b>100</b>	<b>ml</b>
<b>R4</b>	Counterstain	<b>100</b>	<b>ml</b>

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