



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Gram Staining	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

1.0 OBJECTIVE:

To lay down a procedure for Gram Staining.

2.0 SCOPE:

This SOP is applicable for Gram Staining in Microbiology Department.

3.0 RESPONSIBILITY:

Officer / Executive - Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

Ltd. Limited
No. Number
Pvt. Private
QA Quality Assurance
QC Quality Control
SOP Standard Operating Procedure

6.0 PROCEDURE:

6.1 Prepare a thin smear on clear, dry glass slide.

6.2 Allow it to air dry and fix by gentle heat.

6.3 Flood with Gram's Crystal violet for 1 minute. (If over staining results in improper decolourization of known gram negative organisms, use less Crystal violet.)

6.4 Drain the stain.

6.5 Flood the smear with Gram's Iodine; allow it to remain for 1 minute.

6.6 Decolourize with Gram's Decolourizer until the blue dye no longer flows from the smear.

6.7 Wash with water.

6.8 Counter stain with 0.5% w/v Safranin, allow it to remain for 1 minute.

6.9 Wash with water.

6.10 Allow the slide to air dry.

6.11 Let the stained slides air dry and observed under Low Power, High Power and then oil Immersion Lens. For oil immersion lens put a drop of Immersion Oil on the Smear and observe in the Microscope using Oil Immersion Lens.



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6.12 INTERPRETATION:

The Bacteria which retain the Primary Stain Crystal Violet appears dark blue or violet (i.e. not decolorized when stained with alcohol) are referred as Gram positive whereas those that lose the Crystal Violet and Counter stained by Safranin appears red or pink are referred as Gram Negative.

NOTE: Isolate or select the colonies which show different colour and morphology and stained whenever required.

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure-I	Gram Staining Report	

ENCLOSURES: SOP Training Record

8.0 DISTRIBUTION:

- Controlled Copy No. 01 Quality Assurance
- Controlled Copy No.02 Microbiology
- Master Copy Quality Assurance

9.0 REFERENCES:

Experiments in Microbiology, Plant pathology, Tissue culture and Mushroom production by K.R. Aneja (3rd edition).

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision No.	Change Control No.	Details of Changes	Reason for Change	Effective Date	Updated By

