



## 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 Microbiological Laboratory of Quality Control Area.

### 3.0 RESPONSIBILITY:

Officer/Executive - Microbiologist

### 4.0 ACCOUNTABILITY:

Head – QC

### 5.0 PROCEDURE:

- 5.1 Flame the Loop to Red Hot and Place a Drop of Sterile Purified Water with the help of Loop on the Glass Slide.
- 5.2 Flame the Loop again, allows it to cool and take a Small Portion of the Colony to Stain.
- 5.3 Put this colony on to the Previously Deposited Drop of Sterile Purified Water and mix .Finally spread out the Culture on the Slide.
- 5.4 Fix the Smear by Heating Lightly on the opposite side of the Smear, by Passing Quickly over the flame two or three times.
- 5.5 Place the Glass Slide with Smear, on stand and cover it completely with Crystal Violet Solution for 1 minute.
- 5.6 Wash with Purified Water and drain the Excess Water.
- 5.7 Flood the Smear with Iodine Solution and keep for 1 Minute.
- 5.8 Wash with Purified Water and Drain the Excess Water.
- 5.9 Decolorize with gram's decolouriser i.e. 95% ethyl alcohol for 30 seconds.
- 5.10 Wash with Purified Water and Drain the Excess Water.
- 5.11 Cover the Preparation with Safranin solution and keep for 30 seconds.



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**5.12** Wash with Purified Water and drain the Excess Water.

**5.13** Dry the smear with Filter Paper or allow it to dry at Room Temperature.

**5.14** 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.

**5.15 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 and select the colonies which shows difference in colour, shape and texture, colonies having similar colour, shape and texture shall be stained once per day.

**6.0 REFERENCES:**

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

**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      Head Corporate Quality Assurance
- Controlled Copy No. 02      Head Quality Control
- Master Copy                      Quality Assurance Department

**9.0 ABBREVIATIONS:**

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



# PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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### 10.0 REVISION HISTORY:

#### CHANGE HISTORY LOG

Revision No.	Details of Changes	Reason for Change	Effective Date	Updated By
00	New SOP	Nil		



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## ANNEXURE – I

### GRAM STAINING REPORT

**Date of Exposure:**

**Date of Staining:**

S.No.	Name of Area	Plate Code	Morphological Characteristic			Gram Characteristic
			Colour	Shape	Arrangement	

**Remark:**.....

**Performed By:**

**Checked By:**

**Approved By:**

**Sign & Date**

**Sign & Date**

**Sign & Date**