PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure for Gram's Staining	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 Objective

To lay down procedure for Gram's Staining.

2.0 Scope

This Standard Operating Procedure is applicable for formulation plant of ……..

3.0 Responsibility

Executive/Officer - Microbiology : Shall be responsible to follow the procedure for Gram's staining.

Head - QC/Designee : Shall be responsible for the compliance of this SOP.

4.0 Abbreviations and Definitions

QC : Quality Control

SOP : Standard Operating Procedure

Mordant : A substance used to set the dies.

Decolorizing Agent : A substance to decolorize the excess colour of dies.

5.0 Procedure

5.1 Prepare smears of organisms by spreading cell mass from a colony or by taking a loopful from the broth as a thin film on the slide.

5.2 Dry in air and fix the smear by passing the slide rapidly through the Bunsen flame three times.

5.3 Stain with Gram's Crystal Violet as primary stain and allow to stand for about one minute.

5.4 Gently rinse the slide with purified water.

5.5 Put one to two drops of Gram's Iodine on the smear and allow it to act for about 1 minute. Gram's iodine acts as a mordant.

5.6 Gently rinse the slide with purified water.

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5.7 Cover the slide with 95% Alcohol for 10-20 seconds. Alcohol acts as a decolorizing agent.

5.8 Gently rinse the slide with purified water.

5.9 Counter stain with Safranin for about 20-30 seconds.

5.10 Gently rinse the slide with purified water and blot dry.

5.11 Examine under Microscope through the oil-immersion objective.

5.12 During examination identify the cells as Gram negative or Gram positive.

5.13 Gram positive cells retains the Gram's stain and looks purple, while gram negative cells do not retain Gram's stain and looks pink in colour.

6.0 Forms and Records

6.1 Nil

7.0 Reference

7.1 Nil

8.0 Distribution

8.1 Master Copy : Documentation Cell (Quality Assurance)

8.2 Controlled Copies : Quality Control, Quality Assurance

9.0 History

Date	Revision Number	Reason for Revision
	00	New SOP