PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Spore Staining	**Effective Date:**
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1.0 OBJECTIVE

1.1 To lay down the procedure for spore staining.

2.0 SCOPE

2.1 This procedure is applicable for Microbiology Laboratory.

3.0 RESPONSIBILITY

3.1 Microbiologist is responsible for performing spore staining.

4.0 ACCOUNTABILITY

4.1 Head Microbiology

5.0 EHS CONSIDERATIONS

5.1 Wear face Mask and gloves before performing the staining

5.2 Sterilize all biohazard waste before disposal.

6.0 PROCEDURE

6.1 **Requirement**

6.1.1 5% aqueous solution of malachite green.

6.1.2 Safranin (counter stain)

6.1.3 Purified Water

6.1.4 Whatman filter paper

6.1.5 Microscope

6.2 **Methodology:**

6.2.1 Take a clean and dry glass slide.

6.2.2 Transfer the culture to the centre of slide and prepare a smear.

6.2.3 Dry the smear in air and fix with heat.

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6.2.4 Place the slide on a small beaker containing boiling water kept on the hot plate.

6.2.5 Cover the smear with a small piece of whatman filter paper; keep the filter paper saturated with a 5% aqueous solution of malachite green for about 30 minutes.

6.2.6 Wash the slide gently with water.

6.2.7 Add the counter stain safranin to smear for 30 seconds.

6.2.8 Wash the slide with water and blot dry with whatman filter paper.

6.2.9 Observe the slide under microscope 100X using immersion oil.

6.2.10 The spores are stained green whereas the vegetative cells are stained pink.

6.2.11 Observe the location of spore within the cell.[e.g. central spore, sub terminal spore terminal spore with swollen sporangium]

7.0 DEFINITIONS AND ABBREVIATIONS

7.1 Spore Staining: Type of staining technique that utilizes the fact that the spore does not take up dyes readily but, once stained it resists de-colorization. The differentiating agent used may be a dilute solution of an organic acid, an acid dye, or another basic dye.

7.2 Spore: A small, usually single-celled reproductive body that is highly resistant to desiccation and heat and is capable of growing into a new organism, produced especially by certain bacteria, fungi, algae, and non-flowering plants.

8.0 REFERENCE

8.1 NA

9.0 ANNEXURES

9.1 NA

10.0 DISTRIBUTION DETAILS

10.1 Controlled copy of this SOP shall be distributed to Quality Assurance and Microbiology department.

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11.0 REVISION HISTORY

Supersedes SOP No.	Change Control No.	Reason for revision
NA	NA	NA