PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Spore Population Study of Biological Indicator

SOP No.:		**Department:**	Microbiology
		Effective Date:	
Revision No.:	00	**Revision Date:**	
Supersede Revision No.:	Nil	**Page No.:**	1 of 7

1.0. OBJECTIVE:

1.1 To lay down a procedure for the Spore population study of Biological Indicator**.**

2.0. SCOPE:

2.1 This procedure is applicable for the Spore population study of Biological Indicator in Microbiology Laboratory of the Quality control department of ……

3.0. RESPONSIBILITY:

3.1 Officer/Executive – Quality Control

4.0. ACCOUNTABILITY:

4.1 Head of the department is accountable to ensure the compliance of SOP for all aspects.

5.0 DEFINITION:

5.1 NA

6.0. PROCEDURE:

6.1.1 Population determination of ampoules:

6.1.1.1 In order to verify a population of spores in a glass ampoule, the spores must first be recovered from the ampoule. To begin, randomly select four (4) ampoules from the lot to be assayed.

6.1.1.2 Place four (4) ampoules in a sterile 250 ml bottle.

6.1.1.3 Using a sterile glass rod or sterile forceps, crush the ampoules to shards. Ensure the bottle is sitting flat on the bench when striking the ampoule.

6.1.1.4 Rinse the crushing device with 95.2 ml sterile purified water(add 98.8 ml sterile purified water in case of *Bacillus subtilis*) as it is added to the 250 ml bottle.

6.1.1.5 Vortex the bottle for at least one minute to achieve a homogeneous blend.

6.1.1.6 Allow the bottle to stand for five (5) minutes after vortexing to allow air bubbles to dissipate before sonicating. Sonicate the sample for 3-5 minutes.

6.1.1.7 Vortex sample again for approximately 10 seconds. Immediately after vortexing, pipette 10.0 ml from the bottle into a sterile screw cap flat bottom tube to be heat shocked.

6.1.1.8 Heat shock one flat bottom tube containing the 10.0 ml of the dilution in a water or glycol bath preheated to the required temperature for the appropriate length of time. Remove tube and cool in an ice bath (0°to 4°C) for two (2) minutes.

Species	Heat shock Temperature	Heat shock Time
G. stearothermophilus	95 -100°C	15 min
Bacillus subtilis	80 -85°C	10 min

Two dilution series will be made from the heat shocked tube. Vortex the heat-shocked tube at least 10 seconds before a transfer is made. This will avoid settling of the suspension.

6.1.1.9 Transfer two (2) 1.0 ml aliquots to two (2) dilution blank tubes, each containing 9.0 ml of sterile purified water.

6.1.1.10 Vortex each dilution blank tube for at least ten (10) seconds just before making the transfer. Transfer 1.0 ml from each dilution blank tube to a second dilution blank tube containing 9.0 ml of sterile purified water. If a 10^6 population is present repeat this step one more time.

6.1.1.11 Prepare serial dilutions by diluting each time 1 ml of culture suspension with 9 ml of Normal Saline up to 10^{-5} dilution.

6.1.1.12 Vortex each of the last tubes of the dilution series for ten (10) seconds. Pipette 1.0 ml into a 15 x 100 mm Petri plate. Repeat this procedure to achieve two (2) replicate Petri plates from each of the last tubes of the dilution series for a total of four (4) plates. Vortex before pipetting.

6.1.1.13 Pour approximately 20 ml of melted soybean casein digest agar, cooled to 45°to 50°C, into the Petri plate.

6.1.1.14 Swirl the agar filled plates to assure adequate mixing. Allow the agar to solidify. Do not use agar that has been melted and held longer than eight (8) hours.

6.1.1.15 Invert and incubate plates at the appropriate temperature for forty-eight (48) hours.

6.1.1.16 After forty-eight (48) hours of incubation, remove plates and count colonies. Counts should be between 30 –300 CFU per plate.

Species	incubation Temperature
Geobacillus. stearothermophilus	55 -62°C
Bacillus subtilis	30 -35°C

6.1.1.17 After counting the CFU's per plate, calculate the overall average recovered CFU's from all of the plates. Record the data in the annexure – I.

6.1.1.18 Calculate the average population recovered from the carrier lot samples by multiplying the calculated average CFU per plate by the inverse of the dilution factor.

Equation: $\frac{\text{average plate count CFU} \times 1}{\text{dilution factor}}$

6.1.1.19 To confirm the population stated on the certificate, the percent recovery of the certified population must be within –50% , +300% in accordance with USP and ISO 11138. Take the average population determined by the assay and divide by the population value stated on the certificate.

$$\text{Percent recovery} = \frac{\text{calculated average population of samples}}{\text{stated population on certificate}} \times 100$$

6.1.1.20 **NOTE:** If recovery is not confirmed within –50%, +300% of the certificate value, examine possible calculation and/or procedural errors. If the variation between plates is greater than 30 CFU's, re-examine technique used.

6.1.2 Population determination of spore strip:

6.1.2.1 In order to verify a population of spores on a paper carrier, the spores must first be recovered from the carrier. To begin, randomly select four (4) inoculated paper carriers from the lot to be assayed.

6.1.2.2 Place each carrier in a sterile screw cap flat bottom tube with four (4) and add 5.0 ml of sterile purified water.

6.1.2.3 Vortex each tube 4-7 minutes, until the paper carrier is macerated to a pulp. Add an additional 5.0 ml sterile purified water to each tube. Vortex each tube again for one (1) minute.

6.1.2.4 Heat shock the four (4) tubes containing 10.0 ml sterile purified water, glass beads, and macerated carriers in a bath preheated to the required temperature for the appropriate length of time. Remove tubes and cool in an ice bath (0°to 4°C) for two (2) minutes.

Species	Heat shock Temperature	Heat shock Time
G. stearothermophilus	95 -100°C	15 min
B. pumilus	80 -85°C	10 min
B. atrophaeus	65 -70°C	10 min

To ensure homogenous suspension for accurate plate counts, it is extremely important to make each serial transfer immediately after vortexing. A dilution series will be made from each of the four (4) heat shocked tubes. Vortex each tube separately before a transfer is made from that specific tube. This will avoid the settling of the suspension.

6.1.2.5 From each tube, transfer a 1.0 ml aliquot to a blank tube containing 9.0 ml of sterile purified water.

6.1.2.6 Vortex the dilution blank tube for at least ten (10) seconds. Transfer 1.0 ml from each dilution blank tube to a second dilution blank tube containing 9.0 ml of sterile purified water. If a 10^6population is present repeat this step one more time.

6.1.2.7 Prepare serial dilutions by diluting each time 1 ml of culture suspension with 9 ml of Normal Saline up to 10^{-4} dilution.

6.1.2.8 Vortex the last tube from each dilution series for ten (10) seconds. Pipette 1.0 ml into a Petri plate. Repeat this procedure to achieve two (2) replicate Petri plates each dilution series for a total of eight (8) plates. Vortex before pipetting.

6.1.2.9 Pour approximately 20 ml of melted soybean casein digest agar, cooled to 45°to 50°C, into the Petri plate.

6.1.2.10 Swirl the agar filled plates to assure adequate mixing. Allow the agar to solidify. Do not use agar that has been melted and held longer than eight (8) hours.

6.1.2.11 Invert and incubate plates at the appropriate temperature for forty-eight (48) hours.

6.1.2.12 After forty-eight (48) hours of incubation, remove plates and count colonies. Counts should be between 30 –300 CFU per plate.

Species	incubation Temperature
G. stearothermophilus	55 -62°C
B. pumilus	30 -35°C
B. atrophaeus	30 -35°C

6.1.2.13 After counting the CFU's per plate, calculate the overall average recovered CFU's from all of the plates. Record the data in the following table.

6.1.2.14 Calculate the average population recovered from the carrier lot samples by multiplying the calculated overall average CFU per plate by the inverse of the dilution factor.

$$\text{Equation: } \frac{\text{Overall average plate count CFU} \times 1}{\text{dilution factor}}$$

6.1.2.15 To confirm the population stated on the certificate, the percent recovery of the certified population must be within –50% , +300% in accordance with USP and ISO 11138. Take the average population determined by the assay and divide by the population value stated on the certificate.

$$\text{Percent recovery} = \frac{\text{Calculated average population of samples}}{\text{stated population on certificate}} \times 100$$

6.1.2.16 If recovery is not confirmed within –50%, +300% of the certificate value, examine possible calculation and/or procedural errors. If the variation between plates is greater than 30 CFU's, re-examine technique used.

6.2 Precaution

6.2.1 Do not use agar that has been melted and held longer than 8 hours.

6.2.2 All analysis perform under LAF of MLT room.

6.2.3 To avoid inaccurate plate counts, it is important to perform the serial transfer using 2.0 ml pipette or whichever pipette has the larger bore size. This will help avoid clogging of the pipette tip with cotton fibers.

6.3 Limits/ Acceptance Criteria:

6.3.1 To confirm the population stated on the certificate, the percent recovery of the certified population must be within –50% to +300% in accordance with USP and ISO 11138.

7.0. ABBREVIATION:

Abbreviation used	Full form of abbreviation used
QC	Quality Control
Ltd.	Limited
SOP	Standard Operating Procedure
CFU	Colony Forming Unit
IPA	Iso Propyl Alcohol
SCDA	Soyabean Casein Digest Agar

8.0 ANNEXURES :

Annexure No.	Title of Annexure	Format No.
Annexure-I	Population Determination Report of Biological Indicator	

9.0 DISTRIBUTION:

- Controlled Copy No. 01 Quality Control (Microbiology)
- Master Copy Quality Assurance Department

10.0 REFERENCES:

- IH

11.0 REVISION HISTORY:

Revision No.	Change Control No.	Details of Changes	Reason of Changes	Effective Date	Done By
00	Not Applicable	Not Applicable	New SOP		

ANNEXURE –I

POPULATION DETERMINATION REPORT OF BIOLOGICAL INDICATOR

Name of BI : …… Certified spore concentration : ……

Lot size : …… Lot Number : ……

Mfg/Supplier : …… In house Media lot No. : ……

Date of testing : …… Date of observation : ……

Incubation Temp. : …… Incubation period : ……

Incubator ID : …… Analyzed By : ……

Media Used: Soyabean Casein Digest Agar

Tube No.	Dilution	Observed cfu per plate			Overall average plate count CFU
		Plate I	Plate II	Mean cfu	

$$\text{CFU per plate by the inverse of the dilution factor} = \frac{\text{Overall average plate count CFU} \times 1}{\text{dilution factor}}$$

$$\text{Percent recovery} = \frac{\text{Calculated average population of samples}}{\text{Stated population on certificate}} \times 100$$

Limit : To confirm the population stated on the certificate, the percent recovery of the certified population must be within –50% , +300% .

Remarks: The above lots of Biological Indicator Complies/Does Not comply as per specification.

Microbiologist:
(Sign & Date)

Checked by:
(Sign & Date)