PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Qualification and Usage of Biological Indicator

SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 3	

PURPOSE:

The purpose of this SOP is to lay down the procedure for qualification and usage of biological indicator.

SCOPE:

This SOP describes the procedure for qualification and usage of biological indicator for the validation of steam sterilizer in microbiology laboratory.

RESPONSIBILITY:

Officer/Executive - Quality Control.

ACCOUNTABILITY:

Head – QC Department

PROCEDURE:

1.0 The *Bacillus stearothermophilus* (*Geobacillus stearothermophilus*) spore strips ATCC 7953 are used for the validation study of steam sterilizer.

2.0 Every lot of the biological indicators shall be qualified before their use in validation or qualification of sterilization cycles.

3.0 Different consignments with the same lot number shall not be required to re-qualify.

4.0 Each consignment must be accompanied with certificate of analysis from the manufacturer and shall be checked for following details.

- Strain Name and Strain No.
- Spore population.
- D-Value.
- Manufacturing & expiry date.
- Storage condition.

5.0 If any discrepancy with respect to above parameters is observed, it shall be informed to manufacturer / supplier.

6.0 Following tests shall be performed to qualify the biological indicator lot.

7.0 **Procedure for Total viable spore count /Spore population determination.**

7.1 Take three biological indicator paper strips and aseptically remove three spore strips from the pack by tearing off the paper cover.

7.2 Separately place each strip individually in a test tube containing 10 ml chilled sterilized purified water and glass beads, then vortex the content till the strip breaks and formation of pulp occurs.

7.3 Heat the tube containing the suspension in water bath at 95°C to 100°C for 15 minutes.

7.4 Cool rapidly in an ice water bath at 0 to 4°C.

7.5 Vortex the tubes for 15 to 20 second. Make 1:10 fold serial dilutions using sterile saline to get 30 – 300 cfu/ml.

7.6 Pipette out 1.0 ml from the test tube and transfer into two petri dish and mark dilution factor on each petridish. Like wise prepare for another petriplates.

7.7 Add to each plate 15 to 20 ml of Soyabean casein digest agar medium that has been cooled to 45° to 50°C, mix evenly and allow for solidifying.

7.8 Incubate the plates in inverted position at 55°C to 60°C for 72 hours.

7.9 Examine the plates after 72 hours and record the number of CFU in each plate.

7.10 Calculate average number of spores per specimen by multiplying the CFU with dilution factor.

8.0 Acceptance Criteria: The count in all three spore strips must meet the acceptance limit of 50 – 150% of label claim (COA).

9.0 Growth promotion characteristics.

9.1 This test shall be done to evaluate the capability of the organism to grow within the specified time when specified conditions for the growth are met.

9.2 One biological indicator from the received lot shall be aseptically inoculated in sterilized Soybean-Casein Digest Medium in laminar air flow station.

9.3 The tip of the outer wrap shall be cut with incinerated scissors and the strip shall be withdrawn with the help of incinerated forceps.

9.4 The biological indicator strip shall be immersed into sterilized SCDM.

9.5 The inoculated tubes with the strips shall be incubated at 55-60ºC for 48 hours.

9.6 The tubes/ampoules shall be checked for the growth at every 24 hr interval.

9.7 Acceptance Criteria shall be luxuriant growth should be observed within 48 hours.

10.0 Identification :

10.1 The biological indicator G.stearothermophilus organism complies substantially with the morphological and cultural characteristics of strain of G.stearothermophilus ATCC No. 7953.

10.2 Identification shall be done by gram staining by microscopy.

10.3 Cultural Characteristics: G. stearothermophilus first incubated at 55-60 0 C in SCDM for 24 hours, and then further streaked on SCDA. Then incubate two different plates one at 55 - 60°C and other at 30 - 35°C.

10.4 The plates incubated at 30 - 35°C should not show the growth while plates incubated at 55 - 60°C should show the growth.

10.5 The growth observed tubes/ampoules from clause 5.10.3 shall be used for the gram staining reactions.

10.6 Under Microscopic examination, *Geobacillus stearothermophilus* consists of gram positive rods with oval endospores in sub terminally swollen cells.

10.7 The results shall be documented in Qualification of Biological Indicators, Annexure-I.

10.8 If the Biological indicator fails in one or more parameters, the results shall be reviewed and shall be communicated to the manufacturer/supplier.

10.9 The failed lot of biological indicator shall be rejected and shall not be used for the validation exercises.

11.0 Frequency: The qualification of biological indicator is to be carried out for each new lot of Biological Indicator received and before any qualification of steam sterilizer.

ANNEXURE(S):

Annexure –I : Qualification of Biological Indicator.

REFERENCES:

USP 31

Revision No.	Effective Date	Reason for review	Signature & Date	Remarks