PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE				
Title: SOP for Testing of Biological Indicators				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 6	

1.0 PURPOSE:

To lay down procedure for testing of Biological Indicators used for sterilization validation.

2.0 SCOPE:

This SOP is applicable for the testing of Biological Indicators (BI) on receipt (spore population verification and organism characterization) and testing Biological Indicator exposed to sterilization validations.

3.0 RESPONSIBILITY:

Trained quality control microbiologist is responsible to perform testing as described in SOP.

4.0 PROCEDURE:

4.1 General Instructions

4.1.1 Perform all activities under bio safety cabinet or laminar air flow.

4.1.2 Handle biological indicators carefully.

4.1.3 Decontaminate all used glassware, solutions and spore suspensions before disposal.

4.2 Testing of Biological Indicators on Receipt

4.2.1 On receipt of biological indicators verify and ensure the lot received has minimum spore population of 1 x 10^6 spores / carrier (strip or ampoule) and D -Value not less than 1.5 minutes.

4.2.2 Record the above information along with name of the organism, manufacturer's name and manufacturing and expiry dates in Annexure – I.

4.2.3 <u>Sample Preparation of Biological Indicator Paper Carriers</u>

4.2.3.1 Randomly select three biological indicators from the lot to be tested.

4.2.3.2 With the help of sterile forceps, remove the paper carrier from the biological indicator container (strip or self-contained container) and transfer them to test tube containing chilled 30 ml sterile purified water with glass beads. If required cut the paper carrier into pieces.

4.2.3.3 Vortex the tube for about 10 minutes until the strip is macerated into fine pulp.

4.2.3.4 Take an aliquot of 10 ml and proceed for heat shock and enumeration as per 4.2.5.

4.2.4 Sample Preparation of Biological Indicator in Glass ampoules / SS Discs.

4.2.4.1 Randomly select three biological indicators from the lot to be tested and place them in a sterile 100 ml screw cap bottle.

4.2.4.2 With the help of sterile rod or forceps, crush the ampoules in to pieces.

4.2.4.3 Rinse the crushing devise with sterile purified water as it is added to bottle. Make up the volume to 100 ml, i.e., if the volume liquid in each biological indicator is 0.3 ml then add 99.1 ml of sterile purified water to make up to 100 ml.

4.2.4.4 In case of SS discs, just add 100 ml sterile purified water.

4.2.4.5 Vortex the bottle for not less than 1 minute to achieve homogenous blend.

4.2.4.6 Allow the bottle to stand for at least 5 minutes without disturbing to allow air bubbles to dissipate prior to sonication.

4.2.4.7 Sonicate the sample for 3 to 5 minutes at 45 – 60 kHz.

4.2.4.8 Vortex again for at least 1minute after sonication and take an aliquot of 10 ml and proceed for heat shock and enumeration as per 4.2.5.

4.2.5 Enumeration of Spore Population

4.2.5.1 Heat shock the tubes in a water bath at 95°C – 100°C for 15 minutes, starting the timing when temperature reaches 95°C in case of *Geobacillus stearothermophilus* and at 80°C – 85°C for 15 minutes, starting the timing when temperature reaches 80°C in case of *Bacillus atrophaeus / subtilis*.

4.2.5.2 Remove the tube from water bath and cool rapidly in ice-water bath at 0°C – 4°C.

4.2.5.3 Vortex the tubes and transfer two 1 ml aliquots to separate test tubes containing 9 ml sterile purified water.

4.2.5.4 From each tube prepare ten fold serial dilutions up to a suitable dilution to get countable colonies. For example if the labeled spore population is 10^6 then prepare dilutions up to 10^{-6}. Vortex the tubes for at least 10 seconds before proceeding for next dilutions.

4.2.5.5 Pipette 1 ml from each of at least last 3 dilutions of both series into appropriately labeled sterile 90 mm petri plates in duplicate. Vortex before pipetting.

4.2.5.6 Into each plate pour approximately 20 ml of sterile molten Soyabean Casein Digest Agar cooled to 45 to 50°C.

4.2.5.7 Swirl the plates to assure adequate mixing and allow the agar to solidify.

4.2.5.8 Invert and incubate plates at 55°C - 60°C for *Geobacillus stearothermophilus* and at 30°C - 35°C for *Bacillus atrophaeus / subtilis*.

4.2.5.9 Examine the plates after 24 and 48 hours and record the number of colonies for each plate. Select the dilution showing 30 to 300 colonies after 48 hours to calculate results.

4.2.5.10 Calculate the average number of spores per specimen from the results, using the appropriate dilution factor and number of units used for analysis.

4.2.6 Characterization of Organism

4.2.6.1 From one of the first three prepared dilutions, take a loop full of dilution and streak on two Soyabean Casein Digest Agar plates.

4.2.6.2 Incubate one plate at 30 – 35°C and one plate at 55 – 60°C for 24 hours.

4.2.6.3 After completion of incubation, check for growth and perform gram staining.

4.2.7 Acceptance Criteria

4.2.7.1 Spore population enumeration is valid if the log number of spores per carrier at 48 hours is equal to or greater than the log number of spores after 24 hours.

4.2.7.2 The requirements of spore population enumeration test are met if the log of average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count by 0.48 (recovery of 50 to 300%) and has a minimum spore population of 1 x 10^6 spores per carrier.

4.2.7.3 In case of *Geobacillus stearothermophilus*, the organism should show growth at 55 – 60°C and no growth should be observed at 30 – 35°C. On staining it should show Gram-positive rods with oval endospores in sub-terminally swollen cells.

4.2.7.4 In case of *Bacillus atrophaeus / subtilis*, the organism should show growth at 30 – 35°C and no growth should be observed at 55 – 60°C. On staining it should show Gram-positive rods and endospores are oval and central and the cells are not swollen.

4.3 Testing of Biological Indicators Used in Sterilization Validation

4.3.1 The testing of biological indicators used in sterilization validation should be incubated within four hours after unloading.

4.3.2 Testing of Biological Indicator Strips

4.3.2.1 Transfer the biological indicators along with one unexposed indicator (positive control) from the same lot to the microbiological testing lab.

4.3.2.2 Using a permanent marker pen label the test tubes containing 10 ml sterile soybean casein digest medium with validation identification number, strip number and date of test (inoculation). Label one tube as positive control and one tube as negative control.

4.3.2.3 Peel the individual strip container and with the help of sterile forceps, remove the strip and transfer the strip to appropriately labeled tube. Ensure that the strip is completely immersed in the medium.

4.3.2.4 After transferring all test strips, transfer positive control strip to tube labeled as positive control.

4.3.2.5 Incubate all the tubes at 55°C - 60°C for *Geobacillus stearothermophilus* and at 30°C - 35°C for *Bacillus atrophaeus / subtilis* for 7 days.

4.3.2.6 Observe the tubes every day and record the results after 7-day incubation period. Positive control usually gives growth within 24 to 48 hours and as soon as growth is observed in positive control, it should be recorded and then decontaminated and discarded.

4.3.3 Testing of Self-Contained Biological Indicators / Biological Indicator Ampoules

4.3.3.1 Press the self-contained biological indicator container such that the glass ampoule/tube containing the medium is release inside the container and BI strip/disc comes in full contact with the medium. Similarly press one unexposed BI unit (Positive Control). Keep one BI unit as is without breaking the media container (Negative Control).

4.3.3.2 In case of BI ampoules along with exposed ampoule take one negative control ampoule (provided by manufacturer with no added spores) exposed to sterilization and one unexposed ampoule (positive control) from the same lot.

4.3.3.3 Incubate all the units at 55°C - 60°C for *Geobacillus stearothermophilus* and at 30°C - 35°C for *Bacillus atrophaeus / subtilis* for 48 hours.

4.3.3.4 Observe the units every day and record the results. Positive control usually gives growth within 24 to 48 hours and as soon as growth is observed in positive control, it should be recorded and then decontaminated and discarded.

Note: One positive control and one negative control can be used if BIs from multiple sterilization charges are inoculated & incubated on the same day.

4.3.4 Acceptance criteria:

4.3.4.1 The test is valid if the growth is observed in positive controls and no growth is observed in negative controls.

4.3.4.2 The sterilization cycle/pattern under test is validated if no growth is observed in all the biological indicators.

5.0 ABBREVIATIONS AND DEFINITIONS

SOP	Standard Operating Procedure
QCM	Quality Control Microbiology
QAD	Quality Assurance Department
Rev.	Revision
No.	Number
%	Percent
°C	Degree centigrade
mL	Milli litre
CFU	Colony Forming Unit
BI	Biological Indicator
LAF	Laminar Air Flow
BSC	Bio Safety Cabinet

Biological Indicator: Preparation of a specified microorganism that provides a defined and stable resistance to a specific sterilization process. Generally, spores of microorganisms are used for BIs since they provide a higher resistance to the sterilization than the vegetative cells.

BI Strip: A paper strip acting as carriers that have been inoculated with spore suspensions. Usually such strips are packaged in glassine envelope.

Self Contained BI: A BI that is packed in container that also contain growth medium for recovery of microorganisms that have been subjected to sterilization.

BI Ampoule: Suspension of spores in growth medium contained in glass ampoules. These are generally used for sterilization validation of liquids.

***Geobacillus stearothermophilus*:** Species of whose spores are used as biological

indicators for sterilization by Moist Heat Sterilization.

***Bacillus atrophaeus / subtilis*:** Species of whose spores are used as biological indicators for sterilization by Dry Heat Sterilization or Ethylene Oxide.

6.0 REFERENCE DOCUMENTS

USP <55> Biological Indicators - Resistance Performance Tests

7.0 ANNEXURE / ATTACHMENTS

Annexure I: Form 1- Testing of Biological Indicators- Spore Population Enumeration and Characterization.

Annexure II: Form 2 – Testing of Biological Indicators – Sterilization Validation.

8.0 REVISION LOG:

Revision Number	Effective Date	Reason for Revision