STANDARD OPERATING PROCEDURE	
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1.0 OBJECTIVE

1.1 To lay down the procedure for qualification and incubation of biological indicator.

2.0 SCOPE

2.1 This procedure is applicable for Microbiology Laboratory.

3.0 RESPONSIBILITY

3.1 Microbiologist is responsible for qualification and incubation of biological indicator.

4.0 ACCOUNTABILITY

4.1 Head Microbiology

5.0 EHS CONSIDERATIONS

5.1 NA

6.0 PROCEDURE

6.1 All the activities are to be conducted in a laminar air flow station using aseptic techniques.

6.2 Pool three numbers of biological indicators supplied by the manufacturer into a tube containing 100 ml of sterilized chilled purified water.

6.3 Blend the solution for 3-5 minutes by constantly vortexing for a homogenous suspension.

6.4 **For biological indicator strips used in steam heat sterilization process:**

6.4.1 Heat the blended suspension tube at 95-100ºC for 15 minutes in water bath, starting the time when temperature reaches 95ºC.

6.4.2 Cool rapidly in an ice water bath at 0-4ºC.

6.4.3 Serially dilute 1 ml of aliquot in sterilized purified water to yield preferably 30-300 colonies.

6.4.4 Pour plate in duplicate in soybean casein digest agar medium within 20 minutes & incubate in an inverted position at 55-60º C for 48 hours.

6.5 **For biological indicator strips used in dry heat sterilization process:**

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6.5.1 Heat the tube at 80-85º C for 10 minutes in water bath, starting the time when temperature reaches 80º C.

6.5.2 Serially dilute 1 ml of aliquot in sterilized purified water to yield preferably 30-300 colonies.

6.5.3 Pour plate duplicate in soybean casein digest agar medium within 20 minutes & incubate in an inverted position at 30-35º C for 48 hours.

6.6 **For BI ampoules used in steam heat sterilization process:**

6.6.1 Randomly select 3 ampoules from the lot to be assayed.

6.6.2 Break the ampoules using sterile forceps or sterile stainless steel rod into a 250 ml sterilized flask. Depending upon the ampoule volume, make up the flask to 100 ml with sterilized purified water.

6.6.3 For e.g., if the ampoule volume is 0.3 ml, then 3 ampoules volume will be 0.9 ml, and then make up volume will be 99.1 ml.

6.6.4 The suspension shall be vortexes for 1 minute.

6.6.5 Sonicate the suspension for 5 minutes.

6.6.6 From the 100 ml spore suspension, withdraw 10 ml of the suspension and dispense into a sterilized test tube. Rinse the pipette 2-3times to eject out any spores in the pipette tip.

6.6.7 Heat the blended suspension tube at 95-100 º C for 15 minutes in water bath, starting the time when temperature reaches 95º C.

6.6.8 Cool rapidly in an ice water bath at 0-4º C.

6.6.9 Serially dilute 1 ml of aliquot in sterilized purified water to yield preferably 30-300 colonies.

6.6.10 Pour plate in duplicate in soyabean casein digest agar medium within 20 minutes & incubate in an inverted position at 55-60º C for 48 hours.

6.7 **Serial dilution:**

6.7.1 From the blended stock solution transfer aseptically 10 ml into a sterilized test tube (Tube No.1: Dilution-10^{-2}).

6.7.2 From the tube No.1 transfer aseptically 1 ml in 9 ml of sterilized purified water (Tube No.2: (Dilution-10^{-3}).

6.7.3 From the tube No.2 transfer aseptically 1 ml in 9 ml of sterilized purified water (Tube No.3: (Dilution-10^{-4}).

6.7.4 From the tube No.3 transfer aseptically 1 ml in 9 ml of sterilized purified water (Tube No.4: (Dilution-10^{-5}).

6.7.5 From the tube No.4 transfer aseptically 1 ml in 9 ml of sterilized purified water (Tube No.5: (Dilution-10^{-6}).

6.7.6 Vortex each dilution tubes for at least 20 seconds.

6.7.7 Make each serial transfer immediately after vortexing.

6.8 **Plate pouring:**

6.8.1 Pour 1 ml of aliquot in duplicate from dilutions 10^{-4}, 10^{-5}, 10^{-6} respectively in sterile petriplates.

6.8.2 Add 20-25 ml soyabean casein digest agar medium within 20 minutes.

6.8.3 Tilt & rotate to mix the content informingly.

6.8.4 Leave it for solidification. Incubate the plates at specified condition in inverted position.

6.9 **Counting & calculation:**

6.9.1 Count the colonies of those plates, which are showing colonies in range of 30-300 CFU & take an average count.

6.9.2 Count the CFU at 24 hrs and as well as at 48 hrs incubation period.

6.9.3 Determine the spore population of each biological indicator considering dilution factor.

6.9.4 Spore population = Average colonies (CFU) x reciprocal of dilution/ No. of strips or ampoules used.

6.10 **Frequency of testing:**

6.10.1 During the receipt of new lot and whenever required.

6.10.2 Qualification can be done from outside laboratory also.

6.11 **Acceptance Criteria:**

6.11.1 50% to +200% of label claim.

6.12 Record the receiving and issuance details in Annexure I.

6.13 Record the results in Annexure III.

6.14 Clean the area thoroughly with disinfectant at the end of the test.

6.15 **Processed BI incubation procedure:**

6.15.1 Processed BI (only strips) shall be inoculated in SCDM media tube (10 ml) under LAF.

6.15.2 Processed BI shall be incubated within 04 hours of sterilization cycle end time.

6.15.3 BI containing *Bacillus atrophaeus*, should be incubated for 07 days at a 30° to 35°C or as per manufacturer instructions.

6.15.4 BI containing *G. stearothermophilus*, should be incubated for 07 days at a 55° to 60°C or as per manufacturer instructions.

6.15.5 Record the results in Annexure II.

7.0 DEFINITIONS AND ABBREVIATIONS

7.1 BI : Biological indicator

8.0 REFERENCE

8.1 USP <55> Biological indicator resistance performance tests.

9.0 ANNEXURES

9.1 Annexure I : Biological indicator consumption record

9.2 Annexure II : Biological indicator incubation report

9.3 Annexure III : Biological indicator qualification record

10.0 DISTRIBUTION DETAILS `

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

11.0 REVISION HISTORY

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

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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ANNEXURE I
BIOLOGICAL INDICATOR CONSUMPTION RECORD

Biological Indicator Type										
Date	**Opening Balance Qty.**	**Qty. Received**	**Make**	**Lot No.**	**Qty. issued**	**Quantity used for**	**Closing Balance Qty.**	**Issued By**	**Received By**	**Reviewed By**

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ANNEXURE II
BIOLOGICAL INDICATOR INCUBATION REPORT

Date :	**BI Type:**
Sterilization cycle details:	**BI Lot No. :**
Quantity of BI:	**A.R. No.:**
Media Lot no.:	**LAF ID:**
Incubator ID:	**Incubation Temperature:**
Incubation started on:	**Incubation completed on :**

S.No.	Biological Indicator No.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1.								
2.								
3.								
4.								
5.								
6.								
7.								
8.								
9.								
10.								
Media Blank								
Positive control								
Observed by								
Checked by								

NG=No Growth, G=Growth

Remarks: The sample complies / does not comply as per specifications.

Analyzed By: Reviewed By:

Date: Date:

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ANNEXURE III
BIOLOGICAL INDICATOR QUALIFICATION RECORD

Date of receipt		**Name of Biological indicator**	
Date of Mfg.		**Type of Biological Indicator**	
Date of Expiry:		**Lot No.**	
Manufacturer / Supplier		**Spore Population (As per COA)**	
Spore population determination (Acceptance criteria:- 50 to +200 % of Label Claim)			
No. of indicators / ampoules used		**Media used**	SCDA
Vol. of spore suspension used		**Prepared media Lot No.**	
Incubation condition		**Analyzed by**	
Dilution used for testing			

Observation after	**24hrs**	**48hrs**
Plate – 1 CFU		
Plate – 2 CFU		
Average CFU		
Observed by		
Average spore population per indicator (Strip/Ampoule) = Average colonies (CFU) x reciprocal of dilution / No. of strips/ Ampoules		

Remarks: The biological indicator lot is qualified / disqualified for the usage in validation purpose.

Observed By:
Date:

Reviewed By:
Date: