PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Receipt, Usage and Qualification of Biological Indicator	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down a procedure for Receipt, Usage and Qualification of Biological Indicator.

2.0 SCOPE:

This SOP is applicable for Receipt, Usage and Qualification of Biological Indicator in Microbiology Section of Quality Control.

3.0 RESPONSIBILITY:

Officer / Executive- Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATION:

BI	Biological Indicator
CFU	Colony Forming Unit
Ltd.	Limited
No.	Number
QA	Quality Assurance
QC	Quality Control
SCA	Soybean casein digest agar
SOP	Standard Operating Procedure

6.0 PROCEDURE:

6.1 On the receipt of the material check the physical condition of pack labeling details, certificates & Expiry date.

6.2 Store it in cool and dry place or as per manufacturer recommendation and maintain the stock as per **Annexure-II**, Titled **"Stock Maintenance of Biological Indicators"**.

6.3 Spore Paper Strip shall be used for porous load sterilization cycle (vacuum and steam pulse cycle) and Spore Ampoule shall be used for liquid sterilization cycle (Gravity sterilization cycle).

6.4 QUALIFICATION OF SPORE PAPER STRIP:

6.4.1 Take at least four spore paper strip of biological indicator and put in 100 ml of sterilized purified chilled water at 2- 8°C, and vortex for 3 to 5 minutes or above to achieve a homogenous suspension.

6.4.2 For heat shock treatment to the biological indicator, transfer 10 ml of aliquot to test tube and heat the tube in steam pot/ water bath at 95°C to 100°C for 15 minutes and start the time when the temperature reached 95°C.

6.4.3 Heat shock treatment to the biological indicator for Dry Heat sterilizer, Spore Paper Carrier; heat the tube in steam pot/ water bath at 80°C to 85°C for 10 minutes starting the timing when the temperature reaches 80°C.

6.4.4 Cool the test tube rapidly containing suspension of spores in an ice water at 0°C to 4°C.

6.4.5 Transfer 1 ml aliquots to 9 ml of sterilized purified water and make serial dilutions to yield 30 to 300 colonies on each plate in a pair, where the BI has a low spore concentration, it may be necessary to modify the dilution series and to use more plates at each dilution.

6.4.6 Add 1 ml of each selected dilution in two pre-sterilized Petri plates then within 20 minutes pour 20-25 ml sterilized (SCA) Soyabean casein Digest Agar (cool up to 45°C, checks with IR gun)

6.4.7 Mix to attain a homogenous suspension with media and allow solidifying at room temperature.

6.4.8 For thermophilic biological indicator, incubate the plates in an inverted position at 55°C to 60°C for atleast 48 hrs.

6.4.9 For nonthermophilic biological indicator to incubate the plates in inverted position at 30°C to 35°C for atleast 48 hours or at required temperature specified by manufacture.

6.4.10 After incubation by using colony counter, count the cfu per plate and calculate the average no. of spores per test sample from the results, using the appropriate dilution factor.

6.4.11 Record the result in **Annexure-I**, Titled **"Spore Count Report of Biological Indicator"**.

6.4.12 Acceptance Criteria

The viable spore count shall be between 50% and 300% of the manufacturer's stated value.

6.5 QUALIFICATION OF SPORE AMPOULE:

6.5.1 Take at least four spore ampoule of biological indicator and transfer the spore suspension to 100 ml of sterilized purified water chilled to 2- 8°C and vortex for 3 to 5 minutes or above to achieve a homogenous suspension.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Receipt, Usage and Qualification of Biological Indicator	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

6.5.2 For heat shock treatment to the biological indicator, transfer 10 ml of aliquot to test tube and heat the tube in steam pot/ water bath at 95°C to 100°C for 15 minutes starting the timing when the temperature reaches 95°C.

6.5.3 Cool the test tube rapidly containing suspension of spores in an ice water at 0°C to 4°C.

6.5.4 Transfer 1 ml aliquots to 9 ml of sterilized purified water and make serial dilutions to yield 30 to 300 colonies on each plate in a pair, where the BI has a low spore concentration, it may be necessary to modify the dilution series and to use more plates at each dilution.

6.5.5 add 1 ml of each selected dilution in two pre-sterilized Petri plates then within 20 minutes pour 20-25 ml sterilized (SCA) Soyabean casein Digest Agar (cool up to 45°C, checks with IR gun).

6.5.6 Mix to attain a homogenous suspension with media and allow solidifying at room temperature.

6.5.7 For thermophilic biological indicator, incubate the plates in an inverted position at 55°C to 60°C for atleast 48 hrs.

6.5.8 After incubation count the cfu per plate by using colony counter and calculate the average no. of spores per test sample from the results, using the appropriate dilution factor

6.5.9 Record the result in **Annexure-I**, Titled **"Spore Count Report of Biological Indicator"**.

6.5.10 Acceptance Criteria:

The viable spore count shall be between 50% and 300% of the manufacturer's stated value.

MORPHOLOGICAL CHARACTERISTICS OF BIOLOGICAL INDICATORS:

S.No.	Name of culture	Incubation Condition		Physical Characteristics			Biochemical Test	
		Temp.	Period	Morphology	Staining Method	Characteristics	Catalase Test	VPT
1.	*Geobacillus stearothermophilus* ATCC 7953	55-60^{0}C	≥48 hrs.	Large Irregular Colonies	Gram Staining	Gram + ve, Rods	Week Positive Catalase Reaction	-ve
2.	*Bacillus atrophaeus* ATCC 9372	30-35^{0}C	≥48 hrs.	Large Irregular Colonies	Gram Staining	Gram + ve, Rods	Positive Catalase reaction	+ve
3.	*Bacillus subtilis*	30-35^{0}C	≥48 hrs.	Large Irregular Colonies	Gram Staining	Gram + ve, Rods	Positive Catalase reaction	+ve

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Receipt, Usage and Qualification of Biological Indicator	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

6.6 USAGE:

6.6.1 Take an appropriate number of BI from the box.

6.6.2 Place this BI in the most challenging areas defined as specified protocol of the Autoclave and process the load as usual.

6.6.3 After completion of process/cycle, open the sterilizer door, wait for 5 minutes for cool and remove the biological indicator from the Autoclave.

6.6.4 In case of BI ***Geobacillus stearothermophilus* 7953** in glass ampoule, incubate as such.

6.6.5 For activation of the media, place the indicator in an upright position in a plastic crusher. Gently squeeze the glass ampoule. This will allow the media to come in contact with the spore strip.

6.6.6 Biological indicator shall be incubated within 4 hours at specified temperature.

6.6.7 Ampoule/strip BI of *Geobacillus stearothermophilus* Incubate at 55-60˚C for 7 days or desired incubation time as per BI Manufacturer.

6.6.8 Ampoule/strip BI of *Bacillus subtilis/bacillus atrophaeus* Incubate at 30-35˚C for 7 days or desired incubation time as per BI Manufacturer.

6.6.9 Record the result in **Annexure-III**, Titled **"Observation Report of Biological Indicator"**.

6.6.10 For Negative control, incubate the Negative control BI as such without squeeze the glass ampoule at specified temperature and time or desired incubation time as per BI Manufacturer.

6.6.11 Acceptance Criteria: The appearance of a yellow colour indicates bacterial growth. No colour change indicates proper sterilization or as per BI Manufacturer.

6.7 DESTRUCTION OF BIOLOGICAL INDICTORS:

6.7.1 After completion of incubation period or end of the test collect used Biological Indictors from incubation room and put in discarding area for destruction.

6.7.2 Destruction of used Biological Indictors shall be done in vertical autoclave as per respective SOP for discarding or as per BI's manufacturer instructions.

6.7.3 During BI qualification used all pipette tips, tubes and plates shall be decontaminated as per SOP, Titled **"Procedure for operation and Discarding of media in vertical Autoclave (Make-ketan)".**

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Receipt, Usage and Qualification of Biological Indicator	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

6.7.4 Biological Indictors after expiry date or damage during usage shall be discarded in vertical autoclave as per SOP, Titled **"Procedure for operation and Discarding of media in vertical Autoclave (Make-ketan)"** or as per Biological Indictors manufacturer instructions.

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Spore Count Report of Biological Indicator	
Annexure- II	Stock Maintenance of Biological Indicator	
Annexure- III	Observation Report of Biological Indicator	

ENCLOSURES: SOP Training Record

8.0 DISTRIBUTION:

- Controlled Copy No. 01 — Quality Assurance
- Controlled Copy No. 02 — Microbiology
- Master Copy — Quality Assurance

9.0 REFERENCES:

USP AND ISO 11138 Guidelines.

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision No.	Change Control No.	Details of Changes	Reason for Change	Effective Date	Updated By

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Receipt, Usage and Qualification of Biological Indicator	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE – I

SPORE COUNT REPORT OF BIOLOGICAL INDICATOR

Name of Biological Indicator		**Spore Population as per COA**	
Batch No.		**Observed Spore Population**	
ATCC No.		**Manufacturer Name**	
Mfg. Date		**Exp. Date**	
Incubation start date & time		**Incubation completion Date & time**	
Tested by /date			

Date of Observation	**Serial dilution**											
	10^{-2}		**10^{-3}**		**10^{-4}**		**10^{-5}**		**10^{-6}**		**10^{-7}**	
	Plates		**Plates**		**Plates**		**Plates**		**Plates**		**Plates**	
	1st	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
Average Count→												

-Ve Media Control ……..……

Calculation:

$$\text{Result} = \frac{\text{Observed count X dilution factor}}{\text{No. of BI}}$$

$$\% \text{ Recovery} = \frac{\text{Obtained result X 100}}{\text{Label claim of BI}}$$

Remarks: The above sample complies/does not comply as per acceptance criteria.

Done by: (Sign & Date) **Reviewed By: (Sign & Date)**

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Receipt, Usage and Qualification of Biological Indicator	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE – II

STOCK MAINTENANCE OF BIOLOGICAL INDICATOR

Name of BI Organism		**ATCC No.:**	
Make:		**Batch No.**	
Mfg. Date		**Exp. Date**	
Date of received		**Quantity Received**	
Received by			

Date of Issued	Issued Quantity	Balance Quantity	Purpose	Issued by/date	Received By/date	Checked By/date

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Receipt, Usage and Qualification of Biological Indicator	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE – III

OBSERVATION REPORT OF BIOLOGICAL INDICATOR

Cycle Name		**Cycle No.**	
Name of BI		**Spore Population claim by manufacturer**	
In-house recovered BI Spore population		**Name of area**	
ATCC No.		**Make**	
Lot No.		**Type of BI**	
Mfg. Date		**Exp. Date**	
Incubation Temp.		**Date of incubation**	
Start time of Incubation		**Completion time of incubation**	
Incubated By		**Incubator ID No.**	

Observation of BI																		
No. of BI → / **Date ↓**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**-ve**	**Observed by/ date**	**Checked by /date**

Remarks: The above observation complies/does not comply as per acceptance criteria.