



PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Recovery of Labeled Spore Count of Biological Indicators	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

1. **Purpose:** The purpose of this SOP is to define the procedure for recovery of labeled spore count of biological indicator used for the validation of Autoclave.
2. **Scope:** This SOP is applicable for recovery of labeled spore count of biological indicator used for the validation of Autoclave in microbiology section.
3. **References, Attachments & Annexures:**
 - 3.1. **References:** In-house
 - 3.2. **Attachments:**
 - 3.2.1. Attachment-1: Biological indicator recovery labeled spore count template
 - 3.3. **Annexures:** NA
4. **Responsibilities:**
 - 4.1. **Microbiologist:**
 - 4.1.1. To perform the activity as per SOP.
 - 4.1.2. To maintain all the records as per SOP.
 - 4.2. **QC Head or designee:**
 - 4.2.1. To check the SOP.
 - 4.2.2. To give training to all concerned persons before implementation of SOP.
 - 4.2.3. To ensure proper documentation as per SOP.
 - 4.3. **Quality Assurance:**
 - 4.3.1. To check the SOP.
 - 4.3.2. To ensure the implementation of system as per SOP.
 - 4.4. **Quality, Plant and Regulatory head or designee:**
 - 4.4.1. To review and approve the SOP.
5. **Distributions:**
 - 5.1. Quality Assurance
 - 5.2. Quality Control (Microbiology)
6. **Abbreviations & Definitions of terms:**



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6.1. Abbreviations:

- 6.1.1. & : And
- 6.1.2. % : percent
- 6.1.3. °C : Degree Centigrade
- 6.1.4. cfu : Colony forming unit
- 6.1.5. ml : Milli liter
- 6.1.6. No. : Number
- 6.1.7. Sr. No. : Serial Number
- 6.1.8. Sec : Second

6.2. Definitions of terms: None

7. Procedure:

7.1. Precautions:

- 7.1.1. Handle biological indicators carefully.
- 7.1.2. Wear sterile garments and sterile hand gloves during analysis of biological indicators.

7.2. Recovery of Labelled Spore Count of Biological Indicators:

- 7.2.1. Perform the recovery of labelled spore count of one strips/ampoule of *Geobacillus stercorophilus* spores from the lot/batch, which is to be used for validation purpose.
- 7.2.2. Transfer the strip's/ampoule spores aseptically into a sterile container of 250ml containing 100ml of chilled and sterilized purified water. Shake the container by vortex shaker for approximately 15 minutes to obtain homogenised suspension of the spores.
- 7.2.3. Transfer 10ml aliquot of the suspension in the sterile, screw capped/cotton plugged test tube and provide heat shock by putting the tube in water bath maintained at 95-100°C for 15 minutes, then cool it immediately by placing the tube in a container filled with ice cubes.
- 7.2.4. Shake the tube by palming or vortex to obtain homogenised suspension of the spores.
- 7.2.5. Transfer 1ml aliquot further in test tubes containing 10ml sterile purified water and make serial dilutions.
- 7.2.6. Take 1ml aliquot from the each dilution in duplicate petriplates, so as to get yield preferably 30-300 cfu/plate.
- 7.2.7. Pour sterilized and molten (cooled up to 45-50°C) Soyabean Casein Digest Agar medium into petriplates, swirl them and allow solidifying.
- 7.2.8. Incubate the plates in inverted position at 55°C to 60°C for 48 hours. Examine the plates after 24 and 48 hours.
- 7.2.9. Count the colonies per plate and calculate the average number per strips/ampoule by using dilution factor. The test is valid if the log number of spores per carrier at 48 hours is equal to or greater than the log number after 24 hours in each case.



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7.2.10. Use the strips/ampoule for validation purpose if the recovery of labelled spore count is not more than 300% of the label claim and not less than 50% of the label claim.

7.2.11. Note the results in attachment-1 for recording of initial count of biological indicator.

7.3. Acceptance criteria:

7.3.1. The log number of spores per carrier at 48 hours is equal to or greater than the log number after 24 hours in each case.

7.3.2. The recovery of labelled spore count of biological indicator spores should be in between 50% to 300% of the label claim count after the completion of incubation period.

7.4. Frequency:

7.4.1. This study for biological indicator spores strips/ampoule shall be done on the arrival of new lot/batch before use in the validation/revalidation of the autoclave.



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Attachment-1 Biological Indicator Recovery labeled spore count template

Test Organism:	Growth Medium: Soyabean Casein Digest Agar																				
Batch/Lot No.:	Media Lot No.:																				
Manufactured Date:	Incubation Conditions: 55°C to 60°C for 48 hours																				
Expiry Date:	Dilution Fluid: Sterile purified water																				
Date of Testing:	Date of Result:																				
Claimed count:																					
Observation:																					
<table border="1"><thead><tr><th rowspan="2">Time (Hours)</th><th rowspan="2">Dilution Factor</th><th colspan="2">cfu/plate</th><th rowspan="2">Mean cfu/plate</th><th rowspan="2">cfu/Strip/Ampoule</th></tr><tr><th>Plate No.1</th><th>Plate No.2</th></tr></thead><tbody><tr><td>After 24</td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>After 48</td><td></td><td></td><td></td><td></td><td></td></tr></tbody></table>		Time (Hours)	Dilution Factor	cfu/plate		Mean cfu/plate	cfu/Strip/Ampoule	Plate No.1	Plate No.2	After 24						After 48					
Time (Hours)	Dilution Factor			cfu/plate				Mean cfu/plate	cfu/Strip/Ampoule												
		Plate No.1	Plate No.2																		
After 24																					
After 48																					
Conclusion:																					
Acceptance criteria:																					
The log number of spores per carrier at 48 hours is equal to or greater than the log number after 24 hours in each case.																					
The initial count of biological indicator spores should be in between 50% to 300% of the label claim count.																					
Analyzed by :	Checked by :																				
Date :	Date :																				

8. History:

Version No.	Effective Date



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