



**STANDARD OPERATING PROCEDURE**

<b>Department:</b> Microbiology	<b>SOP No.:</b>
<b>Title:</b> Method for Verification of Spore population of Biological Indicators	<b>Effective Date:</b>
<b>Supersedes:</b> Nil	<b>Review Date:</b>
<b>Issue Date:</b>	<b>Page No.:</b>

**1.0 OBJECTIVE:**

To lay down a procedure for determining the spore population of Biological indicators which are commercially available and are used in validation process of Autoclave and Dry heat sterilizers.

**2.0 RESPONSIBILITY:**

Microbiologist / Technician

**3.0 ACCOUNTABILITY:**

Executive

**4.0 PROCEDURE:**

**4.1 REQUIREMENT**

Biological indicator carrier strips/ Ampoules, Cyclomixer, Sonicator, Strong glass bottle, Soyabean caseing digest Agar, Sterile tubes containing 9 ml distilled water, Water bath, 3 mm glass beads and other tersting accessories.

**4.2 TESTING PROCEDURE FOR BIOLOGICAL INDICATOR STRIPS**

4.2.1 Randomly select four Biological Indicator from the lot to be assayed.

4.2.2 Place each carrier in a sterile test tube with (3) 6 mm glass beads and 5 ml of sterile distileed water.

4.2.3 Vortex untill the paper is macerated to pulp, about 4-7 mins.

4.2.4 Add 5 ml of sterile distilled water and vortex for about 4-7 mins.

4.2.5 Heat shock:

4.2.5.1 For Biological indicator of steam sterilization, heat the tube containing the suspension in a water bath at 95-100°C for 15 minutes. Time considered should be when the temperature reaches 95°C.

4.2.5.2 For Biological indicator of dry heat sterilization, heat the tube containing the suspension in a water bath at 80-85°C for 10 minutes. Time considered should be when the temperature reaches 80°C.

4.2.6 Remove the tubes and cool rapidly in an ice bath at 0-4°C.

4.2.7 A serial dilution shall be made from each tube with 9 ml of sterile distilled water, so as to attain 10<sup>-6</sup> dilution.



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- 4.2.8 Vortex each heat-shocked tube at least for 10 seconds before proceeding to the next dilution.
- 4.2.9 Vortex the tubes of  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  for at least 10 seconds before pipetting out 1.0 ml each into two petri dishes (i.e in duplicate).
- 4.2.10 Place 1 ml of each selected dilution in each of 2 petri plates.
- 4.2.11 Pour approximately 20 ml of SCDA precooled to 40-45°C in to petri dishes.
- 4.2.12 Swirl to assure adequate mixing and allow the agar to solidify.
- 4.2.13 For biological indicator of steam sterilization, incubate the plates in an inverted position at 55-60°C for 48 hours.
- 4.2.14 For biological indicator of dry heat sterilization, incubate the plates in inverted position at 30-35°C for 48 hours.
- 4.2.15 Count the number of colonies from each plate and calculate the average number of spore per specimen/ carrier strips using the appropriate dilution factor.

**4.3 TESTING PROCEDURE FOR BIOLOGICAL INDICATOR AMPOULES**

- 4.3.1 Randomly select four ampoules from the lot to assayed.
- 4.3.2 Place all four ampoules in to a sterile 250 ml strong glass bottle.
- 4.3.3 Crush the ampoules to shards using either a sterile stainless steel rod or sterile forceps.
- 4.3.4 Rinse the crushing device with the sterile water for injection as it is added to the 250 ml strong bottle.
- 4.3.5 For Magna Ampoules:
  - 4.3.5.1 Fill volume is 1.2 ml per ampoule.
  - 4.3.5.2 There are four ampoules for a total of 4.8 ml.
  - 4.3.5.3 Add 95.2 ml of water to bring the total volume to 100 ml.
- 4.3.6 Vortex sample for not less than one minute.
- 4.3.7 Prior to sonication allow Stron glass container to sit for five minutes to allow air bubble to dissipate.
- 4.3.8 Sonicate the sample for 5 minutes.
- 4.3.9 Vortex the sample for not less than one minute.
- 4.3.10 Pipette out 10.0 ml aliquot from this tube according to the test organism.



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4.3.11 In a water bath, heat shock this tube according to the test organism.

4.3.12 Follow Point No. 4.2.7 for further test.

**4.4 FREQUENCY:**

4.4.1 Carry out the analysis for each lot of newly received spore strips.

4.4.2 If the newly received lot is not used within 6 months, the analysis shall be carried out before usage.

4.5 **LIMITS:** The count should not be less than the label claim.

**5.0 REASON FOR REVISION:**

Harmonization of format.

**6.0 TRAINING:**

Trainer -- Executive – Quality Assurance

Trainees -- Microbiologist and QA Inspectors

Period -- One day

**7.0 DISTRIBUTION:**

Certified Copy No. 1 : Head of Department – Quality Control

Certified Copy No. 2 : Microbiology Department

Certified Copy No. 3 : Record file

Reference copy No. 4 : Display Near autoclave.

Original Copy : Head – QA.

**8.0 ANNEXURE**

Annexure 1 : Spore Population verification Record.

**9.0 REFERENCES:**

As per supplier Specification.

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**ANNEXURE -1**

**SPORE POPULATION VERIFICATION RECORD**

Date of analysis:

Date of Report:

Name of specimen:

Lot no. of specimen:

No. of specimen analyzed:

Label claim:

Heat shock Time: From \_\_\_\_\_ To \_\_\_\_\_

Temperature: \_\_\_\_\_

Party COA received: YES/NO.

Analytical data:

S.No.	Dilution	No. of colonies		Average no. of colonies
		Plate 1	Plate 2	

Calculation:

No. of spores per carrier strip / Ampoule =  $\frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Volume of sample plated} \times \text{No. of specimen}}$

No. of spore per carrier strip / Ampoule =

**Result:**

The specimen Complies / Doesn't Complies as per label claim.



# PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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