STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Method for Verification of Spore Population of Biological Indicators	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

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 RESPONSIBILTY

Microbiologist / Technician

3.0 ACCOUNTABILITY

Executive

4.0 PROCEDURE

4.1 REQUIREMENT

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

4.2.3 Vortex until 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^{-6} dilution.

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.

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4.3.10 Pipette out 10.0 ml aliquot from this tube according to the test organism.

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:

Harmonisation 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 – Quality Assurance.

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 = $\frac{\text{No. of colonies x dilution factor}}{\text{Volume of sample plated x No. of specimen}}$

No. of spore per carrier strip / Ampoule =

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