STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Determining the total viable spore count of Biological Indicators	**Effective Date:**
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Issue Date:	**Page No.:**

1.0 OBJECTIVE

1.1 To lay down the Procedure for determining the total viable spore count of Biological Indicators.

2.0 SCOPE

2.1 This SOP is applicable for determining the total viable spore count of Biological indicators.

3.0 RESPONSIBILITY

3.1 Microbiologist

4.0 ACCOUNTABILITY

4.1 Qc-Manager

5.0 PROCEDURE

5.1 Take three biological indicator paper strips(*Bacillus stearothermophilus* or *Bacillus subtilis*)

5.2 Tear off the paper cover and transfer strips into a glass tube containing 30ml sterile water with the help of a sterile or incinerated forceps. Perform the activity in the Laminar airflow.

5.3 Gently vortex the tube for 5 minutes so as to achieve a homogenous suspension.

5.4 Heat the tube containing the suspension in water bath at 95° C to 100° C for 15 minutes (For biological indicator used for steam sterilization), and at 80° C to 85° C for 10 minutes (For biological indicator used for dry heat sterilization).

5.5 Cool rapidly in an ice water bath (0^0 C to 4^0 C).

5.6 Prepare serial dilutions of the culture suspension as per the procedure given below:

5.6.1 Transfer 1.0 ml aliquot of culture suspension to a tube containing 9 ml of 0.9% sterile saline solution and vortex for 5 minutes. This constitutes 1:10 dilution.

5.6.2 From the 1:10 dilution, transfer 1 ml aliquot to a tube containing 9 ml of 0.9% sterile saline solution and vortex for 5 minutes. This constitutes 1:100 dilution.

5.6.3 From the 1:100 dilution, transfer 1 ml aliquot to a tube containing 9 ml of 0.9% sterile saline solution and vortex for 5 minutes. This constitutes 1:1000 dilution.

5.6.4 From the 1:1000 dilution, transfer 1 ml aliquot to a tube containing 9 ml of 0.9% sterile saline solution and vortex for 5 minutes. This constitutes 1:10000 dilution.

5.7 From each dilution, pour 1.0 ml of the culture suspension in two petriplates and mark the dilution factor on each plate.

5.8 Add 15 to 20 ml of soyabean casein digest agar medium to each plate having culture suspension.

5.9 Swirl gently to mix and allow them solidify.

5.10 Incubate the plates in an inverted position at 55 to 60ºC for 24 – 48 hours for *Bacillus staerothermophilus* and for *Bacillus subtilis* 30-35ºC for 24 – 48 hours.

5.11 After incubation, count and record the number of CFU in each plate.

5.12 Calculate number of spores per specimen by multiplying the CFU with dilution factor.

5.13 Divide the results so obtained by three to get the population of one strip.

5.14 Record the details as per the Annexure–I.

5.15 Acceptance Criteria: The average spore count should not be less than 0.3 log and should not be more than 0.48 log of the labeled spore population.

5.16 FOR BIOLOGICAL INDICATOR AMPOULES:

5.16.1 Take three biological indicator Ampoules. (*Bacillus stearothermophilus* or *Bacillus subtilis*).

5.16.2 Break the Ampoule and transfer the contents into a glass tube containing 30ml sterile purified water with the help of a sterile forceps.

5.16.3 Gently vortex the tube for 5 minutes so as to achieve a homogenous suspension.

5.16.4 Heat the tube containing the suspension in water bath at 95° C to 100° C for 15 minutes [For biological indicator used for steam sterilization], and at 80° to 85° C for 10 minutes [For biological indicator used for dry heat sterilization].

5.16.5 Cool rapidly in an ice water bath (0^0 C to 4^0 C).

5.16.6 Prepare serial dilutions of the culture suspension as per the procedure given below:

5.16.7 Transfer 1.0 ml aliquot of culture suspension to a tube containing 9 ml of 0.9% sterile saline solution and vortex for 5 minutes. This constitutes 1:10 dilution.

5.16.8 From the 1:10 dilution, transfer 1 ml aliquot to a tube containing 9 ml of 0.9% sterile saline solution and vortex for 5 minutes. This constitutes 1:100 dilution.

5.16.9 From the 1:100 dilution, transfer 1 ml aliquot to a tube containing 9 ml of 0.9% sterile saline solution and vortex for 5 minutes. This constitutes 1:1000 dilution.

5.16.10 From the 1:1000 dilution, transfer 1 ml aliquot to a tube containing 9 ml of 0.9% sterile saline solution and vortex for 5 minutes. This constitutes 1:10000 dilution.

5.16.11 From each dilution, pour 1 ml of the culture suspension in two petriplates and mark the dilution factor on each plate.

5.16.12 Add 15 to 20 ml of soyabean casein digest agar medium to each plate having culture suspension.

5.16.13 Swirl gently to mix and allow them solidify.

5.16.14 Incubate the plates in an inverted position at 55 to 60ºC for 24 – 48 hours for *Bacillus staerothermophilus* and for *Bacillus subtilis* 30-35ºC for 24 – 48 hours.

5.16.15 After incubation, count and record the number of CFU in each plate.

5.16.16 Calculate average number of spores per specimen by multiplying the CFU with dilution factor.

5.16.17 Divide the results so obtained by three to get the population of one ampoule.

5.16.18 Record the details as per the Annexure-I.

5.17 Acceptance Criteria: The average spore count should not be less than 0.3 log and should not be more than 0.48 log of the labeled spore population.

6.0 ABBREVIATIONS

6.1 SOP - Standard Operating Procedure

6.2 CFU Colony Forming Unit

7.0 ANNEXURES

7.1 Annexure-I Efficiency of Biological Indicators