PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	**SOP No.:**
Title: Determining the population and Resistance performance of Biological indicators	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1. Purpose:

The purpose of this SOP is to lay down the procedure for determining the population and resistance performance of biological indicators.

2. Scope:

This procedure is applicable for determining the population resistance performance of biological indicators.

3. Responsibility: Microbiologist.

4. Accountability : Head of Quality Control

5. Material and Equipments: Biological Indicators.

6. Procedure:

6.1. Determining the population of Biological indicators:

6.1.1. Take three biological indicator paper strips.

6.1.2. Tear off the paper cover and transfer strips into a glass tube containing 30-ml sterile purified water with the help of a sterile or incinerated forceps.

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

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

6.1.5. Cool rapidly in a ice water bath.

6.1.6. Transfer 1.0 ml aliquot in a sterile tube containing 9 ml of sterile water and make more serial dilutions (01 to 10) in sterile saline suspension.

6.1.7. Pour 1.0 ml of the sample from each dilutions in two petri dishes and mark the dilution factor on each petri dish.

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

6.1.9. Swirl gently to mix and allow it to solidify.

6.1.10. Incubate the plates inverted at 55°C ± 2°C (for steam sterilization) and at 30°C to 35°C (for dry heat sterilization).

6.1.11. Examine the plates after 48 hours and record the number of CFU in each plate.

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

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

6.1.14. Record the details as per the Annexure I.

6.1.15. Release the COA for the usage of biological indicators to the plant duly approved by the Head QA as per the Annexure II.

6.1.16. **Acceptance Criteria**: The average spore count should not be less than 0.3 and should not be more than 0.48 log of the labeled spore population. (As per USP 26)

6.2. Resistance performance of Biological indicators:

6.2.1. Remove three specimens of the relevant biological indicator from their original individual containers.

6.2.2. Pulp the paper into component fibers by placing the test specimens in a sterile 250 ml cup containing 100 ml of chilled sterilized purified water.

6.2.3. Shake the sample for 15 to 30 minutes so as to get a homogeneous suspension.

6.2.4. Transfer 10 ml aliquot of the suspension to a sterile test tube.

6.2.5. For the Biological Indicator of the steam steriliser heat the tube containing the suspension in a water bath at 95 °C to 100 °C for fifteen minutes.

6.2.6. Start the timing when the temperature reaches 95 °C.

6.2.7. For the BI of the Dry Heat sterilizer heat the tube containing the suspension in a water bath at 80°C to 85 °C for ten minutes.

6.2.8. Start the timing when the temperature reaches 80°C.

6.2.9. Cool rapidly in a ice water bath.

6.2.10. Transfer two 1.0 ml of the sample in different tubes and prepare serial dilutions in purified water.

6.2.11. The dilutions should be selected in a way such that it should yield 30 to 300 colonies, but should not be less than 6 colonies in each pair of plates.

6.2.12. Place 1.0 ml of the samples into 90 mm sterile petriplates.

6.2.13. Each plate add 20 ml of Soyabean Caesin Digest Agar Medium.

6.2.14. Swirl it slowly taking care not to spill the media out of the plate to attain a homogeneous suspension and allow it to solidify.

6.2.15. Incubate the plates in an inverted position at 55 °C to 60 °C for Biological Indicators for steam sterilization and at 30°C to 35°C for Biological Indicator for Dry Heat Sterilizer.

6.2.16. Observe the plates after 24 hrs and 48 hrs of incubation.

6.2.17. Calculate the average number of spores per specimen from the results using the appropriate dilution factor and record the details as Annexure I.

6.2.18. The test is valid if the log number of spores per carrier at 48 hrs is equal to or greater than the log number of spores after 24 hrs in each case.

6.3. FREQUENCY:

6.3.1. When ever a new lot of Biological Indicators are received.

7. Distribution and control:

7.1. *Master copy*:

Master copy should keep in lock and key in QA department

7.2. Controlled copy:

Controlled copy should keep with HOD of Quality control department.

7.3. Reference copy :

Reference copy should be present in departmental file.