STANDARD OPERATING PROCEDURE

Title: Handling of Biological Indicator				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 39	

1.0 OBJECTIVE

To lay down procedure for the testing methodology and use of biological indicators in monitoring / validation of equipment such as Moist heat sterilizer, Dry heat sterilizer and Terminal sterilizer.

2.0 SCOPE

This SOP is applicable for all the biological indicators used in manufacturing area.

3.0 RESPONSIBILITY

Prepared by - Executive Microbiology

Checked by - Assistant Manager Microbiology / QC

Approved by - Head QA, QC

4.0 PROCEDURE

4.1 Receiving and storage of biological indicators

4.1.1 On receipt of the biological indicators check visually for contamination in case of ampoules. Check the lot number and expiry date of the biological indicators.

4.1.2 Ensure that the certificate of analysis is received along with the lot of biological indicators and check the certificate for the following information.

4.1.2.1 The name of the organism along with the ATCC number from which the spores are derived.

4.1.2.2 The batch number/lot number.

4.1.2.3 The total viable spore count or mean population per unit of biological indicator.

4.1.2.4 D - Value and the method used to determine D - value.

4.1.2.5 Z - Value.

4.1.2.6 Manufacturing date.

4.1.2.7 Expiry date.

4.1.2.8 Storage specifications.

4.1.2.9 Disposal procedure.

4.1.2.10 Bacteriological medium that will meet the requirement for growth promoting ability.

4.1.2.11Conditions under which reproducible resistance characteristics are obtained.

4.1.2.12 Survival time and kill time under specified conditions.

4.1.3 Storage of biological indicators is to be done as per the manufacturer instructions.

4.1.4 Receiving and usage of biological indicators record is to be maintain in the record sheet as per annexure - I.

4.2 Types of biological indicators

4.2.1 For Moist Heat Sterilization: (Strips and ampoules)

4.2.1.1 *Geobacillus stearothermophilus* ATCC 7953

4.2.2 For Dry Heat Sterilization: (Strips)

4.2.2.1 *Bacillus subtilus var.niger* ATCC 9372.

4.2.3 For Low Temperature Steam Sterilization of solution: (ampoules)

4.2.3.1 *Bacillus subtilus* 5230, ATCC 35021.

4.3 Determination of total viable spore counts of biological indicator ampoules for steam sterilization

4.3.1 Allocate the A.R.Number for the each batch of biological indicators to be rested for total viable spore count.

4.3.2 Randomly pick 3 ampoules from each batch of biological indicator received. Under aseptic conditions, cut open these ampoules with ampoule cutter. Pool the contents in a sterile 250 mL conical flask.

4.3.3 Add chilled sterilized purified water to make 100-mL and mix for 3 to 5 minutes to achieve a homogeneous suspension.

4.3.4 Transfer a 10-mL aliquot to a sterile screw cap tube (16 x 125-mm) and place the tube in a water bath at 95°C to 100°C for 15 minutes. Cool rapidly in an ice water bath (0°C to 4°C).

4.3.5 Transfer 1-mL aliquot to suitable tubes, in duplicate and prepare ten-fold serial dilution in sterile purified water to yield 30 to 300 colonies when plated. The serial dilutions are prepared as per the label claim.

4.3.6 Place 1 mL of each selected dilution into 4 sterile Petri plates. Within 20 minutes, add to each plate 20-mL of soybean casein digest agar medium, which has been melted and cooled to 45°C to 50° C. Mix well, and allow solidifying.

4.3.7 Incubate the two plates (set - 1) in inverted position at 55°C to 60°C for 48 hours. Examine the plates for evidence of contamination with other microorganisms.

4.3.8 Incubate the other two plates (set - 2) in inverted position at 30°C to 35°C for 48 hours. Examine the plates for evidence of contamination with other microorganisms.

4.3.9 Count the number of colonies after 24 and 48 hours, using the number of colonies after 48 hours to calculate the final results (The test is valid if the log number of spores per ampoules after 48 hours is equal to or greater than the log number of spores after 24 hours).

4.3.10 No colonies should be observed at temperature 30°C to 35°C.

4.3.11 Calculate the average of the number of viable spores per ampoule. Calculate the log of average number of viable spores per ampoule.

4.3.12 Calculate the lower limit for total viable spore count by determining the log of the labeled spore count per ampoule and subtracting 0.3 from the value.

4.3.13 Calculate the upper limit for the total viable spore count by summing 0.48 to the log value of the labeled spore count per ampoule.

4.3.14 The requirements of the test are met if the log average number of viable spores per ampoule is not less than 0.3 log labeled spore count per container and does not exceed the log labeled spore count per ampoule by 0.48.

Example for calculation of total viable spore count is as follows.

Labeled spore count per ampoule (BI) = 1.2×10^6

Average spore count per ampoule (BI) = 2.2×10^6

Lower limit = Log of labeled spore count per ampoule (BI) - 0.3

Lower limit = 6.079 - 0.3

Lower limit = 5.779

Upper limit = Log of labeled spore count per ampoule (BI) + 0.48

Upper limit = 6.079 + 0.48

Upper limit = 6.559

Log average spore count per ampoule (BI) = 6.342

4.3.15 After completion of testing record the observations of total viable spore counts in the record sheet as Annexure - II.

4.3.16 Retest for total viable count per ampoule should be carried out after one year.

4.4 Determination of total viable spore counts of biological indicator strips for steam sterilization

4.4.1 Allocate the A.R.Number for the each batch of biological indicators to be rested for total viable spore count.

4.4.2 Randomly pick 3 strips from each batch of biological indicator received. Under aseptic conditions, remove the strips from the individual envelops, and pulp the paper into component fibers by placing them in a sterile 250 mL cup of a suitable blender containing 100 mL of chilled sterilized purified water and blend for 3 to 5 minutes to achieve a homogeneous suspension.

OR

Randomly pick 3 strips from each batch of biological indicator received. Under aseptic conditions, remove the strips from the individual envelope. Place these strips in 250-mL sterile conical flask containing 100 mL of chilled sterilized purified water and sterile glass beads. Vortex until the paper carrier is macerated to achieve a homogeneous suspension.

4.4.3 Transfer a 10 mL aliquot to a sterile screw cap tube (16 x 125-mm) and place the tube in a water bath at 95°C to 100°C for 10 minutes (Heat shock) starting the time when temperature reach at 95°C.

4.4.4 Cool rapidly in an ice water bath (0°C to 4°C).

4.4.5 Transfer 1-mL aliquot to suitable tubes, in duplicate and prepare ten-fold serial dilution in sterile purified water to yield 30 to 300 colonies but not less than 6 when plated. The serial dilutions are prepared as per the label claim.

4.4.6 Place 1.0 mL of each selected dilution into 4 sterile Petri plates. Within 20 minutes, add to each plate 20-mL of Soyabean Casein Digest Agar Medium, which has been melted and cooled to 45°C to 50°C. Mix well and allow solidifying.

4.4.7 Incubate the two plates (set -1) in an inverted position at 55°C to 60°C for 48 hours. Examine the plates for evidence of contamination with other microorganisms.

4.4.8 Incubate the other two plates (set - 2) in an inverted position at 30°C to 35°C for 48 hours. Examine the plates for evidence of contamination with other microorganisms.

4.4.9 Count the number of colonies after 24 and 48 hours, using the number of colonies after 48 hours to calculate the final results (The test is valid if the log number of spores per strip after 48 hours is equal to or greater than the log number of spores after 24 hours).

4.4.10 No colonies should be observed at temperature 30°C to 35°C.

4.4.11 Calculate the average of the number of viable spores per strip. Calculate the log of average number of viable spores per strip.

4.4.12 Calculate the lower limit for total viable spore count by determining the log of the labeled spore count per strip and subtracting 0.3 from the value.

4.4.13 Calculate the upper limit for the total viable spore count by summing 0.48 to the log value of the labeled spore count per strip.

4.4.14 The requirements of the test are met if the log average number of viable spores per strip is not less than 0.3 log labeled spore count per strip and does not exceed the log labeled spore count per strip by 0.48.

4.4.15 After completion of testing record the observations of total viable spore counts in the record sheet as Annexure - III.

4.4.16 Retest for total viable count per strip should be carried out after six month.

4.5 Determination of total viable spore counts of biological indicator strips for dry heat sterilization

4.5.1 Allocate the A.R.Number for the each batch of biological indicators to be rested for total viable spore count.

4.5.2 Randomly pick 3 strips from each batch of biological indicator received. Under aseptic conditions, remove the strips from the individual envelops, and pulp the paper into component fibers by placing them in a sterile 250 mL cup of a suitable blender containing 100 mL of chilled sterilized purified water and blend for 3 to 5 minutes to achieve a homogeneous suspension.

OR

Randomly pick 3 strips from each batch of biological indicator received. Under aseptic conditions, remove the strips from the individual envelope. Place these strips in 250-mL sterile conical flask containing 100 mL of chilled sterilized purified water and sterile glass beads. Vortex until the paper carrier is macerated to achieve a homogeneous suspension

4.5.3 Transfer a 10-mL aliquot to a sterile screw cap tube (16 x 125-mm) and place the tube in a water bath at 80°C to 85°C for 10 minutes (Heat shock) starting the time when temperature reach at 80°C.

4.5.4 Cool rapidly in an ice water bath (0°C to 4°C).

4.5.5 Transfer 1-mL aliquot to suitable tubes, in duplicate and prepare ten-fold serial dilution in sterile purified water to yield 30 to 300 colonies but not less than 6 when plated. The serial dilutions are prepared as per the label claim.

4.5.6 Place 1.0 mL of each selected dilution into 4 sterile Petri plates. Within 20 minutes, add to each plate 20-mL of Soyabean casein digest agar medium, which has been melted and cooled to 45°C to 50° C. Mix well, and allow solidifying

4.5.7 Incubate the two plates (set -1) in an inverted position at 30°C to 35°C for 48 hours. Examine the plates for evidence of contamination with other microorganisms.

4.5.8 Incubate the other two plates (set -2) in an inverted position at 55°C to 60°C for 48 hours. Examine the plates for evidence of contamination with other microorganisms.

4.5.9 Count the number of colonies after 24 and 48 hours, using the number of colonies after 48 hours to calculate the final results (The test is valid if the log number of spores per strip after 48 hours is equal to or greater than the log number of spores after 24 hours).

4.5.10 No colonies should be observed at temperature 55°C to 60°C.

4.5.11 Calculate the average of the number of viable spores per strip. Calculate the log of average number of viable spores per strip.

4.5.12 Calculate the lower limit for total viable spore count by determining the log of the labeled spore count per strip and subtracting 0.3 from the value.

4.5.13 Calculate the upper limit for the total viable spore count by summing 0.48 to the log value of the labeled spore count per strip.

4.5.14 The requirements of the test are met if the log average number of viable spores per strip is not less than 0.3 log labeled spore count per strip and does not exceed the log labeled spore count per strip by 0.48.

4.5.15 After completion of testing record the observations of total viable spore counts in the record sheet as Annexure - IV.

4.5.16 Retest for total viable count per strip should be carried out after one year.

4.6 Determination of total viable spore counts of biological indicator ampoules for low temperature steam sterilization for solutions

4.6.1 Allocate the A.R.Number for the each batch of biological indicators to be rested for total viable spore count.

4.6.2 Randomly pick 4 ampoules from each batch of biological indicator received. Under aseptic conditions, cut open these ampoules with ampoule cutter. Pool the contents in a sterile 250 mL conical flask.

4.6.3 Add sterilized purified water to make a total volume 100-mL, (For e.g., if the ampoule has a fill of 0.3 mL, then add 98.7 mL of sterile purified water for a total volume 10.0 mL.) .

4.6.4 Vortex the sample and mix for not less than one minute to achieve a homogeneous suspension.

4.6.5 Sonicate the flask for not less than three minutes but not more than five minutes and vortex again.

4.6.6 Transfer a 10-mL aliquot from the conical flask to a sterile screw cap tube (16 x 125-mm).

4.6.7 Place the tube in a water bath at 65°C to 70°C for 15 minutes.

4.6.8 After fifteen minutes remove the tube and cool rapidly in an ice bath (0°C to 4°C).

4.6.9 Vortex the heat shocked tube for at least ten seconds.

4.6.10 Transfer 1-mL aliquot to suitable tubes, in duplicate and prepare ten-fold serial dilution in sterile purified water to yield 30 to 300 colonies but not less than 6 when plated. The serial dilutions are prepared as per the label claim.

4.6.11 Place 1 mL of each selected dilution into 4 sterile Petri plates. Within 20 minutes, add to each plate 20-mL of soybean casein digest agar medium, which has been melted and cooled to 45°C to 50° C. Mix well, and allow solidifying.

4.6.12 Incubate the two plates (set - 1) in inverted position at 30°C to 35°C for 48 hours. Examine the plates for evidence of contamination with other microorganisms.

4.6.13 Incubate the other two plates (set - 2) in inverted position at 55°C to 60°C for 48 hours. Examine the plates for evidence of contamination with other microorganisms.

4.6.14 Count the number of colonies after 24 and 48 hours, using the number of colonies after 48 hours to calculate the final results (The test is valid if the log number of spores per ampoules after 48 hours is equal to or greater than the log number of spores after 24 hours).

4.6.15 No colonies should be observed at temperature 55°C to 60°C.

4.6.16 Calculate the average of the number of viable spores per ampoule. Calculate the log of average number of viable spores per ampoule.

4.6.17 Calculate the lower limit for total viable spore count by determining the log of the labeled spore count per ampoule and subtracting 0.3 from the value.

4.6.18 Calculate the upper limit for the total viable spore count by summing 0.48 to the log value of the labeled spore count per ampoule.

4.6.19 The requirements of the test are met if the log average number of viable spores per ampoule is not less than 0.3 log labeled spore count per container and does not exceed the log labeled spore count per ampoule by 0.48.

Example for calculation of total viable spore count is as follows.

Labeled spore count per ampoule (BI) = 1.2×10^6

Average spore count per ampoule (BI) = 2.2×10^6

Lower limit = Log of labeled spore count per ampoule (BI) - 0.3

Lower limit = 6.079 - 0.3

Lower limit = 5.779

Upper limit = Log of labeled spore count per ampoule (BI) + 0.48

Upper limit = 6.079 + 0.48

Upper limit = 6.559

Log average spore count per ampoule (BI) = 6.342

4.6.20 After completion of testing record the observations of total viable spore counts in the record sheet as Annexure - VIII.

4.6.21 Retest for total viable count per ampoule should be carried out after one year.

4.7 Use of biological indicator ampoules for monitoring / validation of moist heat sterilizer

4.7.1 Biological indicator (ampoules) is to be used for monitoring the sterilization cycles during validation of the moist heat sterilizer as per validation protocol.

4.7.2 Allocate A.R. number for each sterilization cycle monitored by biological indicators (ampoules).

4.7.3 Place the biological indicator (ampoules) at different positions within the sterilizer chamber and carry out the sterilization cycle, as per validation protocol.

4.7.4 After completion of sterilization cycle remove the biological indicator (ampoules) from the sterilizer chamber.

4.7.5 Incubate the above exposed biological indicator (ampoules) at 55°C to 60°C for a period of 24 to 48 hours or recommended by the manufacturer along with an unexposed biological indicator (ampoule) as a positive control.

4.7.6 After completion of incubation record the observations of biological indicator (ampoules) in the record sheet as Annexure - V.

4.8 Use of biological indicator strips for monitoring / validation of moist heat sterilizer

4.8.1 Biological indicator (strips) is to be used for monitoring the sterilization cycles during validation of the moist heat sterilizer as per validation protocol.

4.8.2 Allocate A.R. number for each sterilization cycle monitored by biological indicators (strips).

4.8.3 Place the biological indicator (strips) at different positions within the sterilizer chamber and carry out the sterilization cycle, as per validation protocol.

4.8.4 After completion of sterilization cycle remove the biological indicator (strips) from the sterilizer chamber.

4.8.5 Transfer the biological indicator strips in sterile 10 mL nutrient broth or Soyabean casein digest medium with the help of a sterile forceps, under laminar airflow. Incubate the tubes at 55°C to 60°C for a period of 24 to 48 hours or recommended by the manufacturer with an unexposed biological indicator (strip) as a positive control.

4.8.6 Incubate the 10 mL of medium with out inoculating the biological indicator strip as a negative control for media.

4.8.7 After completion of incubation record the observations of biological indicator (strips) in the record sheet as Annexure - VI.

4.9 Use of biological indicator strips for monitoring / validation of dry heat sterilizer

4.9.1 Biological indicator (strips) is to be used for monitoring the sterilization cycles during validation of the dry heat sterilizer as per validation protocol.

4.9.2 Allocate A.R. number for each sterilization cycle monitored by biological indicators (strips).

4.9.3 Place the biological indicator (strips) at different positions within the sterilizer chamber and carry out the sterilization cycle, as per validation protocol.

4.9.4 After completion of sterilization cycle remove the biological indicator (strips) from the sterilizer chamber.

4.9.5 Transfer the biological indicator (strips) in sterile 10 mL nutrient broth or Soyabean casein digest medium with the help of a sterile forceps, under laminar airflow. Incubate the tubes at 30°C to 35°C for a period of 24 to 48 hours or recommended by the manufacturer with an unexposed biological indicator (strip) as a positive control.

4.9.6 Incubate the 10 mL of medium with out inoculating the biological indicator strip as a negative control for media.

4.9.7 After completion of incubation record the observations of biological indicator (strips) in the record sheet as Annexure - VII.

4.10 Use of biological indicator ampoules for monitoring of low temperature steam sterilization of solutions

4.10.1 Biological indicator (ampoules) is to be used for monitoring of low temperature steam sterilization of solutions as per validation protocol.

4.10.2 Allocate A.R. number for each sterilization cycle monitored by biological indicators (ampoules).

4.10.3 Place the biological indicator (ampoules) at different positions within the sterilizer chamber and carry out the sterilization cycle, as per validation protocol.

4.10.4 After completion of sterilization cycle remove the biological indicator (ampoules) from the sterilizer chamber.

4.10.5 Incubate the above exposed biological indicator (ampoules) at 35°C to 39°C for a period of 24 to 48 hours or recommended by the manufacturer with an unexposed biological indicator (ampoule) as a positive control.

4.10.6 After completion of incubation record the observations of biological indicator (ampoules) in the record sheet as Annexure - IX.

4.11 Acceptance criteria

4.11.1 Acceptance criteria is given as per Table - I, Table - II, Table - III and Table - IV.

Table - I

Acceptance criteria For Biological Indicator (Ampoules) For Moist Heat Sterilization

Incubation Conditions		Observation	Conclusion
Exposed indicator incubated at 55°C - 60°C	For a period of 24 to 48 hours or recommended by the manufacture	Purple colour remains purple	Satisfactory sterilization
Exposed indicator incubated at 55°C - 60°C		Purple colour change to yellow colour	Inadequate/faulty sterilization
Exposed indicator incubated at 55°C - 60°C		Purple colour change to reddish brown colour	Excessive sterilization
Unexposed indicator incubated at 55°C - 60°C (Positive control)		Purple colour change to yellow colour	Organisms present in the biological indicator is viable and satisfactory

Table - II

Acceptance criteria For Biological Indicator (Strips) For Moist Heat Sterilization

Incubation Conditions		Observation	Conclusion
Medium containing exposed indicator & incubated at 55°C- 60°C	For a period of 24 to 48 hours or recommended by the manufacture	No turbidity or no growth observed	Satisfactory sterilization
Medium containing exposed indicator & incubated at 55°C - 60°C		Turbidity or growth observed	Inadequate/faulty sterilization
Medium containing unexposed indicator & incubated at 55°C - 60°C (Positive control)		Turbidity or growth observed	Organisms present in the biological indicator is viable and satisfactory
Incubate the media with out inoculating biological indicator as a negative control		No turbidity or no growth observed	Medium is free from microorganism /contamination

Table - III

Acceptance criteria For Biological Indicator (Strips) For Dry Heat Sterilization

Incubation Conditions		Observation	Conclusion
Medium containing exposed indicator incubated at 30°C - 35°C	For a period of 24 to 48 hours or recommended by the manufacture	No turbidity or no growth observed	Satisfactory sterilization
Medium containing exposed indicator incubated at 30°C - 35°C		Turbidity or growth observed	Inadequate/faulty sterilization
Medium containing unexposed indicator incubated at 30°C - 35°C (Positive control)		Turbidity or growth observed	Organisms present in the biological indicator is viable and satisfactory
Incubate the media with out inoculating biological indicator as a negative control		No turbidity or no growth observed	Medium is free from microorganism /contamination

Table - IV

Acceptance criteria For Biological Indicator (Ampoules) for Low Temperature Steam Sterilization of Solutions

Incubation Conditions		Observation	Conclusion
Exposed indicator incubated at 35°C - 39°C	For a period of 24 to 48 hours or recommended by the manufacture	Red/orange colour remains red/orange	Satisfactory sterilization
Exposed indicator incubated at 35°C - 39°C		Red/orange colour change to yellow colour	Inadequate/faulty sterilization
Exposed indicator incubated at 35°C - 39°C		Red/orange colour change to reddish brown colour	Excessive sterilization
Unexposed indicator incubated at 35°C - 39°C (Positive control)		Red/orange colour change to yellow colour	Organisms present in the biological indicator is viable and satisfactory

5.0 SAFETY & PRECAUTIONS

After use or expired biological indicator is to be discarded by using a validated disposal procedure by autoclave.

STANDARD OPERATING PROCEDURE

Title: Handling of Biological Indicator				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	13 of 39	

6.0 REVISION HISTORY

Revision No.	Reason for Revision	Superseded from & date
00	First Issue	--------

7.0 REFERENCES

Not applicable

8.0 ABBREVIATIONS

SOP : Standard Operating Procedure

QC : Quality Control

QA : Quality Assurance

^{0}C : Degree centigrade

mL : Milliliter

mm : Millimeter

BI : Biological Indicator

ATCC : American Type Culture Collection

9.0 ANNEXURES

Annexure - I : Biological indicator usage record.

Annexure - II : Record sheet for observation of total viable spore count of biological indicator (ampoule) for moist heat sterilization.

Annexure - III : Record sheet for observation of total viable spore count of biological indicator (strips) for moist heat sterilization.

Annexure - IV : Record sheet for observation of total viable spore count of biological indicator (strips) for dry heat sterilization.

Annexure - V : Record sheet for observation of biological indicator (ampoule) used for moist heat sterilization.

STANDARD OPERATING PROCEDURE

Title: Handling of Biological Indicator				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	14 of 39	

Annexure - VI : Record sheet for observation of biological indicator (strip) used for moist heat sterilization.

Annexure - VII: Record sheet for observation of biological indicator (strip) used for dry heat sterilization.

Annexure - VIII: Record sheet for observation of total viable spore count of biological indicator (ampoule) for low temperature steam sterilization for solution.

Annexure - IX: Record sheet for observation of total viable spore count of biological indicator (ampoule) for low temperature steam sterilization for solution.

ANNEXURE - I

BIOLOGICAL INDICATOR USAGE RECORD

Name of the Organism: _____ Strain No: _____

Date of Receipt	Qty. Received	Batch No./ Lot No.	Mfg. Date	Expiry Date	Date of Issue	Qty. Issue	Qty. in Stock	Done By	Checked By

ANNEXURE - II

RECORD SHEET FOR OBSERVATION OF TOTAL VIABLE SPORE COUNT OF BIOLOGICAL INDICATOR (AMPOULE) FOR MOIST HEAT STERILIZATION

Detail of biological indicator:

Name of the Organism: _____ Strain No: _____

Batch No./ Lot No. _____ Manufacturing Date: _____

Expiry Date: _____ A.R.No: _____

Testing Done On: _____ Testing Done By: _____

Label Claim: _____(Spore per Ampoule)

Testing Procedure:

Randomly pick ____ ampoules from the above batch received and under aseptic conditions cut them and pool the content into _____ mL of chilled sterilized purified water. Mix well _____ minutes to achieve a homogeneous suspension.

10 mL aliquot of the above-homogenized suspension is incubated in water bath at _____ for _____ minutes and cooled rapidly the same in an ice water bath at _____.

Prepare in duplicate ten-fold serial dilution in sterile purified water as per the label claim.

Place _____ mL of each selected dilution into 4 sterile Petri plates. Within 20 minutes, add to each plate _____ of Soyabean casein digest agar, which has been melted and cooled to _____.

Mix well, and incubate in inverted position at _____for _____.

Count the number of colonies and note down in the following table.

Observation Table:

Incubation started on: _____

Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Spore Per mL	Spore /Ampoule
Set -1 (55 – 60°C) After 24 hours								
Set -1 (55 - 60°C) After 48 hours								
Set -2 (30 - 35°C) After 24 hours								
Set -2 (30 -35°C) After 48 hours								

Incubation completed on: _____

Observation:

- Spores/Ampoule = Spores/mL X dilution factor
- Set - 1: Average Spore/ Ampoule =
- Set - 2: Spores/ Ampoule =

Calculation:

- Labeled Spore count per Ampoule -
- Average Spore Count per Ampoule –
- Lower Limit = Log labeled spore count per Ampoule - 0.3

- Upper limit = Log labeled spore count per Ampoule + 0.48
- Log of average spore count per Ampoule -

Result:

- The total viable spore counts per ampoule are found with in/without acceptance criteria.

Acceptance Criteria:

- The requirements of the test are met if the log average number of viable spores per ampoule is not less than 0.3 log labeled spore count per ampoules and does not exceed the log labeled spore count per ampoules by 0.48.

Media Details:

Name of the medium used: _____

Load number of the medium used: _____

Tested by:
(Sign & Date)

Checked by:
(Sign & Date)

ANNEXURE - III

RECORD SHEET FOR OBSERVATION OF TOTAL VIABLE SPORE COUNT OF BIOLOGICAL INDICATOR (STRIP) FOR MOIST HEAT STERILIZATION

Detail of biological indicator:

Name of the Organism: _____ Strain No: _____

Batch No./ Lot No. _____ Manufacturing Date: _____

Expiry Date: _____ A.R.No: _____

Testing Done On: _____ Testing Done By: _____

Label Claim: _____(Spore per Strip)

Testing Procedure:

Randomly pick ____ strips from the above batch received and under aseptic conditions and place the strips into _____ mL of chilled sterilized purified water containg glass beads. Mix well in a vortex up to achieve a homogeneous suspension.

10 mL aliquot of the above-homogenized suspension is incubated in water bath at _____ for _____ minutes and cooled rapidly the same in an ice water bath at _____.

Prepare in duplicate ten-fold serial dilution in sterile purified water as per the label claim.

Place _____ mL of each selected dilution into 4 sterile Petri plates. Within 20 minutes, add to each plate _____ of Soyabean casein digest agar, which has been melted and cooled to _____.

Mix well, and incubate in inverted position at _____ for _____.

Count the number of colonies and note down in the following table.

Observation Table:

Incubation started on: _____

Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Spore Per mL	Spore /Strip
Set -1 (55 - 60°C) After 24 hours								
Set -1 (55 - 60°C) After 48 hours								
Set -2 (30 - 35°C) After 24 hours								
Set -2 (30 - 35°C) After 48 hours								

Incubation completed on: _____

Observation:

- Spores/Strip = Spores/mL X dilution factor
- Set - 1: Average Spore/ Strip =
- Set - 2: Spores/ Strip =

Calculation:

- Labeled Spore count per Strip -
- Average Spore Count per Strip –
- Lower Limit = Log labeled spore count per Strip - 0.3

- Upper limit = Log labeled spore count per Strip + 0.48
- Log of average spore count per Strip -

Result:

- The total viable spore counts per strip are found with in/without acceptance criteria.

Acceptance Criteria:

- The requirements of the test are met if the log average number of viable spores per strip is not less than 0.3 log labeled spore count per strip and does not exceed the log labeled spore count per strip by 0.48.

Media Details:

Name of the medium used: _____

Load number of the medium used: _____

Tested by:
(Sign & Date)

Checked by:
(Sign & Date)

ANNEXURE - IV

RECORD SHEET FOR OBSERVATION OF TOTAL VIABLE SPORE COUNT OF BIOLOGICAL INDICATOR (STRIP) FOR DRY HEAT STERILIZATION

Detail of biological indicator:

Name of the Organism: _____ Strain No: _____

Batch No./ Lot No. _____ Manufacturing Date: _____

Expiry Date: _____ A.R.No: _____

Testing Done On: _____ Testing Done By: _____

Label Claim: _____ (Spore per Strip)

Testing Procedure:

Randomly pick ____ strips from the above batch received and under aseptic conditions and place the strips into _____ mL of chilled sterilized purified water containg glass beads. Mix well in a vortex up to achieve a homogeneous suspension.

10 mL aliquot of the above-homogenized suspension is incubated in water bath at _____ for _____ minutes and cooled rapidly the same in an ice water bath at _____.

Prepare in duplicate ten-fold serial dilution in sterile purified water as per the label claim.

Place _____ mL of each selected dilution into 4 sterile Petri plates. Within 20 minutes, add to each plate _____ of Soyabean casein digest agar, which has been melted and cooled to _____.

Mix well, and incubate in inverted position at _____ for _____.

Count the number of colonies and note down in the following table.

Observation Table:

Incubation started on: _____

Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Spore Per mL	Spore /Strip
Set -1 (30 - 35°C) After 24 hours								
Set -1 (30 - 35°C) After 48 hours								
Set -2 (55 - 60°C) After 24 hours								
Set -2 (55 - 60°C) After 48 hours								

Incubation completed on: _____

Observation:

- Spores/Strip = Spores/mL X dilution factor
- Set - 1: Average Spore/ Strip =
- Set - 2: Spores/ Strip =

Calculation:

- Labeled Spore count per Strip -
- Average Spore Count per Strip -

- Lower Limit = Log labeled spore count per Strip - 0.3
- Upper limit = Log labeled spore count per Strip + 0.48
- Log of average spore count per Strip -

Result:

- The total viable spore counts per strip are found with in/without acceptance criteria.

Acceptance Criteria:

- The requirements of the test are met if the log average number of viable spores per strip is not less than 0.3 log labeled spore count per strip and does not exceed the log labeled spore count per strip by 0.48.

Media Details:

Name of the medium used: _____

Load number of the medium used: _____

Tested by:
(Sign & Date)

Checked by:
(Sign & Date)

ANNEXURE - V

RECORD SHEET FOR OBSERVATION OF BIOLOGICAL INDICATOR (AMPOULE) USED FOR MOIST HEAT STERILIZATION

Detail of Sterilization cycle:

Sterilization Cycle Detail: _____

Date of Sterilization Cycle: _____ A.R.No: _____

Detail of biological indicator:

Name of the Organism: _____ Strain No: _____

Batch No./ Lot No. _____ Manufacturing Date: _____

Expiry Date: _____ Incubation Temperature & Time: _____

Testing Done On: _____ Testing Done By: _____

Incubation Started On: _____ Incubator No.: _____

Location No. ↓ Date →	Observation						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day

STANDARD OPERATING PROCEDURE

Title: Handling of Biological Indicator				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	26 of 39	

Location No. ↓ **Date** →	**Observation**						
	1st Day	**2nd Day**	**3rd Day**	**4th Day**	**5th Day**	**6th Day**	**7th Day**
Observation Done By							

Incubation Completed On: _____

Result: Sterililization Cycle is found satisfactory/ not satisfactory.

Key Words:

+ ve = Purple colour change to yellow colour.

– ve = Purple colour remains purple.

X = Purple colour change to reddish brown colour.

Observation Guide for Biological Indicators (Ampoules):

Incubation Conditions		Observation	Conclusion
Exposed indicator incubated at 55°C - 60°C	For a period of 24 to 48 hours or recommended by the manufacture	Purple colour remains purple	Satisfactory sterilization
Exposed indicator incubated at 55°C - 60°C		Purple colour change to yellow colour	Inadequate/faulty sterilization
Exposed indicator incubated at 55°C - 60°C		Purple colour change to reddish brown colour	Excessive sterilization
Unexposed indicator incubated at 55°C - 60°C (Positive control)		Purple colour change to yellow colour	Organisms present in the biological indicator is viable and satisfactory

Tested by:
(Sign & Date)

Checked by:
(Sign & Date)

ANNEXURE - VI

RECORD SHEET FOR OBSERVATION OF BIOLOGICAL INDICATOR (STRIPS) USED FOR MOIST HEAT STERILIZATION

Detail of Sterilization cycle:

Sterilization Cycle Detail: _____

Date of Sterilization Cycle: _____ A.R.No: _____

Detail of biological indicator:

Name of the Organism: _____ Strain No: _____

Batch No./ Lot No. _____ Manufacturing Date: _____

Expiry Date: _____ Incubation Temperature & Time: _____

Testing Done On: _____ Testing Done By: _____

Incubation Started On: _____ Incubator No.: _____

Location No. ↓ Date →	Observation						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day

STANDARD OPERATING PROCEDURE

Title: Handling of Biological Indicator				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	29 of 39	

Location No. ↓ **Date** →	**Observation**						
	1st Day	**2nd Day**	**3rd Day**	**4th Day**	**5th Day**	**6th Day**	**7th Day**
Observation Done By							

Incubation Completed On: _____

Result: Sterililization Cycle is found satisfactory/ not satisfactory

Media Details:

Name of the medium used: _____

Volume of media used for each indicator strip: _____

Load number of the medium used: _____

Key Words:

+ ve = No turbidity or no growth observed.

– ve = Turbidity or growth observed.

Observation Guide for Biological Indicators (Strips):

Incubation Conditions		Observation	Conclusion
Medium containing exposed indicator & incubated at 55°C- 60°C	For a period of 24 to 48 hours or recommended by the manufacture	No turbidity or no growth observed	Satisfactory sterilization
Medium containing exposed indicator & incubated at 55°C - 60°C		Turbidity or growth observed	Inadequate/faulty sterilization
Medium containing unexposed indicator & incubated at 55°C - 60°C (Positive control)		Turbidity or growth observed	Organisms present in the biological indicator is viable and satisfactory
Incubate the media with out inoculating biological indicator as a negative control		No turbidity or no growth observed	Medium is free from microorganism /contamination

Tested by:
(Sign & Date)

Checked by:
(Sign & Date)

RECORD SHEET FOR OBSERVATION OF BIOLOGICAL INDICATOR (STRIPS) USED FOR DRY HEAT STERILIZATION

Detail of Sterilization cycle:

Sterilization Cycle Detail: _____

Date of Sterilization Cycle: _____ A.R.No: _____

Detail of biological indicator:

Name of the Organism: _____ Strain No: _____

Batch No. / Lot No. _____ Manufacturing Date: _____

Expiry Date: _____ Incubation Temperature & Time: _____

Testing Done On: _____ Testing Done By: _____

Incubation Started On: _____ Incubator No.: _____

Location No. ↓ **Date** →	**Observation**						
	1st Day	**2nd Day**	**3rd Day**	**4th Day**	**5th Day**	**6th Day**	**7th Day**

Location ↓	**Observation**

STANDARD OPERATING PROCEDURE

Title: Handling of Biological Indicator			
SOP No.:		**Department:**	Microbiology
		Effective Date:	
Revision No.:	00	**Revision Date:**	
Supersede Revision No.:	Nil	**Page No.:**	32 of 39

No. Date →	**1st Day**	**2nd Day**	**3rd Day**	**4th Day**	**5th Day**	**6th Day**	**7th Day**
Observation Done By							

Incubation Completed On: _____

Result: Sterililization Cycle is found satisfactory/ not satisfactory.

Media Details:

Name of the medium used: _____

Volume of media used for each indicator strip: _____

Load number of the medium used: _____

Key Words:

+ ve = No turbidity or no growth observed.

– ve = Turbidity or growth observed.

Observation Guide for Biological Indicators (Strips):

Incubation Conditions		**Observation**	**Conclusion**
Medium containing exposed indicator incubated at 30°C - 35°C	For a period of 24 to 48 hours or recommended by the manufacture	No turbidity or no growth observed	Satisfactory sterilization
Medium containing exposed indicator incubated at 30°C - 35°C		Turbidity or growth observed	Inadequate/faulty sterilization
Medium containing unexposed indicator incubated at 30°C - 35°C (Positive control)		Turbidity or growth observed	Organisms present in the biological indicator is viable and satisfactory
Incubate the media with out inoculating biological indicator as a negative control		No turbidity or no growth observed	Medium is free from microorganism /contamination

Tested by:
(Sign & Date)

Checked by:
(Sign & Date)

RECORD SHEET FOR OBSERVATION OF TOTAL VIABLE SPORE COUNT OF BIOLOGICAL INDICATOR (AMPOULE) FOR LOW TEMPERATURE STEAM STERILIZATION FOR SOLUTION

Detail of biological indicator:

Name of the Organism: _____ Strain No: _____

Batch No./ Lot No. _____ Manufacturing Date: _____

Expiry Date: _____ A.R.No: _____

Testing Done On: _____ Testing Done By: _____

Label Claim: _____(Spore per Ampoule)

Testing Procedure:

Randomly pick ____ ampoules from the above batch received and under aseptic conditions cut them and pool the content into _____ mL of sterilized purified water. Mix well _____ minutes to achieve a homogeneous suspension.

10 mL aliquot of the above-homogenized suspension is incubated in water bath at _____ for _____ minutes and cooled rapidly the same in an ice bath at _____.

Prepare in duplicate ten-fold serial dilution in sterile purified water as per the label claim.

Place _____ mL of each selected dilution into 4 sterile Petri plates. Within 20 minutes, add to each plate _____ of Soyabean casein digest agar, which has been melted and cooled to _____.

Mix well, and incubate in inverted position at _____for _____.

Count the number of colonies and note down in the following table.

Observation Table:

Incubation started on: _____

Dilutions	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**Spore Per mL**	**Spore /Ampoule**
Set -1 (30 - 35°C) After 24 hours								
Set -1 (30 - 35°C) After 48 hours								
Set -2 (55 - 60°C) After 24 hours								
Set -2 (55 - 60°C) After 48hours								

Incubation completed on: _____

Observation:

- Spores/Ampoule = Spores/mL X dilution factor
- Set - 1: Average Spore/ Ampoule =
- Set - 2: Spores/ Ampoule =

Calculation:

- Labeled Spore count per Ampoule -
- Average Spore Count per Ampoule –
- Lower Limit = Log labeled spore count per Ampoule - 0.3

- Upper limit = Log labeled spore count per Ampoule + 0.48
- Log of average spore count per Ampoule -

Result:

- The total viable spore counts per ampoule are found with in/without acceptance criteria.

Acceptance Criteria:

- The requirements of the test are met if the log average number of viable spores per ampoule is not less than 0.3 log labeled spore count per ampoules and does not exceed the log labeled spore count per ampoules by 0.48.

Media Details:

Name of the medium used: _____

Load number of the medium used: _____

Tested by:
(Sign & Date)

Checked by:
(Sign & Date)

ANNEXURE - IX

RECORD SHEET FOR OBSERVATION OF BIOLOGICAL INDICATOR (AMPOULE) USED FOR LOEW TEMPERATURE ATEAM STERILIZATION FOR SOLUTION

Detail of Sterilization cycle:

Sterilization Cycle Detail: _____

Date of Sterilization Cycle: _____ A.R.No: _____

Detail of biological indicator:

Name of the Organism: _____ Strain No: _____

Batch No./ Lot No. _____ Manufacturing Date: _____

Expiry Date: _____ Incubation Temperature & Time: _____

Testing Done On: _____ Testing Done By: _____

Incubation Started On: _____ Incubator No.: _____

Location No. ↓ Date →	Observation						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day

STANDARD OPERATING PROCEDURE

Title: Handling of Biological Indicator			
SOP No.:		**Department:**	Microbiology
		Effective Date:	
Revision No.:	00	**Revision Date:**	
Supersede Revision No.:	Nil	**Page No.:**	38 of 39

Location No. ↓ **Date** →	**Observation**						
	1st Day	**2nd Day**	**3rd Day**	**4th Day**	**5th Day**	**6th Day**	**7th Day**
Observation Done By							

Incubation Completed On: _____

Result: Sterililization Cycle is found satisfactory/ not satisfactory.

Key Words:

+ ve = Red/orange colour change to yellow colour.

– ve = Red/orange colour remains Red/orange.

X = Red/orange colour change to reddish brown colour.

Observation Guide for Biological Indicators (Ampoules):

Incubation Conditions		Observation	Conclusion
Exposed indicator incubated at 35°C - 39°C	For a period of 24 to 48 hours or recommended by the manufacture	Red/orange colour remains red/orange	Satisfactory sterilization
Exposed indicator incubated at 35°C - 39°C		Red/orange colour change to yellow colour	Inadequate/faulty sterilization
Exposed indicator incubated at 35°C - 39°C		Red/orange colour change to reddish brown colour	Excessive sterilization
Unexposed indicator incubated at 35°C - 39°C (Positive control)		Red/orange colour change to yellow colour	Organisms present in the biological indicator is viable and satisfactory

Tested by:
(Sign & Date)

Checked by:
(Sign & Date)