STANDARD OPERATING PROCEDURE

Department: Microbiology	**SOP No.:**
Title: Procedure for Validation of Steam Sterilizer	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 Objective

To lay down a procedure for Validation of Steam Sterilizers.

2.0 Scope

This Standard Operating Procedure is applicable for formulation plant.

3.0 Responsibility

Executive /Officer-Microbiology : Shall be responsible for following the Procedure for Validation of Steam Sterilizers as per this SOP.

Head - QC/Designee : Shall be responsible for the compliance of this SOP

4.0 Abbreviations and Definitions

QC : Quality Control

SOP : Standard Operating Procedure

RTD : Resistance Temperature Detectors

D Value : The D Value is the time (in minutes) required to reduce the microbial population by 90% or 1 log cycle (i.e. to a surviving fraction of 1/10)

z Value : The z value is the temperature required for one log reduction in the D value.

F_o Value : The F_o Value is the equivalent time that a monitored article is exposed to the desired temperature, e.g. 121°C for steam sterilization.

5.0 Procedure

Note: Executive/Officer-Microbiology shall inform the external agency selected for validation as per the schedule for performing the validation as per the plan mentioned below.

5.1 Empty Chamber Heat Distribution Studies

5.1.1 Purpose

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure for Validation of Steam Sterilizer	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

To establish the uniformity of temperature distribution in the Steam Sterilizer chamber and identify the location of the "coolest point".

5.1.2 **Test Instrument**

Calibrated temperature data logger with minimum 10 calibrated RTD probes (Resolution ± 0.1 °C)

5.1.3 **Procedure**

5.1.3.1 Conduct the test three times and ensure that it has a cold start each time.

5.1.3.2 Keep the chamber empty for all cycles.

5.1.3.3 Fix minimum10 RTDs in the chamber, through the access port to cover the throughout sterilizer chamber, ensure that one probe should be fix near to the drain point. The reference number allocated to each probe shall be mentioned in reports.

5.1.3.4 Use Teflon tape to secure probes in position. Ensure that the tips do not touch any metallic surface.

5.1.3.5 Attach the probes to the multiple probe temperature data logger.

5.1.3.6 Close the door(s) of Steam Sterilizer/ Autoclave.

5.1.3.7 Set the cycle parameters as established in Cycle development studies. Set the Sterilization Time to 15 / 30 minutes.

5.1.3.8 Set the data logger to display the temperature of all the probes and record the temperature for every one minute interval.

5.1.3.9 Operate the Steam Sterilizer as per SOP titled, Operating Procedure for Steam Sterilizer. Start the sterilization cycle and data logger at the same time.

5.1.3.10 Analyze the results and locate the coolest point, as the position in the proximity of the probe, showing the minimum temperature, during the complete cycle.

5.2 **Loaded Chamber Heat Distribution Studies**

5.2.1 **Purpose**

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure for Validation of Steam Sterilizer	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

To determine the heat distribution at maximum load in sterilizer. To establish that the heat distribution at the "slowest to heat point" of the specific load, at the established sterilization phase cycle time.

5.2.2 **Test Instrument**

Calibrated temperature data logger with minimum 10 No. calibrated RTD probes (Resolution 0.1 °C)

5.2.3 **Procedure**

5.2.3.1 Conduct the test three times and ensure that it has a cold start each time.

5.2.3.2 Keep the chamber loaded with full load for all cycles.

5.2.3.3 Fix minimum10 RTDs in the chamber, through the access port to cover the throughout sterilizer chamber, ensure that one probe should be fix near to the drain point. The reference number allocated to each probe shall be mentioned in reports.

5.2.3.4 Use Teflon tape to secure probes in position. Ensure that the tips do not touch any metallic surface.

5.2.3.5 Attach the probes to the multiple probe temperature data logger.

5.2.3.6 Close the door (s) of Steam Sterilizer/ Autoclave.

5.2.3.7 Set the cycle parameters as established in Cycle development studies. Set the Sterilization time to 15/30 minutes.

5.2.3.8 Set the data logger to display the temperature of all the probes, and record the temperature after every one minute interval.

5.2.3.9 Operate the Steam Sterilizer/ Autoclave as per SOP titled "Operating Procedure for Autoclave"/ "Operating Procedure for Steam Sterilizer". Start the sterilization cycle and data logger at the same time.

5.2.3.10 Analyze the results and locate the coolest point, as the position in the proximity of the probe, showing the minimum temperature, during the complete cycle.

5.3 **Loaded Chamber Heat Penetration Study**

5.3.1 **Purpose**

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure for Validation of Steam Sterilizer	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

To determine the heat distribution at maximum load in sterilizer to establish that the steam penetration at the "slowest to heat point" of the specific load, at the established sterilization phase cycle time, meets the specified F_o Value (NLT 12 min).

5.3.2 **Test Instrument**

Calibrated temperature data logger with minimum 10 No. calibrated RTD probes (Resolution ± 0.1 °C)

5.3.3 **Procedure**

5.3.3.1 Carry out the test using the maximum load intended to be sterilised using the Steam Sterilizer. Each experiment shall have a cold start each time.

5.3.3.2 Set the operation values of parameters as established during cycle development studies and the Sterilization Time as 15/30 minutes.

5.3.3.3 Load the Steam Sterilizer Chamber and record the Load pattern diagram in report.

5.3.3.4 Dip the RTDs in Medium or place such that the sensor is in contact with the material to be sterilised. Use Teflon tape to secure RTD probes in position.

5.3.3.5 The mapping of probes should be done to ensure proper representation of the full load. Allocate a reference number to each probe and state in the diagram.

5.3.3.6 Attach the RTD probes to the multiple probe data logger.

5.3.3.7 Close the doors of Steam Sterilizer/Autoclave.

5.3.3.8 Set the data logger to display the temperature of all the probes, after every 1 minute and record the same manually.

5.3.3.9 Operate the Steam Sterilizer/ Autoclave as per SOP titled Operating Procedure for Steam Sterilizer. Start the sterilization cycle and data logger at the same time.

5.3.3.10 Record the temperature of all the probes.

5.3.3.11 Record and analyze the results to locate the slowest to heat point as the position in the proximity of the RTD, showing the minimum temperature, during the cycle.

5.3.3.12 Calculate the F_o value at the "slowest to heat" point, using the following formula:

$$F_o = \frac{t \times (T_o - T_r)}{z}$$

Where t = time interval of temperature measurement (1 minutes here)

T_o = exposure temperature at any given instant

T_r = process reference temperature (121.5/121 ° C)

z = A resistance value of 10 ° C assumed for the Steam Sterilizer

5.3.3.13 Repeat the experiment three times to ensure reproducibility of location of slowest to heat point, and F_o Value at coolest point, in the specific load.

5.3.3.14 On the basis of the results of the calculated F_o Value increase or decrease the Sterilization Cycle Time to achieve a desired Fo Value.

5.3.3.15 Repeat the experiment three times to assure the reproducibility of F_o value at coolest point in the steam sterilizer chamber.

5.4 Suitability by Heat labile physical indicator

5.4.1 Purpose

To establish an assurance level for completion of sterilization using chemical indicators.

5.4.2 Indicator used

OK (Proper sterilization strip) or Steam Clox Indicator

5.4.3 Procedure

5.4.3.1 Place strip of OK (Proper sterilization) / Steam Clox Indicator in the chamber of autoclave.

5.4.3.2 Run Steam Sterilizer/Autoclave at 121.5/121ºC and 15 lbs pressure as per SOP.

5.4.3.3 After completion of sterilization cycle remove the physical indicators from the autoclave.

5.4.3.4 Observe the colour change in the indicator the colour should change from pink to green in Steam Clox and from cream to black in Proper Sterilization OK indicator.

5.4.3.5 Attach the indicator with the record after check and write the date along with checked by sign.

5.5 **Biological Challenge Test**

5.5.1 **Purpose**

To study the destruction of a resistant microbial challenge by a Moist Heat Cycle, at the Sterilization Cycle Time established by F_o studies.

5.5.2 **Test Spores**

Prospore vials of *Geobacillus stearothermophilus*

5.5.3 **Procedure**

5.5.3.1 Carry out the test using the loads for which F_o (thermal) has been established.

5.5.3.2 Place four vials of self contained *Geobacillus stearothermophillus* at four different locations by covering the coolest locations like drain point and put stickers on each indicating date and time of validation, equipment ID and location of ampoule inside the Autoclave.

5.5.3.3 Run a complete Steam Sterilizer cycle at the cycle parameters established during F_o studies for the particular load.

5.5.3.4 After completion of the sterilization cycle remove biological indicator from the Autoclave.

5.5.3.5 Retain one vial without sterilization as positive controls.

5.5.3.6 Cool the ampoules to about 60°C and incubate all the vials at 55 to 60°C for 48 hours.

5.5.3.7 Sterilization cycle is considered to be perfect complete and validated if the colour change in the vials are as follows up to the completion of incubation period-

Sterilized vials - Clear and Purple in Colour

Unsterilized Vials - Turbid and yellow in Colour

5.5.3.8 Enter all the observations in the format maintained for calibration of equipment as Annexure-1.

5.6 **Steam Penetration Study by Bowie-Dick Test**

5.6.1 **Purpose**

To study of steam penetration evenly in steam sterilizer chamber.

5.6.2 **Test Kit Used**

Bowie-Dick type test pack

5.6.3 **Procedure**

5.6.3.1 Keep the Bowie-Dick type test pack in the lower shelf of steam sterilizer near drain on SS stand set the Bowie- Dick cycle for 8 minutes.

5.6.3.2 Run the cycle as per the SOP for operation of steam Sterilizer for 8 minutes.

5.6.3.3 After completion of cycle remove the test pack from chamber, remove the cover and paper sheets around test paper.

5.6.3.4 Observe the Bowie- Dick test paper for even colour change of the indicator from yellow to blue which is distributed on test paper.

5.6.3.5 Record the result in Annexure-2 and attach this test paper with Annexure.

5.6.3.6 The test should be considered satisfactory if a uniform colour change throughout the indicator.

5.6.3.7 If there is a failure check the previous load and the previous results.

5.7 **Frequency of calibration:**

Source	Parameter	Standard	Acceptance Criteria	Frequency
External	Heat distribution	Calibrated Thermocouple	± 3.0º C	Yearly
External	Heat Penetration	Calibrated Thermocouple	± 3.0º C	Yearly

STANDARD OPERATING PROCEDURE

Department: Microbiology	**SOP No.:**
Title: Procedure for Validation of Steam Sterilizer	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

In House	Physical Indicator	OK/ Steam Clox	Colour change	Every Cycle
In House	Biological Challenge Test	*Geobacillus stearothermophilus* Prospore vials	No turbidity after 48 hrs. incubation	Half Yearly
In House	Bowie-Dick Test	3M/ Any Standard Company	Colour Change	Half Yearly

5.8 **Documentation:**

5.8.1 Outside agency shall submit a detailed report for validation of autoclave containing all data of heat distribution and heat penetration studies.

5.8.2 Microbiologist shall fill the details of calibration status on the calibration tag pasted on the equipment.

5.8.3 Report any discrepancy observed during calibration to Head - Quality Control and notify the defect to the maintenance department or service engineer of the equipment and Affix an Under Maintenance label on the equipment.

6.0 Forms and Records

6.1 Validation Report of steam sterilizer by Biological Indicator : Annexure-I

6.2 Result of Bowie-Dick Type test Kit : Annexure-II

7.0 Distribution

7.1 Master Copy : Documentation Cell (Quality Assurance)

7.2 Controlled Copies : Quality Control, Quality Assurance

8.0 History

Date	Revision Number	Reason for Revision

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	**SOP No.:**
Title: Procedure for Validation of Steam Sterilizer	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE I

VALIDATION REPORT OF STEAM STERLIZER BY BIOLOGICAL INDICATOR

Validation Report No. :

Equipment Name :	**Indicator Used :**
Equipment ID :	**Lot No. :**
Location :	**Exp Date :**
Sterilization Parameter :	**Z-value :**
Date of Incubation :	**D-value :**
Date of Observation :	**Survives Time :**
Incubation Temperature:	**Killed Time :**
Load Pattern :	**Prospore Count :**
	Mfg By :

S.No.	**Location Details**	**Observation**		
		Date		
1.				
2.				
3.				
4.				
5.	+ve Control			

Note: 1. Test Ampoule shows Turbidity/Change of colour to Yellow - **Test Invalid**
2. Positive control Ampoule shows Turbidity/Change of colour to Yellow -**Test Valid**
3. Test Ampoule retains its Purple colour/No Turbidity -**Test Valid**

Remarks: The Sterilizations Cycle is OK / Not OK.

MICROBIOLOGIST
(Date/Sign)

CHECKED BY
(Date/Sign)

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure for Validation of Steam Sterilizer	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE II

RESULTS OF BOWIE-DICK TYPE TEST KIT

Date of Sterilization		Ster.-Hold Time	: 8 minutes
Sterilization Cycle No.	:	Autoclave ID No.	:
BOWIE-DICK TYPE TEST KIT			
Manufacturer	:	Mfg date	:
Lot Number	:	Exp. date	:

Observation :

Conclusion: Steam penetration is **SATISFACTORY / NOT SATISFACTORY**

Acceptance Criteria: The colour of Bowie-Dick test paper should be changed from yellow to blue/ black evenly.