PERFORMANCE QUALIFICATION PROTOCOL FOR SUPER HEATED WATER SPRAY STERILIZER

EQUIPMENT ID No.	
LOCATION	**LOADING AREA**
DATE OF QUALIFICATION	
SUPERSEDE PROTOCOL No.	**NIL**

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PROTOCOL CONTENTS

PERFORMANCE QUALIFICATION PROTOCOL FOR SUPER HEATED WATER SPRAY STERILIZER

1.0 PROTOCOL APPROVAL:

PREPARED BY:

DESIGNATION	NAME	SIGNATURE	DATE
OFFICER / EXECUTIVE (QUALITY ASSURANCE)			

REVIEWED BY:

DESIGNATION	NAME	SIGNATURE	DATE
OPERATING MANAGER (QUALITY ASSURANCE)			
HEAD (ENGINEERING			
HEAD (PRODUCTION)			

APPROVED BY:

DESIGNATION	NAME	SIGNATURE	DATE
HEAD (QUALITY ASSURANCE)			

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2.0 OBJECTIVE:

- This protocol is designed to establish & provide documentary evidence, to assure that the **"Super Heated Water Spray Sterilizer"** supplied by **M/s Machin Fabrik** is suitable for sterilizing the Loaded Material.
- In addition, this validation study is intended to assure the sterility of the Loaded Material, when the equipment is operated in accordance with the established standard operating procedure to maintain reliability and repeatability.
- To confirm the suitability of the Standard Operating Procedures for all routine activities associated with the system.

3.0 SCOPE:

- The Protocol covers all aspects of Performance Qualification for the Super Heated Water Spray Sterilizer (PC/LB/SHS-001) installed in the LVP Line.

4.0 RESPONSIBILITY:

The Validation Group, comprising of a representative from each of the following Departments, shall be responsible for overall compliance of this Protocol:

DEPARTMENTS	RESPONSIBILITIES
Quality Assurance	• Preparation, Approval and Compilation of the Performance Qualification Protocol. • Protocol Training. • Co-ordination with Quality Control, Production and Engineering to carryout Performance Qualification activity. • Monitoring of Performance Qualification.
Quality Control	• Review of Performance Qualification Protocol. • Analytical Support (Microbiological Testing / Analysis)
Production	• Review of Performance Qualification Protocol. • To co-ordinate and support Performance Qualification activity.
Engineering	• Review of Performance Qualification Protocol. • To co-ordinate and support Validation activity. • Responsible for Trouble shooting during execution (If occurs).

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5.0 EQUIPMENT DETAILS:

Equipment Name	**Super Heated Water Spray Sterilizer**
Equipment ID No.	
Size	**1750 Dia X 4500 LG mm**
Chamber Volume	**10800 Liters**
Manufacturer's Name	**M/s Machin Fabrik**
Place of Installation	**LVP Line**

6.0 SYSTEM DESCRIPTION:

- The Super Heated Water Spray Sterilizer is an unique Sterilization System make by Machin Fabrik and it is used to perform the sterilization of LDPE Bottles and LDPE Ampoules by heating water above 100.0^{o}C and still maintaining it in liquid phase.

6.1 STERILIZATION MECHANISM:

- Steam is introduced in the tube side of the heat exchanger.
- The chamber is pressurized gradually by introducing compressed air.
- As the temperature of water in the chamber increases and reaches the sterilization temperature, the control system in place controls this temperature for the Sterilization period.
- When the sterilization hold period is over, the circulating water is cooled by introducing cooling water through the tubes of the heat exchanger.
- When the chamber temperature reaches at set point, the sterilized material is then unloaded in the unloading side.
- The Super Heated Water Spray Sterilizer process consists of following phases: -
- Heat up
- Sterilization hold
- Cooling

PERFORMANCE QUALIFICATION PROTOCOL FOR SUPER HEATED WATER SPRAY STERILIZER

7.0 REASON FOR QUALIFICATION:

- Installation of New System.
- Any major modification in the existing system. □
- Change of Location. □
- Periodic requalification
- **(Specific Reason)** ----------

8.0 SITE OF STUDY:

L-Block, LVP Line.

9.0 FREQUENCY OF QUALIFICATION :

- Half Yearly

10.0 PRE-QUALIFICATION REQUIREMENTS :

10.1 TEST EQUIPMENT:

S.No.	Test Instrument
1.0	Duly Calibrated Data logger with Calibrated PT-100 sensors.
2.0	Biological Indicator 10^6spore i.e. *Bacillus Subtilis* must be checked for spore population
3.0	All parts of Sterilizer used for measurement like temp. sensors, pressure gauges, timers must be calibrated

10.2 TEST EQUIPMENT CALIBRATION:

Review the calibration status for the test equipment (Data Logger with PT-100 sensors) to be utilized. All Equipment / Instrumentation must remain within the Calibration due date for the duration of Validation Study for which the item is used. If a due date potentially occurs during the testing period then the instrument must be recalibrated before it is utilized. Record the Calibration details in Performance Qualification Report.

10.3 CALIBRATION OF TEMPERATURE SENSORS:

- **Pre & Post calibration of Temperature Sensors**

  Pre & Post calibration shall be carried out before starting and after completion of Heat Penetration Cycle.

10.4 PRE & POST CALIBRATION OF TEMPERATURE SENSORS:

A) PROCEDURE:

- Temperature sensors which are to be used for Performance Qualification study shall be qualified in oil bath at 40°C, 80°C, 108°C & 120°C prior to its usage in the qualification.
- Record the Temperature of all the sensors while putting it in oil bath & also record the reading in calibrated thermometer. Record the data for at least five minutes after putting all the sensors to the oil bath there by allowing the temperature to stabilize.
- Record the data for Five Minutes by data logger and attach the print out with report.
- Put individual sensor to the slot of High Temperature reference block which is stabilized at different set of temperature. Record the readings of thermometer & sensor after at least one minute after stabilization of temperature.

  Note: Sensor no. 27 to be used as master sensor to monitor the temperature of oil bath (it is spare as 26 sensor are used in Qualification).
- Record the Temperature for Five Minutes by data logger and attach the print out with report.

B) ACCEPTANCE CRITERIA:

- Temperature should not be fluctuating ± 0.5 °C in oil bath from the calibrated thermometer and Temperature Sensors during the data-logging period.

C) TRAINING OF EXECUTION TEAM:

Provide the training to a team for the execution of protocol before execution of the same. Record of training shall be recorded in Performance Qualification Report.

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11.0 TESTS & CHECKS:

The Super Heated Water Spray Sterilizer shall be considered Qualified on successful completion of the following tests for 100 ml LDPE bottles:

- **Empty Chamber** Heat Distribution Studies (3 cycle) in empty chamber.
- **Minimum Loaded Chamber** Heat Penetration Studies (3 cycle) for each sterilization load of Fixed Loading Pattern.
- **Maximum Loaded Chamber** Heat Penetration Studies (3 cycle) for each sterilization load of Fixed Loading Pattern.
- Bio-challenge studies using *Bacillus Subtilis* Spore Ampoule (containing 10^6 or more spore) during the heat penetration studies.
- Estimation of the F_0 value achieved during the sterilization hold period at each temperature mapping probe.
- Estimation of Desired & Actual Spore Log Reduction after each Loaded Heat Penetration cycle at 108.0°C.
- Minimum Loaded Chamber Bottle Leakage Test (3 cycle) for each of Fixed Loading Pattern.
- Maximum Loaded Chamber Bottle Leakage Test (3 cycle) for each of Fixed Loading Pattern.

To qualify these tests, the equipment should be fulfill the acceptance criteria described in the individual test procedures.

Load Pattern:

1. Heat Distribution Study. (Empty Chamber) (3Cycle)
2. Heat Penetration Study Minimum Load (3 Cycle) {1 Trolley (3960 Bottles)}.
3. Heat Penetration Study Maximum Load (3 Cycle) {4 Trolley (15840 Bottles)}.
4. Bottle Leakage Test Minimum Load (3 Cycle) {1 Trolley (3960 Bottles)}.
5. Bottle Leakage Test Maximum Load (3 Cycle) {4 Trolley (15840 Bottles)}.

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11.1 EMPTY CHAMBER HEAT DISTRIBUTION STUDY

11.1.0 Objective

- To verify Temperature Uniformity throughout the Chamber.
- The sterilizer is capable of attaining a temperature of 108.0°C during the sterilization hold.
- To verify that at any location(s) where the probes are placed, achieving Minimum Sterilization Temperature 108.0°C throughout the Sterilization Temperature Hold will be considered as Cold Spot.

11.1.1 EQUIPMENT / INSTRUMENT USED:

- Duly Calibrated Data logger with calibrated PT-100 sensors.

11.1.2 PROCEDURE:

- Conduct the study with empty chamber cycles with temperature probes and Biological Indicators.
- Transfer the load to sterilizer and connect the 27 probes as per the locations defined in figure.
- Connect the outputs of all the probes to the temperature data logger and close the door of sterilizer.
- Switch ON the MAINS of the control panel and set the parameters –

Parameter	Purpose	Set value
Add water in	For Proper Circulation	30 Sec.
Initial H/E Exhaust	To achieve effective heat distribution	03 min
Set Point 1	----------	95.0^{0}C
Set Point 2	----------	100.0^{0}C
Set Point 3	----------	105.0^{0}C
Rate 1	----------	5.0^{0}C
Rate 2	----------	4.0^{0}C

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Parameter	Purpose	Set value
Rate 3	----------	2.0^{0}C
Sterilization Temperature	Sterilization	108.0 ^{0}C
Sterilization Time	To hold the sterilization period as per the set time	60 min.
Control Band	To control max. & min. level of temperature during sterilization period	0.2 ^{0}C
Overshoot Temperature	To indicate through the alarm when there is excess temp. In the chamber during sterilization hold period.	110.0 ^{0}C
Sterilization Stop Temperature	To stop sterilization hold time in case the chamber temperature falls	107.5 ^{0}C
Sterilization Reset Temperature	To reset the sterilization hold time in case the chamber temperature falls below this value during sterilization period.	107.0 ^{0}C
H/E Exhaust Delay Time	To stabilize the temperature	3 min.
H/E Cooling Exhaust	To exhaust the temperature from chamber & to cool down the water.	3 min.
Slow Cooling & Temperature	To stabilize the cooling & temperature	85 ^{0}C
Cooling End Temperature	To stop the cooling	50 ^{0}C
H/E Drain Time	To remove the water from chamber	5 min.
Process End Pressure	To end the process & allow to unload the material	0.030 Bar

- Simultaneously Insert new chart in chart recorder provided on the control panel of Super Heated Water Spray Sterilizer and adjust the start time and temperature of the instrument.
- Now start the cycle as per SOP for Operating Instruction.
- After attaining temperature 108.0°C, record the chamber temperature for every minute.
- Simultaneously start the recording with data logger and take printouts.
- At the end of the cycle Switch OFF the cycle.
- When pressure becomes 0.00 BAR, open the door with the help of safety gloves.
- Remove the load.

PERFORMANCE QUALIFICATION PROTOCOL FOR SUPER HEATED WATER SPRAY STERILIZER

Location of Sensors inside the Empty Chamber

SENSOR No.	LOCATION IN THE CHAMBER
S1	Outlet of Heat Exchanger with inbuilt sensor T1
S2	Middle of the left side near loading area door with inbuilt sensor T2
S3	Middle of the left side near sump side with inbuilt sensor T3
S4	Middle of the left side near unloading area door with inbuilt sensor T4
S5	Middle of the right side near unloading area door with inbuilt sensor T5
S6	Middle of the right side near loading area door with inbuilt sensor T6
S7	In the drain of the autoclave chamber. T7
S8	Middle of Left side first tray of first trolley
S9	Middle of Right side second tray of first trolley
S10	Middle of Left side third tray of first trolley
S11	Middle of Left side fifth tray of first trolley
S12	Middle of Right side sixth tray of first trolley
S13	Middle of Left side first tray of second trolley
S14	Middle of Right side second tray of second trolley
S15	Middle of Left side third tray of second trolley
S16	Middle of Left side fifth tray of second trolley
S17	Middle of Right side sixth tray of second trolley
S18	Middle of Left side first tray of third trolley
S19	Middle of Right side second tray of third trolley
S20	Middle of Left side third tray of third trolley
S21	Middle of Left side fifth tray of third trolley
S22	Middle of Right side sixth tray of third trolley
S23	Middle of Left side first tray of fourth trolley
S24	Middle of Right side second tray of fourth trolley
S25	Middle of Left side third tray of fourth trolley
S26	Middle of Left side fifth tray of fourth trolley
S27	Middle of Right side sixth tray of fourth trolley

PERFORMANCE QUALIFICATION PROTOCOL FOR SUPER HEATED WATER SPRAY STERILIZER

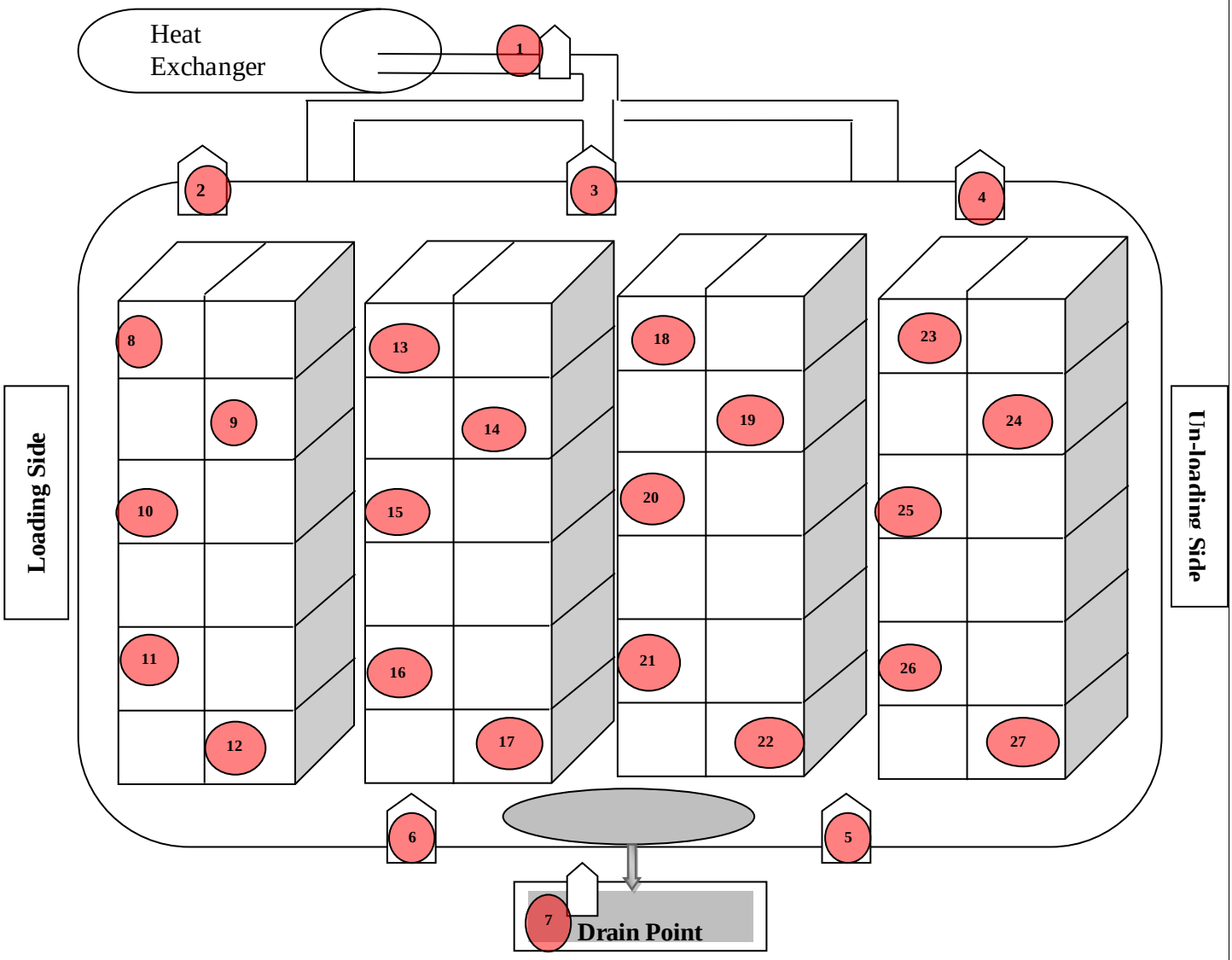

Figure 1: Location of temperature sensors inside the Empty chamber

In Built Sensor → ⬠

External Sensor → ●

11.5.3 ACCEPTANCE CRITERIA:

- There should be uniform distribution of temperature within the range of **108.0°C to 109.5°C** at each probe in sterilizer chamber during sterilization hold period.

11.5.4 OBSERVATIONS :

Record the Observations in Performance Qualification Report.

11.5.5 EVALUATION OF RESULT

- Heat Distribution Test shall be qualified if distribution of heat in the sterilizer chamber during the sterilization hold period is uniform and Temperature uniformity at a given time of Temperature recording between all probes during hold Period is found within ± 1°C.
- If Temperature Uniformity is not achieved, check whether sensor is touching any metal surface.
- After taking proper corrective measures, Heat Distribution shall be re-performed.

11.6 HEAT PENETRATION STUDIES

11.6.0 Objective

- To ensure that the heat is sufficiently penetrating into the innermost portions of the load subjected for Sterilization to achieve desired Temperature of 108.0°C during the whole Sterilization Hold Period.
- To ensure that if Sterilization Temperature (108.0°C) is not achieved throughout the cycle, load configuration or size of the load has to be reviewed and Sterilization Cycles to be repeated.
- To ensure that temperature spread within the range of 108.0°C to 109.5°C during Sterilization Hold Period indicate that, Uniform Heating Process which is achieved in the Empty Chamber Heat Distribution Study is not affected by load. There could be the possibility of lag period for attaining 108.0°C during heat penetration trials as the probes are placed deep into the load.
- Heat Penetration studies shall be carried out with the following different loads:

  Heat Penetration studies.
- Minimum 100 ml LDPE Bottles {1 Trolley (3960 Bottles)}.
- Maximum 100 ml LDPE Bottles {4 Trolley (15840 Bottles)}

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11.7 HEAT PENETRATION STUDY (100ml Bottles Loaded Chamber) Minimum Load:

A) Load Details:

For 100 ml LDPE Bottles: 3960 bottles.

- All trays of one trolley are fully loaded.

B) Load Configuration:

Sensor No.	Location in the Chamber	Justification for Location Selection
1	Outlet of Heat Exchanger with inbuilt sensor T1	To verify the uniform temperature penetration at different location (Top, middle & bottom of trolley) with covers left corners, right corners and center of trolleys in the sterilizer.
2	Middle of the left side near loading area door with inbuilt sensor T2	
3	Middle of the left side near sump side with inbuilt sensor T3	
4	Middle of the left side near unloading area door with inbuilt sensor T4	
5	Middle of the right side near unloading area door with inbuilt sensor T5	
6	Middle of the right side near loading area door with inbuilt sensor T6	
7	In the drain of the autoclave chamber. T7	
8	Inside the 100 ml bottle (with BI), Middle of Left side first tray of first trolley	
9	Inside the 100 ml bottle (with BI), Middle of Right side second tray of first trolley	

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Sensor No.	Location in the Chamber	Justification for Location Selection
10	Inside the 100 ml bottle (with BI), Middle of Left side third tray of first trolley	
11	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of first trolley	
12	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of first trolley	

C) Equipment / Instrument Used:

- Duly Calibrated Data logger with calibrated sensors.
- Biological Indicators

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D) Procedure

- Conduct the study with Loaded Chamber Cycles with Temperature Probes and Biological Indicators.
- Biological indicator shall be qualified for its purity & population as per SOP.
- Transfer the load to sterilizer and connect the probes as per the given locations.
- Connect the outputs of all the probes to the temperature data logger and close the door of sterilizer.
- Set the parameters on PLC.

Set Parameters:

Parameter	Purpose	Set value
Add water in	For Proper Circulation	30 Sec.
Initial H/E Exhaust	To achieve effective heat distribution	03 min
Set Point 1	----------	95.0^{0}C
Set Point 2	----------	100.0^{0}C
Set Point 3	----------	105.0^{0}C
Rate 1	----------	5.0^{0}C
Rate 2	----------	4.0^{0}C
Rate 3	----------	2.0^{0}C
Sterilization Temperature	Sterilization	108.0 ^{0}C
Sterilization Time	To hold the sterilization period as per the set time	60 min.
Control Band	To control max. & min. level of temperature during sterilization period	0.2 ^{0}C
Overshoot Temperature	To indicate through the alarm when there is excess temp. In the chamber during sterilization hold period.	110.0 ^{0}C
Sterilization Stop Temperature	To stop sterilization hold time in case the chamber temperature falls	107.5 ^{0}C
Sterilization Reset Temperature	To reset the sterilization hold time in case the chamber temperature falls below this value during sterilization period.	107.0 ^{0}C

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H/E Exhaust Delay Time	To stabilize the temperature	3 min.
H/E Cooling Exhaust	To exhaust the temperature from chamber & to cool down the water.	3 min.
Slow Cooling & Temperature	To stabilize the cooling & temperature	85 ^{0}C
Cooling End Temperature	To stop the cooling	50 ^{0}C
H/E Drain Time	To remove the water from chamber	5 min.
Process End Pressure	To end the process & allow to unload the material	0.030 Bar

Simultaneously insert new chart in chart recorder provided on the control panel of Steam Sterilizer and adjust the start time and temperature of the instrument.

- Run the cycle as per SOP.
- After attaining temperature 108.0°C, record the chamber temperature and pressure for every minute.
- Simultaneously start the recording with data logger and take the printouts. At the end of the cycle Switch Off.
- When Pressure becomes 0.030 Bar, open the door & remove biological indicator from the Load and send to microbiology for incubation.
- Incubate BI as per SOP on "Exposure and incubation of Biological Indicators". Record observations in the respective format of the SOP.
- Each autoclaved Biological indicator should give the '-ve' result after the incubation.
- Compile data for each loaded cycle in corresponding in Performance Qualification Report.
- Finally calculate the F_0 value & Spore Log Reduction in heat penetration study of each load.
- Finally calculate the F_0 value for heat penetration study.

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Location of Sensors in the chamber

SENSOR No.	LOCATION IN THE CHAMBER
S1	Outlet of Heat Exchanger with inbuilt sensor T1
S2	Middle of the left side near loading area door with inbuilt sensor T2
S3	Middle of the left side near sump side with inbuilt sensor T3
S4	Middle of the left side near unloading area door with inbuilt sensor T4
S5	Middle of the right side near unloading area door with inbuilt sensor T5
S6	Middle of the right side near loading area door with inbuilt sensor T6
S7	In the drain of the autoclave chamber. T7
S8	Inside the 100 ml bottle (with BI), Middle of Left side 1 tray of first trolley
S9	Inside the 100 ml bottle (with BI), Middle of Right side 4 tray of first trolley
S10	Inside the 100 ml bottle (with BI), Middle of Left side 7 tray of first trolley
S11	Inside the 100 ml bottle (with BI), Middle of Left side 10 tray of first trolley
S12	Inside the 100 ml bottle (with BI), Middle of Right side 12 tray of first trolley

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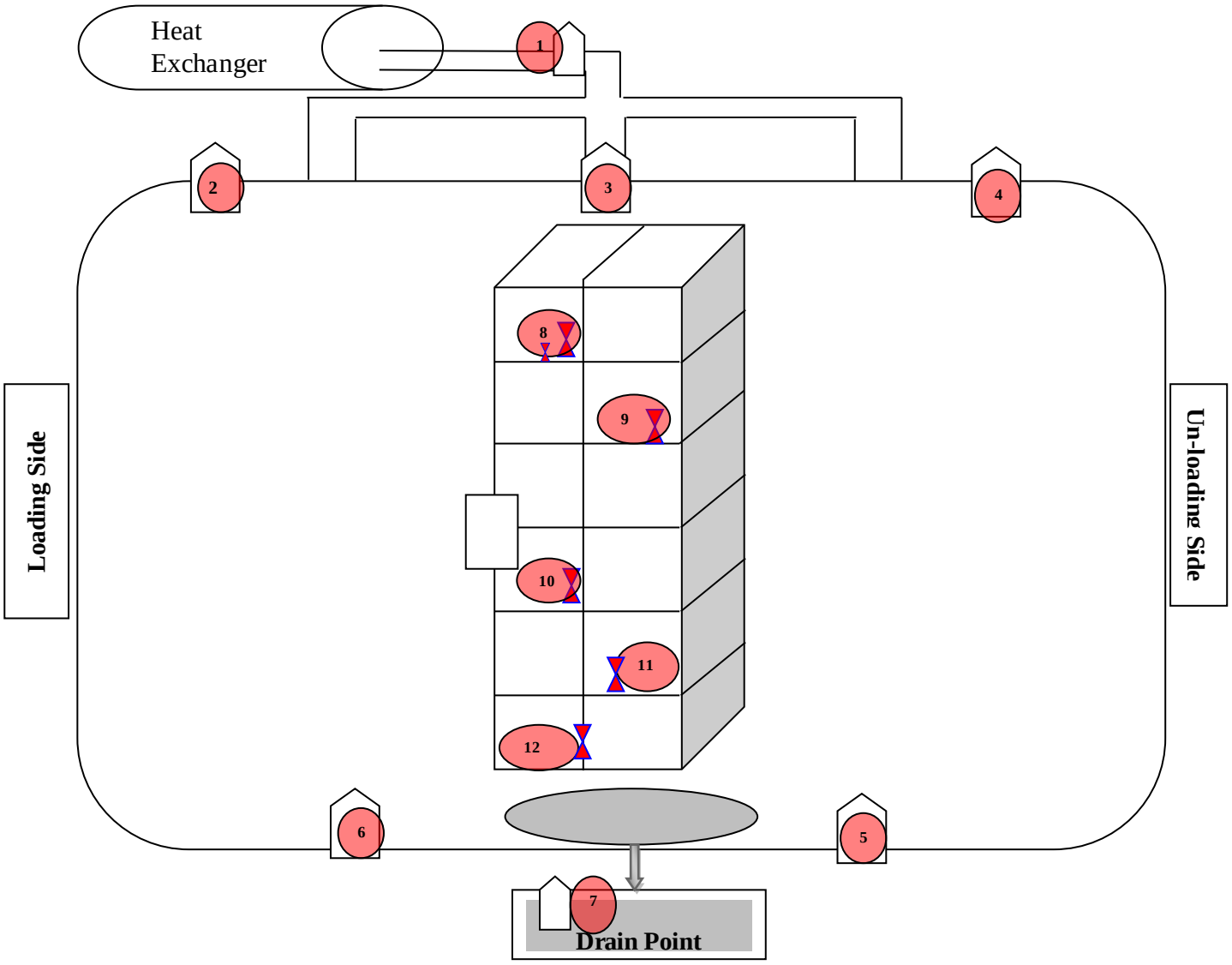

Figure 1: Location of temperature sensors & BI inside the Minimum Load

E) Acceptance Criteria

- There should be uniform distribution of heat in the Sterilizer Chamber during the Sterilization Hold Period and the Temperature at each Temperature Mapping Probe should be within the range of 108.0°C to 109.5°C during the Sterilization Hold Period.
- The chemical indicator of each loaded trolley should change the color from Purple to Gray.
- The calculated Minimum F_0 value should be more than biological F_0 value for the Biological Indicator.
- Each autoclaved Biological indicator should give '-ve' results after the incubation
- SLR **Actual** should be more than SLR **Desired**.

F) Observation :

Record the observations in Performance Qualification Report.

G) Evaluation of Result

Heat Penetration Test shall be qualified if distribution of heat in the Super Heated Water Spray Sterilizer chamber during the sterilization hold period is uniform and Temperature uniformity at a given time of Temperature recording between all probes during hold is found within ± 1°C. If Temperature Uniformity is not achieved, check whether sensor is touching any metal surface. After taking proper corrective measures, Heat Penetration shall be re-performed.

11.8 HEAT PENETRATION STUDY (100ml BOTTLES LOADED CHAMBER) (MAXIMUM LOAD):

A) Load Details:

For 100 ml LDPE Bottles: 15840 bottles.

- All trays of all trolleys are fully loaded.

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B) Load Configuration:

Sensor No.	Location in the Chamber	Justification for Location Selection
1	Outlet of Heat Exchanger with inbuilt sensor T1	This is the location for the reference measurement point of the sterilizer, which controls the sterilization cycle. Hence important to compare the achieved temperature distribution results (Print out of sterilizer) with the results from data logger.
2	Middle of the left side near loading area door with inbuilt sensor T2	
3	Middle of the left side near sump side with inbuilt sensor T3	
4	Middle of the left side near unloading area door with inbuilt sensor T4	
5	Middle of the right side near unloading area door with inbuilt sensor T5	
6	Middle of the right side near loading area door with inbuilt sensor T6	
7	In the drain of the autoclave chamber. T7	
8	Inside the 100 ml bottle (with BI), Middle of Left side first tray of first trolley	To verify the uniform temperature penetration at different location (Top, middle & bottom of trolley) with covers left corners, right corners and center of trolleys in the sterilizer.
9	Inside the 100 ml bottle (with BI), Middle of Right side second tray of first trolley	
10	Inside the 100 ml bottle (with BI), Middle of Left side third tray of first trolley	
11	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of first trolley	
12	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of first trolley	
13	Inside the 100 ml bottle (with BI), Middle of Left side first tray of second trolley	
14	Inside the 100 ml bottle (with BI), Middle of Right side second tray of second trolley	
15	Inside the 100 ml bottle (with BI), Middle of Left side third tray of second trolley	
16	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of second trolley	
17	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of second trolley	
18	Inside the 100 ml bottle (with BI), Middle of Left side first tray of third trolley	
19	Inside the 100 ml bottle (with BI), Middle of Right side second tray of third trolley	
20	Inside the 100 ml bottle (with BI), Middle of Left side third tray of third trolley	
21	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of third trolley	
22	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of third trolley	
23	Inside the 100 ml bottle (with BI), Middle of Left	

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Sensor No.	Location in the Chamber	Justification for Location Selection
	side first tray of fourth trolley	
24	Inside the 100 ml bottle (with BI), Middle of Right side second tray of fourth trolley	
25	Inside the 100 ml bottle (with BI), Middle of Left side third tray of fourth trolley	
26	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of fourth trolley	
27	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of fourth trolley	

C) Equipment / Instrument Used:

- Duly Calibrated Data logger with calibrated sensors.
- Biological Indicators

D) Procedure

- Conduct the study with Loaded Chamber Cycles with Temperature Probes and Biological Indicators.
- Biological indicator shall be qualified for its purity & population as per SOP.
- Transfer the load to sterilizer and connect the probes as per the given locations.
- Connect the outputs of all the probes to the temperature data logger and close the door of sterilizer.
- Set the parameters on PLC.

Set Parameters

Parameter	Purpose	Set value
Add water in	For Proper Circulation	30 Sec.
Initial H/E Exhaust	To achieve effective heat distribution	03 min
Set Point 1	----------	95.0^{0}C
Set Point 2	----------	100.0^{0}C
Set Point 3	----------	105.0^{0}C
Rate 1	----------	5.0^{0}C

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Parameter	Purpose	Set value
Rate 2	----------	4.0^{0}C
Rate 3	----------	2.0^{0}C
Sterilization Temperature	Sterilization	108.0 ^{0}C
Sterilization Time	To hold the sterilization period as per the set time	60 min.
Control Band	To control max. & min. level of temperature during sterilization period	0.2 ^{0}C
Overshoot Temperature	To indicate through the alarm when there is excess temp. In the chamber during sterilization hold period.	110.0 ^{0}C
Sterilization Stop Temperature	To stop sterilization hold time in case the chamber temperature falls	107.5 ^{0}C
Sterilization Reset Temperature	To reset the sterilization hold time in case the chamber temperature falls below this value during sterilization period.	107.0 ^{0}C
H/E Exhaust Delay Time	To stabilize the temperature	3 min.
H/E Cooling Exhaust	To exhaust the temperature from chamber & to cool down the water.	3 min.
Slow Cooling & Temperature	To stabilize the cooling & temperature	85 ^{0}C
Cooling End Temperature	To stop the cooling	50 ^{0}C
H/E Drain Time	To remove the water from chamber	5 min.
Process End Pressure	To end the process & allow to unload the material	0.030 Bar
Print Interval	For proper monitoring	60 sec.

Simultaneously insert new chart in chart recorder provided on the control panel of Steam Sterilizer and adjust the start time and temperature of the instrument.

- Run the cycle as per SOP.
- After attaining temperature 108.0°C, record the chamber temperature and pressure for every minute.
- Simultaneously start the recording with data logger and take the printouts. At the end of the cycle Switch Off.
- When Pressure becomes 0.030 Bar, open the door & remove biological indicator from the Load and send to microbiology for incubation.

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- Incubate BI as per SOP on "Exposure and incubation of Biological Indicators". Record observations in the respective format of the SOP.
- Each autoclaved Biological indicator should give the '-ve' result after the incubation.
- Compile data for each loaded cycle in corresponding in Performance Qualification Report.
- Finally calculate the F_0 value & Spore Log Reduction in heat penetration study of each load.
- Finally calculate the F_0 value for heat penetration study.

Location of Sensors in the chamber

SENSOR No.	LOCATION IN THE CHAMBER
S1	Outlet of Heat Exchanger with inbuilt sensor T1
S2	Middle of the left side near loading area door with inbuilt sensor T2
S3	Middle of the left side near sump side with inbuilt sensor T3
S4	Middle of the left side near unloading area door with inbuilt sensor T4
S5	Middle of the right side near unloading area door with inbuilt sensor T5
S6	Middle of the right side near loading area door with inbuilt sensor T6
S7	In the drain of the autoclave chamber. T7
S8	Inside the 100 ml bottle (with BI), Middle of Left side first tray of first trolley
S9	Inside the 100 ml bottle (with BI), Middle of Right side second tray of first trolley
S10	Inside the 100 ml bottle (with BI), Middle of Left side third tray of first trolley
S11	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of first trolley
S12	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of first trolley
S13	Inside the 100 ml bottle (with BI), Middle of Left side first tray of second trolley
S14	Inside the 100 ml bottle (with BI), Middle of Right side second tray of second trolley
S15	Inside the 100 ml bottle (with BI), Middle of Left side third tray of second trolley
S16	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of second trolley
S17	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of second trolley
S18	Inside the 100 ml bottle (with BI), Middle of Left side first tray of third trolley
S19	Inside the 100 ml bottle (with BI), Middle of Right side second tray of third trolley
S20	Inside the 100 ml bottle (with BI), Middle of Left side third tray of third trolley
S21	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of third trolley
S22	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of third trolley
S23	Inside the 100 ml bottle (with BI), Middle of Left side first tray of fourth trolley
S24	Inside the 100 ml bottle (with BI), Middle of Right side second tray of fourth trolley
S25	Inside the 100 ml bottle (with BI), Middle of Left side third tray of fourth trolley
S26	Inside the 100 ml bottle (with BI), Middle of Left side fifth tray of fourth trolley
S27	Inside the 100 ml bottle (with BI), Middle of Right side sixth tray of fourth trolley

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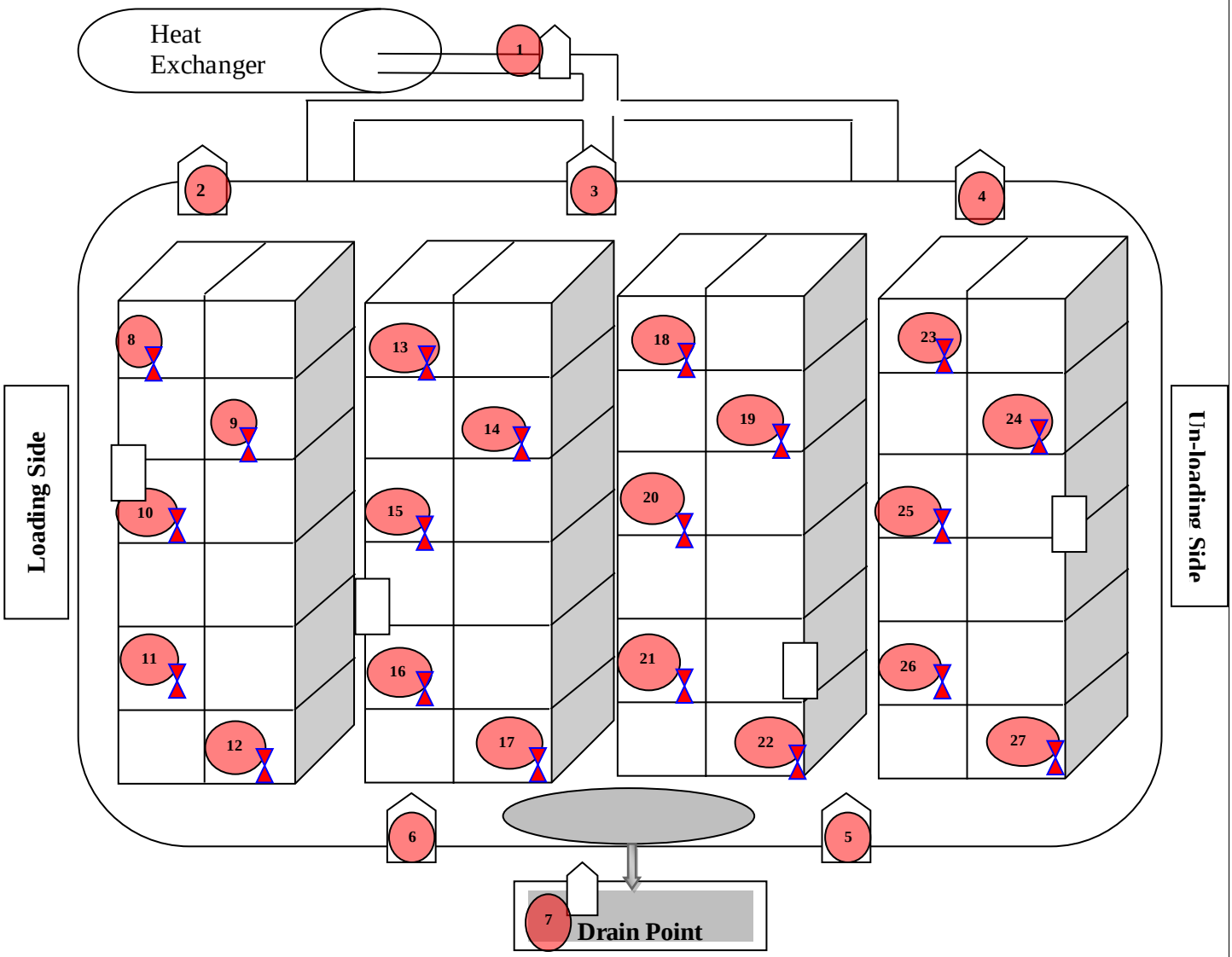

Figure 1: Location of temperature sensors & BI inside the Maximum Load.

E) Acceptance Criteria

- There should be uniform distribution of heat in the Sterilizer Chamber during the Sterilization Hold Period and the Temperature at each Temperature Mapping Probe should be within the range of 108.0°C to 109.5°C during the Sterilization Hold Period.
- The chemical indicator of each loaded trolley should change the color from Purple to Gray.
- The calculated Minimum F_0 value should be more than biological F_0 value for the Biological Indicator.
- Each autoclaved Biological indicator should give '-ve' results after the incubation
- SLR **Actual** should be more than SLR **Desired**.

F) Observation :

Record the observations in Performance Qualification Report.

G) Evaluation of Result

Heat Penetration Test shall be qualified if distribution of heat in the Super Heated Water Spray Sterilizer chamber during the sterilization hold period is uniform and Temperature uniformity at a given time of Temperature recording between all probes during hold is found within ± 1°C. If Temperature Uniformity is not achieved, check whether sensor is touching any metal surface. After taking proper corrective measures, Heat Penetration shall be re-performed.

11.9 BOTTLE LEAKAGE CHALLENGE TEST:

Load details:

- Minimum 100ml LDPE Bottles {1 Trolley (3960 Bottles)}.
- Maximum 100ml LDPE Bottles {4 Trolley (15840 Bottles)}

A. OBJECTIVE:

- Objective of this Test is to verify the leakage of loaded Bottles in the chamber

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B. PROCEDURE:

- Mark 240 Leaked Bottles.
- Load Five leaked Bottles in a different among of good Bottles in each Tray.
- Load all Trays in the Trolley and transfer to the chamber.
- Now start the cycle as per SOP for Operating Instruction.
- Set the following parameters in PLC.

Parameter	Set value
Vacuum	-0.800 Bar
Vacuum Band	0.010 Bar
Vacuum hold time	10 Min.
Pressure	-0.200 Bar
No of pulses	2 Nos.
Process end pressure	-0.030Bar

- Check the pressure of the chamber initially.
- At the end of the cycle Switch OFF the cycle.
- When pressure becomes 0.030 BAR, open the door with the help of safety gloves.
- Remove the load.

C. ACCEPTANCE CRITERIA

- All the marked leak bottles should be empty or shrink after completion of the cycle.

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11.10 BIOLOGICAL CHALLENGE TEST

A) OBJECTIVE:

- To demonstrate the degree of process lethality provided by the Sterilization cycle.

B) EQUIPMENT / INSTRUMENT USED:

- Biological Indicator (spores of ***Bacillus subtilis)***.

C) PROCEDURE

- After determining the worst case items and worst locations i.e. cold spots, challenge these items/locations with biological indicator (spores of *Bacillus subtilis).*
- Carry out the microbial challenge study concurrently with loaded chamber Heat Penetration studies.
- Place, previously population validated biological indicator along with the probes at the same location, within each load type of the specified load pattern, as in the loaded chamber heat penetration studies. Retain two biological indicators as positive control.
- Operate the autoclave as per SOP on operation of Super-Heated Water Spray Sterilizer.
- Record the chamber temperature and pressure for every minute.
- Simultaneously start the recording with data logger and take printouts.
- At the end of the cycle Switch OFF the autoclave.
- Remove the biological indicator with the help of safety gloves and incubate all exposed & unexposed BI.
- After incubation observe the indicator for growth.(As per COA).
- If indicator shows positive results increase holding time and validate the cycle for this period to get minimum Sterility Assurance Level (SAL) 10^{-6}.Run three consecutive cycles.
- Biological Indicator Detail & testing Result are Mention in performance Qualification Report by Manually.

D) ACCEPTANCE CRITERIA:

- Visually observe the ampoules, test +ve when purple color change to yellow color, test -ve when purple color remain as such.
- If no evidence of growth observed in any of the inoculated tube and growth observed in positive control tube, the test meets the criteria to achieve the desired level of sterility.

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11.10.0 ESTIMATION OF F_0 VALUE

A) Numerical F_0 Value:

The actual observations obtained during the heat penetration studies at different temperature sensing locations are complied in the table and the observed temperature shall be subjected for calculation of F_0 values at that particular location. The lethality factor calculations are done by using the following formula and the computed (during the sterilization period) are given in the following table.

$$F_0 = dt \sum 10^{(T-121.1)/Z} \quad \text{\_\_\_\_\_ (a)}$$

F_0=dt ∑ (Sum of lethality factors)

Where,

dt = time interval between successive temperature measurements

T = observed temperature at that particular time (as per the actual temperatures recorded)

Z = change in the heat resistance of *Bacillus subtilis ATCC "5230"* spores as temperature is changed (10^0C).

B) F_0 Value for Biological Indicators:

The biological F_0 value for biological indicator strip exposed during the sterilization can be calculated as follows.

$Fo = D_{121} (\log A - \log B)$ …… (b)

Where,

D_{121}	D value of the biological indicator at 121^0C
A	Experimental Biological indicator concentration or spore population
B	Desired level of sterility (SAL- 10^0)

C) Desired Spore log reduction:

Calculate the desired reduction in spore population by using the formula-

$SLR_{desired} = \log A - \log SAL_{desired}$ ----------(c)

Where,

A= Experimental population of Biological Indicator

$SAL_{desired}$= Desired level of sterility (10^0)

D) Actual Spore log reduction:

Calculate actual reduction in spore population by using the formula-

$SLR_{Actual} = F_0 / D_{121}$---------- (d)

Where,

F_0 = Minimum calculated F_0 value

D_{121}= D value of Biological Indicator

E) Sterility Assurance Level :

= SLR_{Actual} - Initial population of BI

11.10.1 RATIONALE FOR CHALLENGING THE FULL LOAD HEAT PENETRATION CYCLE WITH BIOLOGICAL INDICATOR (WROST CASE)

STUDY	RATIONALE
Full Load Heat Penetration Equipment ID No.: ……	Full Load in SHWSS is the worst case study for Penetration studies as in full load; optimum volume of SHWSS is occupied with bottles / ampoules. The Biological Indicators were used to support Heat Penetration studies by showing that BI of sufficient resistance when placed along with temperature sensors are practically killed to the desired acceptance criteria.

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12.0 CHECKLIST OF ALL TESTS AND CHECKS:

A checklist shall be provided to ensure that all tests or checks required for this protocol have been executed. After execution observations shall be recorded in Performance Qualification Report.

The list includes:

S.No.	TESTS OR CHECKS	ACCEPTANCE CRITERIA
1.	Empty Chamber Heat Distribution Study	There should be uniform distribution of heat in the steam sterilizer chamber during the sterilization hold period and the temperature at each temperature mapping probe should be within the range of 108.0°C to 109.5°C during the Sterilization Hold Period
2.	Minimum Loaded Chamber Heat Penetration Studies with Biological Indicator Placement For 100 ml LDPE bottles	• There should be uniform distribution of temperature within the range of 108.0°C to 109.5°C at each probe in sterilizer chamber during sterilization hold period • All Biological Indicators should show no growth after incubation. • The calculated Minimum F_0 value should be more than Biological F_0 value for the Biological indicator strip. • SLR Actual should be more than SLR Desired.
3.	Maximum Loaded Chamber Heat Penetration Studies with Biological Indicator Placement For 100 ml LDPE bottles	
4.	Bottle Leakage Test (Minimum Load)	All the marked leak bottles should be empty or shrink after completion of the cycle
5.	Bottle Leakage Test (Maximum Load)	

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13.0 REFERENCES:

- Master Validation Plan.
- Schedule – M "Good Manufacturing Practices and Requirements of Premises, Plant and Equipment for Pharmaceutical Products."
- WHO Essential Drugs and Medicines Policy, QA of Pharmaceuticals, Vol-2.Good Manufacturing Practices and Inspection.
- HTM 2010 Part-3 "Validation & Verification"
- PDA Technical Monograph 1.
- SOP for "Operation & Cleaning of Super Heated Water Spray Sterilizer".
- SOP for "Qualification of Biological Indicator".
- SOP for "Exposure & Incubation of Biological Indicator".

14.0 DOCUMENTS TO BE ATTACHED:

- Raw data of Microbiological Analysis
- Calibration Certificates for Data Logger & Temperature Sensor.
- COA of Biological Indicator.
- Data Logger Printouts.
- Super Heated Water Spray Sterilizer PLC Printouts.

15.0 NON COMPLIANCE:

All the Non-compliances of procedure, specifications, and sampling, analysis and documentation activities shall be monitored & recorded.

16.0 DEVIATION FROM PRE-DEFINED SPECIFICATION, IF ANY:

- In case of any deviation observed during PQ, inform to Head QA for necessary action.
- Document the deviation detail in observed deviation section.
- The Head QA will study the impact of deviation. If deviation is acceptable and it does not have an Impact on operation as well as on performance of the machine & prepare final conclusion.

17.0 CHANGE CONTROL, IF ANY:

- If any change control is required during PQ, inform to Head QA for necessary action.
- Document the details observed.
- The Head QA will study the impact of change. If change is acceptable and it does not have an impact on operation as well as on performance of the machine & prepare final conclusion.

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18.0 ABBREVIATIONS:

QA	:	Quality Assurance
QC	:	Quality Control
ID	:	Identification
No.	:	Number
Ltd.	:	Limited
FDA	:	Food and Drug Administration
CFR	:	Code of Federal Regulations
GMP	:	Good Manufacturing Practices
cGMP	:	Current Good Manufacturing Practices
WHO	:	World Health Organization
SOP	:	Standard Operating Procedure
BI	:	Biological Indicator
LDPE	:	Low Density Poly Ethylene
PLC	:	Programmable Logic Control
SHWSS	:	Super-Heated Water Spray Sterilizer
COA	:	Certificate of Analysis