PERFORMANCE QUALIFICATION

PROTOCOL FOR

CIP-SIP MODULE

CAPACITY: 500 LITER

EQUIPMENT ID No.	
LOCATION	**CIP/SIP ROOM**
DATE OF QUALIFICATION	
SUPERSEDED PROTOCOL NO.	**NIL**

PHARMA DEVILS	PERFORMANCE QUALIFICATION PROTOCOL FOR CIP-SIP MODULE (500 LITER)	PROTOCOL No.:

PROTOCOL CONTENTS

PHARMA DEVILS	PERFORMANCE QUALIFICATION PROTOCOL FOR CIP-SIP MODULE (500 LITER)	PROTOCOL No.:

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)			

APPROVED BY:

DESIGNATION	NAME	SIGNATURE	DATE
HEAD (PRODUCTION)			

2.0 OBJECTIVE:

- The objective of this protocol is to establish that CIP-SIP Module meets the following criteria:
- The CIP-SIP Module is performed as per the pre-defined parameter and/ or quality attributes.
- The CIP-SIP Module unit is capable suitable for cleaning of manufacturing vessels, filtration vessels & associated product transfer lines.

3.0 SCOPE:

- The Protocol covers all aspects of Performance Qualification for the CIP-SIP Module installed in the CIP/SIP area.

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, Review Authorization 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	• 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).
External Qualification Agency(if Applicable)	• Performance of Qualification activity as per protocol.

5.0 EQUIPMENT DETAILS:

Equipment Name	CIP/SIP Module
Equipment	
Manufacturer's Name	
Model	
Job No.	
Supplier's Name	Process Equipment
Location of Installation	CIP/SIP Room

6.0 SYSTEM DESCRIPTION:

CIP-SIP Module 500 Ltr is automatic unit used for washing and Sterilizing different capacity of vessel (Capacity from 1000 to 2000 Liter), piping & inline devices.

The CIP-SIP technology involves the use of pure steam, Water for injection high pressure pumps, Vessels and aseptic design principles to ensure that large scale process are free of dirt & organic contaminants.

The complete plant will be operated through PLC provided in the control panel. The HMI will display the various setting for the processes programmed. The annual mode also can be run through HMI.

The sequences logic will have following control philosophy.

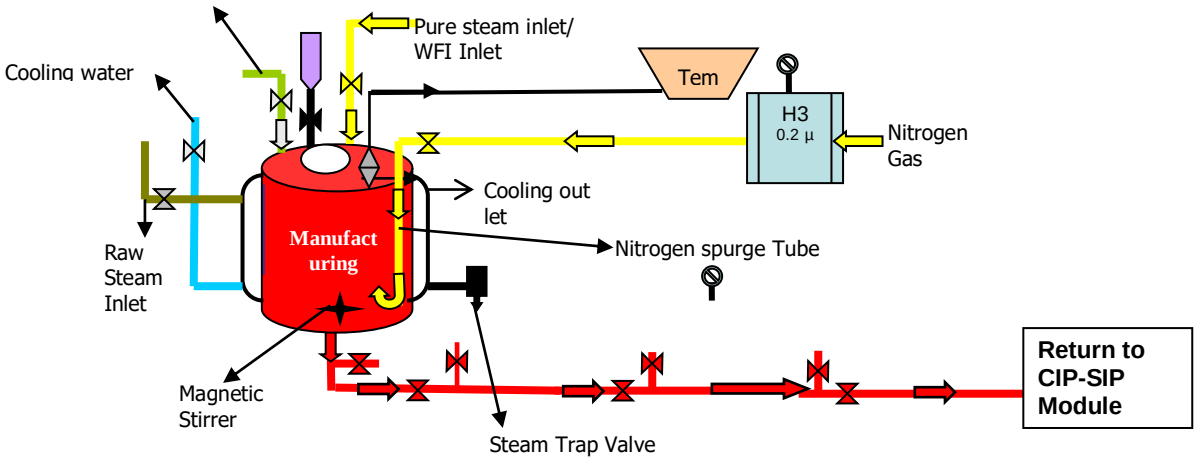
- Purified Water once through Pre wash cycle (Pre rinse) – Fixed
- Purified Water Recirculated wash cycle (Intermediate) – Fixed
- WFI once through rinse cycle(Final rinse) - Fixed

The design of each and every part are carried out considering the safety, required output, optimum utility and energy saving. The different utilities needs to be controlled as required.

The CIP-SIP Module is also used to sterilize in place Manufacturing Vessel, Holding Vessel, product pipeline, and filter housing transfer/circulation pump by passing clean steam and connecting the outlet valve through flexible hose by SIP system.

PHARMA DEVILS	PERFORMANCE QUALIFICATION PROTOCOL FOR CIP-SIP MODULE (500 LITER)	PROTOCOL No.:

Part I



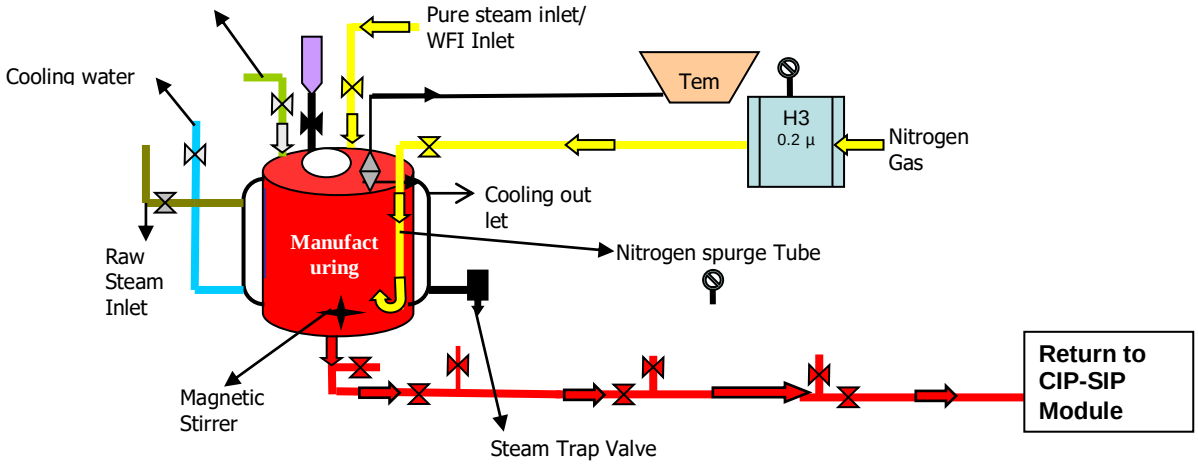
Temperature

Pressure gauge

H3 – HOUSING FOR 0.22 MICRON AIR CARTRIDGE FILTER

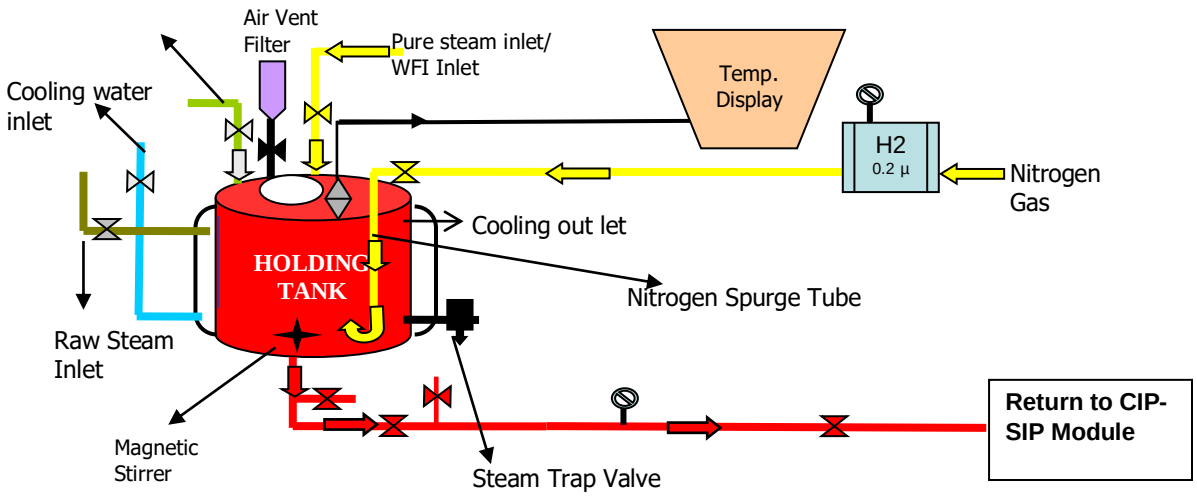
Diaphragm valve

Part-II



PHARMA DEVILS	PERFORMANCE QUALIFICATION PROTOCOL FOR CIP-SIP MODULE (500 LITER)	PROTOCOL No.:

Part-III



Temperature

Pressure gauge

Diaphragm valve

7.0 REASON FOR QUALIFICATION:

- New equipment in CIP/SIP Room.
- After completion of the Operation Qualification of the Equipments, it is imperative to perform the Performance Qualification. The study will establish that the parameters are followed, critical variables are under control and the quality of the output is, as desired.
- Periodically

8.0 SITE OF STUDY:

CIP/SIP room.

9.0 FREQUENCY OF REQUALIFICATION :

- Yearly $\pm$ 1 month as per Validation Master Plan.
- After any major breakdown or after major modification.

10.0 PRE-QUALIFICATION REQUIREMENTS :

Verification for availability, completeness and approval status of all the required relevant documents shall be done and observations shall be recorded in the performance qualification report

10.1 Verification of Documents

Record the observations for documents in the below mentioned table.

S. No.	Document Name	Document / SOP No.	Completed (Yes/No)	Checked By (Engineering) Sign/Date	Verified By (QA) Sign/Date
1.	Executed and approved Design Qualification document				
2.	Executed and approved Installation Qualification document				
3.	Executed and approved Operational Qualification document				
4.	SOP for operation & Cleaning of SIP-CIP Module				
5.	SOP for Preventive Maintenance SIP-CIP Module				

10.2 TEST EQUIPMENT

S.No.	Test Instrument
1.	Duly Calibrated Data logger with calibrated PT-100 sensors.
2.	Biological Indicator 10^6 spores i.e. *Geobacillus stearothermophilus* must be checked for spore population.
3.	Chemical Indicator (Steam Clox).
4.	All parts of CIP/SIP module like temperature sensors, pressure gauges, must be calibrated.

10.3 TEST EQUIPMENT CALIBRATION:

Review the calibration status for the test equipment (Data Logger with PT-100 sensors) to be utilized and record the calibration status Performance qualification report. 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. Calibration of all the instruments used for qualification should be mentioned along with Calibration Certificates.

10.4 CALIBRATION OF TEMPERATURE SENSORS:

Pre & Post Calibration of Temperature Sensors

Pre & Post calibration shall be carried out before starting and after completion of Validation activity.

10.5 Training Record of Validation Team:

- All the persons involved in the execution of qualification activity must be trained in all aspects of the qualification activity including the test methodology, acceptance criteria and safety precautions to be followed during working.
- Training record shall be attached with qualification report.

11.0 TESTS AND CHECKS:

11.1 CIP (CLEAN IN PLACE):

11.1.1 MANUFACTURING VESSEL & CONNECTED LOOP:

- **OBJECTIVE:**

  To demonstrate that the system is to ensure that, the washing cycles are sufficient to remove residual impurities of previously manufactured product from inner surface of the Manufacturing Vessel.

- **PROCEDURE (For 1000 liter) :**

  - Collect 1000 Liter water for injection in Manufacturing Vessel..
  - Add 20 Liters 5 % NaOH solution in the Vessel and start stirring for 10 min.
  - After completion of stirring drain the solution from Manufacturing Vessel.
  - Start CIP cycle as per SOP.
  - After completion of CIP cycle, immediately collect Sample from drain and send to QC For pH Checking. All parameters should meet with acceptance criteria.
  - Take print out from the CIP system for each cycle.
  - Repeat all steps with 10 % & 15 % NaOH solution Also.
  - Each cycle at one times of 5% ,10 %, 15% NaOH Concentration.

- **PROCEDURE (For 2000 liter) :**

  - Collect 2000 Liter water for injection in Mfg. Vessel.
  - Add 40 Liters 5 % NaOH solution in the Vessel and start stirring for 10 min.
  - After completion of stirring drain the solution from Mfg. Vessel.
  - Start CIP cycle as per SOP.
  - After completion of CIP cycle, immediately collect Sample from drain and send to QC For pH Checking. All parameters should meet with acceptance criteria.
  - Take print out from the CIP system for each cycle.
  - Repeat all steps with 10 % & 15 % NaOH solution Also.
  - Each cycle at one times of 5% ,10 %, 15% NaOH Concentration.

- **RESULT RECORDING:**

  - Record the results in Performance Qualification Report

➢ **ACCEPTANCE CRITERIA:**

- Finally rinsed WFI should meet the WFI specification for pH (Limit 5-7) and Conductivity (Limit: less than 1.3 µs/cm).

11.1.2 HOLDING VESSEL (2000 Ltr.) & CONNECTED LOOP:

➢ **OBJECTIVE:**

To ensure that, the washing cycles are sufficient to remove residual impurities of previously manufactured product from inner surface of the Holding Vessel.

➢ **PROCEDURE (For 2000 liter) :**

- Collect 2000 Liter water for injection in holding Vessel.
- Add 40 Liters 5 % NaOH solution in the Vessel .
- After completion of dissolving drain the solution from holding Vessel.
- Start CIP cycle as per SOP.
- After completion of CIP cycle, immediately collect Sample from drain and send to QC for pH, Conductivity Checking. All parameters should meet with acceptance criteria.
- Take print out from the CIP system for each cycle.
- Repeat all steps with 10 % & 15 % NaOH solution Also.
- Each cycle at one times of 5% ,10 %, 15% NaOH Concentration.

➢ **RESULT RECORDING:**

- Record the results in Performance Qualification report

➢ **ACCEPTANCE CRITERIA:**

- Finally rinsed WFI should meet the WFI specification for pH (Limit 5-7) and Conductivity (Limit: less than 1.3 µs).

11.2 SIP (STERILIZATION IN PLACE):

11.2.1 HEAT DISTRIBUTION STUDY FOR MANUFACTURING VESSEL (1000 Ltr.) & CONNECTED LOOP

A. OBJECTIVE:

- The Objective of heat distribution study is to provide a documentary evidence for a uniform temperature distribution in Equipment train Connected with Manufacturing Vessel by using 5 Nos. of temperature probes.

B. EQUIPMENT / INSTRUMENTS

- Duly Calibrated Data logger with calibrated PT-100 sensors
- Biological Indicator 10^6 spores i.e. *Geobacillus stearothermophilus)*

C. PROCEDURE:

- Check the master data logger and probes are calibrated and which is traceable to national standards
- Insert 8 nos. of Temperature probes in following locations, as schematically shown in system drawing for Manufacturing Vessel (Part I).
- Seal the port with clamp to ensure no steam leakage during operation.
- Set the following parameters in PLC & operate CIP/SIP module as per SOP and also start the data logger to record actual temperatures at every 30 second

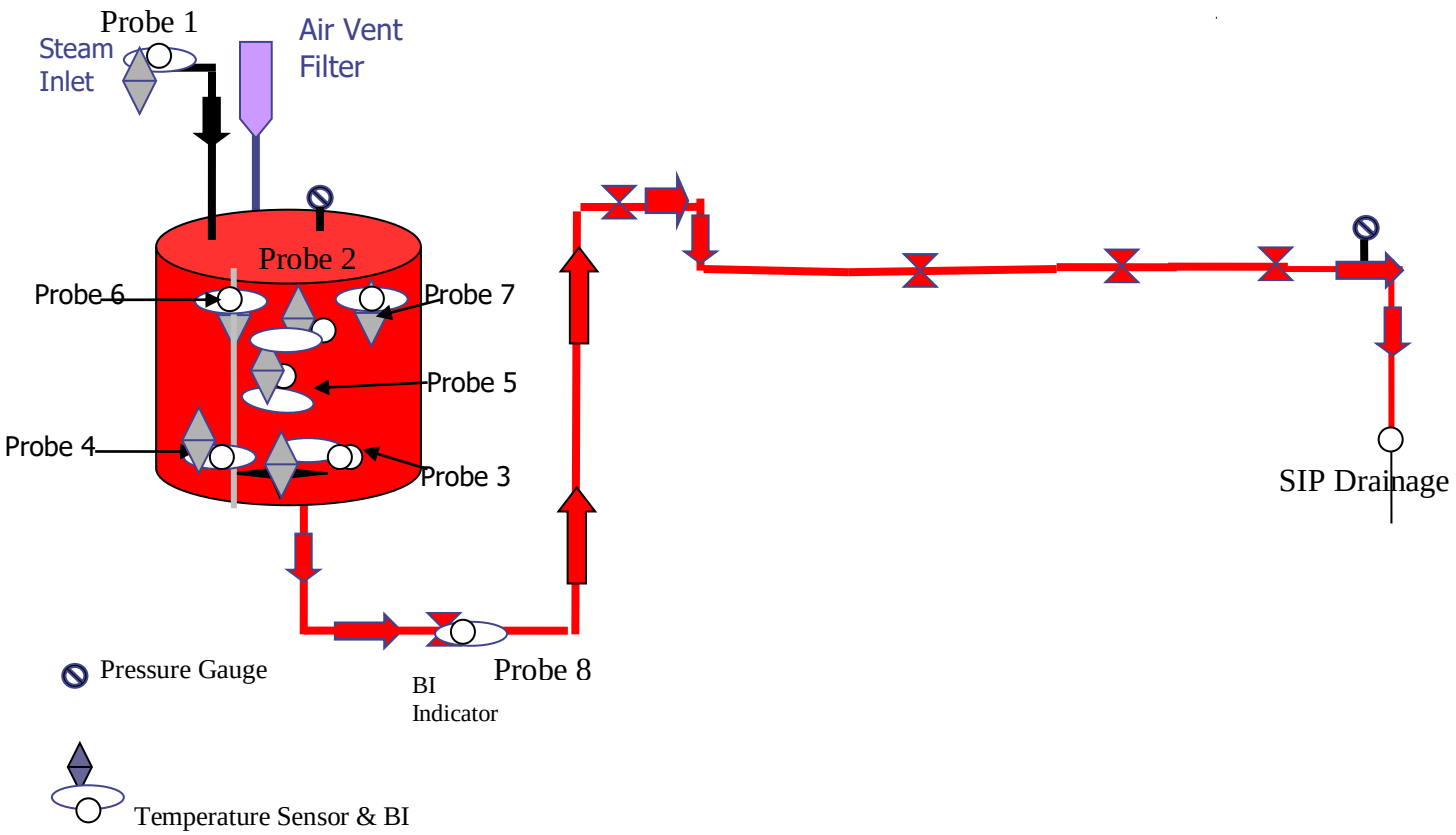
S.No.	Location	No. of Probes	No. of BI.	ID No. Of Probe
1.	Steam Inlet loop from modules	1	1	1
2.	Steam Outlet loop to modules (Drain)	8	8	8
4.	Manufacturing Vessel Inbuilt Sensor	3	3	3
5.	Manufacturing Vessel	2,4,5,6,7	2,4,5,6,7	2,4,5,6,7

- Perform three consecutive SIP cycle for Manufacturing Vessel & Connected Loop (Part I) as per respective SOP at 122.0 ^{0}C and 1.5 bar pressure for 30 minutes.
- Monitor the temperature and pressure throughout the sterilization cycle. Check Temperature on display of data logger at different locations and also as being plotted on the graph. Check and record pressure differentials on the pressure gauges installed at various locations.
- After completion of each sterilization cycle, start drying of product line with filters by selecting filter drying program on the machine and passing sterile filtered air for 30 minutes.
- At the initial stages of the filter drying process, collect steam condensate samples aseptically from the sampling points specified in System drawing and test for the following parameters.
- Repeat above procedure for all manufacturing Vessel having different capacity.

PHARMA DEVILS	PERFORMANCE QUALIFICATION PROTOCOL FOR CIP-SIP MODULE (500 LITER)	PROTOCOL No.:

Temperature Sensor & BI's Location for Manufacturing Vessel (1000 Ltr.) with Connected Loop

Part-I



- Check the all Parameter of SIP Process Mention in table

Set Parameters:	Acceptance Criteria
Leak test Pressure	**1.50 bar**
Stabilization time	**2 Minute**
Leak Test Time	**3 minute**
Leak Rate	**0.20 bar**
Purging time	**030 Second**
Sterilization Pressure	**1.50 Bar**
Pressure Dead Band	**0.02 bar**
Pulsation temperature	**115.0°C**
Sterilization Temperature	**122.0 °C**
Heating ON Temperature	**123.5 °C**
Heating OFF Temperature	**124.0°C**
Sterilization Hold Time	**30 Minute**
Sterilization Fail Temperature	**120.5^{0}C**
Overshoot Temperature	**127 ^{0}C**
Drain Time	**2 Minute**
Cooling Temperature	**80 ^{0}C**

D. RESULTS:

- Record the observations in Performance Qualification Report.

E. ACCEPTANCE CRITERIA:

- There should be uniform distribution of heat through Equipment train Connected with manufacturing Vessel during the sterilization hold period and the temperature at each temperature mapping probe should be within the limit of 122.0^0 C during the Sterilization Hold Period.
- Temperature Uniformity at a given time of Temperature recording between all probes during hold period should not be more than ± 1°C.
- Following parameter should meets for successful Performance qualification of CIP/SIP module.

Test Parameter	Acceptance Criteria
Description	A Clear colorless liquid
PH	Between 5.0 to 7.0
Bacterial Endotoxin	NMT 0.25 EU/ml
Conductivity (at 25^0C)	NMT 1.3 µ s/cm

11.2.2 HEAT DISTRIBUTION STUDY FOR MANUFACTURING VESSEL (2000 Ltr.) & CONNECTED LOOP

F. OBJECTIVE:

- The Objective of heat distribution study is to provide a documentary evidence for a uniform temperature distribution in Equipment train Connected with Manufacturing Vessel by using 5 Nos. of temperature probes.

G. EQUIPMENT / INSTRUMENTS

- Duly Calibrated Data logger with calibrated PT-100 sensors
- Biological Indicator 10^6 spores i.e. *Geobacillus stearothermophilus)*

H. PROCEDURE:

- Check the master data logger and probes are calibrated and which is traceable to national standards
- Insert 10 nos. of Temperature probes in following locations, as schematically shown in system drawing for Manufacturing Vessel (Part I).
- Seal the port with clamp to ensure no steam leakage during operation.

- Set the following parameters in PLC & operate CIP/SIP module as per SOP and also start the data logger to record actual temperatures at every 30 second

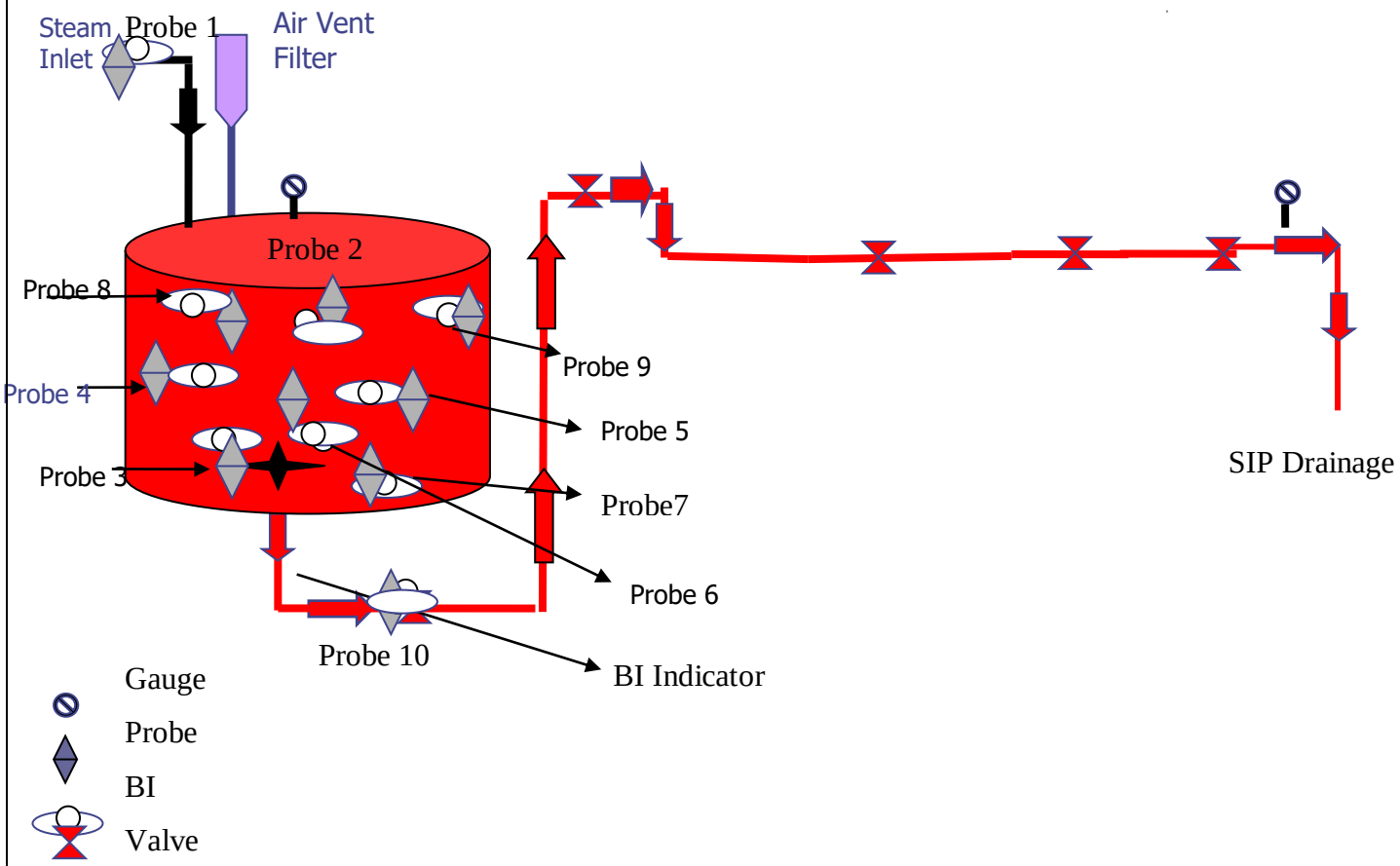
S.No.	Location	No. of Probes	No. of BI.	ID No. Of Probe
1.	Steam Inlet loop from modules	1	1	1
2.	Steam Outlet loop to modules (Drain)	10	10	10
3.	Manufacturing Vessel Inbuilt Sensor	3	3	3
4.	Manufacturing Vessel	2,4,5,6,7,8,9	2,4,5,6,7,8,9	2,4,5,6,7,8,9

- Perform three consecutive SIP cycle for Manufacturing Vessel & Connected Loop (Part I) as per respective SOP at 122.^{0}C and 1.5 bar pressure for 30 minutes.
- Monitor the temperature and pressure throughout the sterilization cycle. Check Temperature on display of data logger at different locations and also as being plotted on the graph. Check and record pressure differentials on the pressure gauges installed at various locations.
- After completion of each sterilization cycle, start drying of product line with filters by selecting filter drying program on the machine and passing sterile filtered air for 30 minutes.
- At the initial stages of the filter drying process, collect steam condensate samples aseptically from the sampling points specified in System drawing and test for the following parameters.
- Repeat above procedure for all manufacturing Vessel having different capacity.
- Check the all Parameter of SIP Process Mention in table

PHARMA DEVILS	PERFORMANCE QUALIFICATION PROTOCOL FOR CIP-SIP MODULE (500 LITER)	PROTOCOL No.:

Temperature Sensor & BI's Location for Manufacturing Vessel with Connected Loop

Part-I



Set Parameters:	Acceptance Criteria
Leak test Pressure	1.50 bar
Stabilization time	2 Minute
Leak Test Time	3 minute
Leak Rate	0.20 bar
Purging time	030 Second
Sterilization Pressure	1.50 Bar
Pressure Dead Band	0.02 bar
Pulsation temperature	115.0°C
Sterilization Temperature	122.0 °C
Heating ON Temperature	123.5 °C
Heating OFF Temperature	124.0°C
Sterilization Hold Time	30 Minute
Sterilization Fail Temperature	120.5^0C
Overshoot Temperature	127 ^{0}C
Drain Time	2 Minute
Cooling Temperature	80 ^{0}C

I. RESULTS:

- Record the observations in Performance Qualification Report.

J. ACCEPTANCE CRITERIA:

- There should be uniform distribution of heat through Equipment train Connected with manufacturing Vessel during the sterilization hold period and the temperature at each temperature mapping probe should be within the limit of 122.°C during the Sterilization Hold Period.
- Temperature Uniformity at a given time of Temperature recording between all probes during hold period should not be more than ± 1°C.
- Following parameter should meets for successful Performance qualification of CIP/SIP module.

Test Parameter	Acceptance Criteria
Description	A Clear colorless liquid
PH	Between 5.0 to 7.0
Bacterial Endotoxin	NMT 0.25 EU/ml
Conductivity (at 25^0C)	NMT 1.3 µ s/cm

11.2.3 HEAT DISTRIBUTION STUDY FOR HOLDING VESSEL (2000 Ltr.) & CONNECTED LOOP

A) OBJECTIVE:

- The Objective of heat distribution study is to provide a documentary evidence for a uniform temperature distribution in Equipment train Connected with Holding Vessel by using 5 Nos. of temperature probes.

B) EQUIPMENT / INSTRUMENTS

- Duly Calibrated Data logger with calibrated PT-100 sensors
- Biological Indicator 10^6 spores i.e. *Geobacillus stearothermophilus)*

C) PROCEDURE:

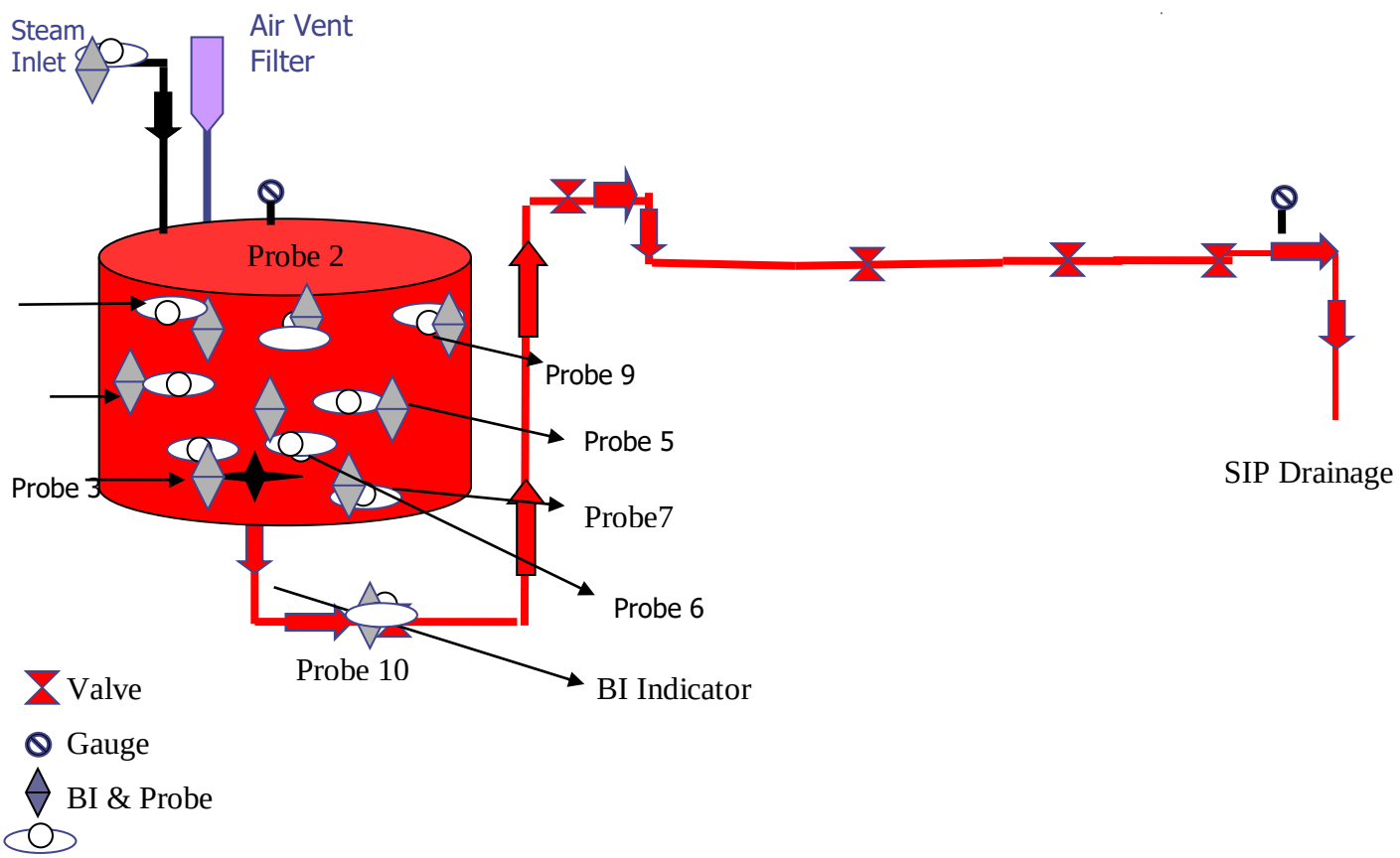
- Check the master data logger and probes are calibrated and which is traceable to national standards
- Insert 10 nos. of Temperature probes in following locations, as schematically shown in system drawing for Manufacturing Vessel
- Seal the port with clamp to ensure no steam leakage during operation.
- Set the following parameters in PLC & operate CIP/SIP module as per SOP and also start the data logger to record actual temperatures at every 30 second.

S.No.	Location	No. of Probes	No. of BI.	ID No. Of Probe
1.	Steam Inlet loop from modules	1	1	1
2.	Steam Outlet loop to modules (Drain)	10	10	10
3.	Holding Vessel Inbuilt Sensor	3	3	3
4.	Holding Vessel	2,4,5,6,7,8,9	2,4,5,6,7,8,9	2,4,5,6,7,8,9

- Perform three consecutive SIP cycle for Holding Vessel & Connected Loop (Part II) as per respective SOP at 122.0^0 C and 1.5 bar pressure for 30 minutes.
- Monitor the temperature and pressure throughout the sterilization cycle. Check Temperature on the temperature gauge at different locations and also as being plotted on the graph. Check and record pressure differentials on the pressure gauges installed at various locations.
- After completion of each sterilization cycle, start drying of product line by selecting filter drying program on the machine and passing sterile filtered air for 30 minutes.
- At the initial stages of the filter drying process, collect steam condensate samples aseptically from the sampling points specified in System drawing and test for the following parameters.
- Take the printout from data logger & CIP/SIP Module
- Repeat above procedure for all Holding Vessel having different capacity.

PHARMA DEVILS	PERFORMANCE QUALIFICATION PROTOCOL FOR CIP-SIP MODULE (500 LITER)	PROTOCOL No.:

TEMPERATURE SENSOR & BI'S LOCATION FOR HOLDING VESSEL (2000 ltr.)



- Check the all Parameter of SIP Process Mention in table.

Set Parameters:	Acceptance Criteria
Leak test Pressure	1.50 bar
Stabilization time	2 Minute
Leak Test Time	3 minute
Leak Rate	0.20 bar
Purging time	030 Second
Sterilization Pressure	1.50 Bar
Pressure Dead Band	0.02 bar
Pulsation temperature	115.0°C
Sterilization Temperature	122.0 °C
Heating ON Temperature	123.5 °C
Heating OFF Temperature	124.0°C
Sterilization Hold Time	30 Minute
Sterilization Fail Temperature	120.5^{0}C
Overshoot Temperature	127 ^{0}C
Drain Time	2 Minute
Cooling Temperature	80 ^{0}C

D) RESULTS:

- Record the observations in Performance Qualification Report.

E) ACCEPTANCE CRITERIA:

- There should be uniform distribution of heat through Equipment train Connected with manufacturing Vessel during the sterilization hold period and the temperature at each temperature mapping probe should be within the limit of 122.°C during the Sterilization Hold Period.
- Temperature Uniformity at a given time of Temperature recording between all probes during hold period should not be more than ± 1°C.

Test Parameter	Acceptance Criteria
Description	A Clear colorless liquid
PH	Between 5.0 to 7.0
Bacterial Endotoxin	NMT 0.25 EU/ml
Conductivity (at 25^0C)	NMT 1.3 µ s/cm

11.3 BIO-CHALLENGE STUDY FOR MANUFACTURING VESSEL (1000 Ltr.) & CONNECTED LOOP

A) OBJECTIVE:

The purpose of Bio-challenge study is to provide documentary evidence that the SIP cycle is capable to achieve microbial inactivation, by using *Geobacillus stearothermophilus* ATCC 7953.

B) PROCEDURE:

- Biological challenge study concurrently carried out with heat distribution study,
- Place the biological indicator in a horizontal position in the following locations as specified in system drawing part I.
- After completion of sterilization cycle remove the biological indicator with the help of safety gloves. Content of the ampoule are hot and under pressure. Allow to cool at room temperature for 10 to 15 minutes.
- Sent the exposed biological indicator to microbiology laboratory for incubation.
- After incubation observe the indicator for growth. (+ve when purple color change to yellow color, - ve when purple color remain as such).
- Place the processed units and one unprocessed unit (control) in a vertical position in an incubator at 55 -60^0C for 48 hours.

- Observe the incubated units after each 24 hrs and record the observation in respective format
- If exposed 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.
- After 2 days incubation, all positive units should be discarded as per SOP.

C) ACCEPTANCE CRITERIA:

- If positive control unit does not show sign of growth consider the test invalid.
- A negative control unit should not show any growth during incubation.
- A failed sterilization cycle is indicated by turbidity or color change toward yellow in exposed biological indicator.
- Test unit that retains its purple color after sterilization indicates that sterilization parameters have been met.

11.4 BIO-CHALLENGE STUDY FOR MANUFACTURING VESSEL (2000 Ltr.)& CONNECTED LOOP

D) OBJECTIVE:

The purpose of Bio-challenge study is to provide documentary evidence that the SIP cycle is capable to achieve microbial inactivation, by using *Geobacillus stearothermophilus* ATCC 7953.

E) PROCEDURE:

- Biological challenge study concurrently carried out with heat distribution study,
- Place the biological indicator in a horizontal position in the following locations as specified in system drawing part I.
- After completion of sterilization cycle remove the biological indicator with the help of safety gloves. Content of the ampoule are hot and under pressure. Allow to cool at room temperature for 10 to 15 minutes.
- Sent the exposed biological indicator to microbiology laboratory for incubation.
- After incubation observe the indicator for growth. (+ve when purple color change to yellow color, - ve when purple color remain as such).
- Place the processed units and one unprocessed unit (control) in a vertical position in an incubator at 55 -60^{0}C for 48 hours.
- Observe the incubated units after each 24 hrs and record the observation in respective format

- If exposed 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.
- After 2 days incubation, all positive units should be discarded as per SOP.

F) ACCEPTANCE CRITERIA:

- If positive control unit does not show sign of growth consider the test invalid.
- A negative control unit should not show any growth during incubation.
- A failed sterilization cycle is indicated by turbidity or color change toward yellow in exposed biological indicator.
- Test unit that retains its purple color after sterilization indicates that sterilization parameters have been met.

11.5 BIO-CHALLENGE STUDY FOR HOLDING VESSEL (2000 Ltr.) & CONNECTED LOOP

A) OBJECTIVE:

The purpose of Bio-challenge study is to provide documentary evidence that the SIP cycle is capable to achieve microbial inactivation, to an SAL 10^{-6} by using *Geobacillus Stearothermophilus* ATCC 7953.

B) PROCEDURE

- During the heat distribution study, place the biological indicator in a horizontal position in the following locations as specified in system drawing part II.
- After completion of sterilization cycle remove the biological indicator with the help of safety gloves. Content of the ampoule are hot and under pressure. Allow to cool at room temperature for 10 to 15 minutes.
- Sent the exposed biological indicator to microbiology laboratory for incubation.
- After incubation observe the indicator for growth. (+ve when purple color change to yellow color, - ve when purple color remain as such).
- Place the processed units and one unprocessed unit (control) in a vertical position in an incubator at 55 -60^0C for 48 hours.
- Observe the incubated units after each 24 hrs and record the observation in respective format

- If exposed 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.
- After 2 days incubation, all positive units should be discarded as per SOP.

G) ACCEPTANCE CRITERIA:

- If positive control unit does not show sign of growth consider the test invalid.
- A negative control unit should not show any growth during incubation .
- A failed sterilization cycle is indicated by turbidity or color change toward yellow in exposed biological indicator.
- Test unit that retains its purple color after sterilization indicates that sterilization parameters have been met.

11.6 F_0 CALCULATION

A) Numerical F_0 Value:

The actual observations obtained during the heat distribution study at different temperature sensing locations are complied in the table and the observed temperature shell be subjected for calculation of F0 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.

$F_0 = dt \sum 10^{(T-121)/Z}$ _____ (a)

$F_0 = dt \sum$ (Sum of lethality factors)

Where,

dt : Time interval between successive temperature measurements (in min).

T : Observed temperature at that particular time (as per the actual temperatures recorded)

Z = change in the heat resistance of Geobacillus stearothermophilus spores as temperature is changed (10^0C or as mentioned in COA).

B) **F_0 Value for Biological Indicators:**

The biological Fo 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^{-6})

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^{-6})

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 the biological indicator at 121^0C.

E) ACCEPTANCE CRITERIA:

The calculated numerical Fo value of SIP cycle should be more than the biological indicator value, which is exposed in SIP validation.

12.0 CHECKLIST OF ALL TESTS AND CHECKS:

This checklist is provided to ensure that all tests or checks required for this protocol to be executed and consisting of following tests.

S.No.	Name of Test or Check	Acceptance Criteria
1.	Test for Efficiency of washing Cycle for Manufacturing Vessel& Connected Loop	PH : Between 5.0 to 7.0 Conductivity :NMT 1.3 µ s/cm
2.	Test for Efficiency of washing Cycle for Manufacturing Vessel& Connected Loop	PH : Between 5.0 to 7.0 Conductivity :NMT 1.3 µ s/cm
3.	Test for Efficiency of washing Cycle for Holding Vessel & Connected Loop	PH : Between 5.0 to 7.0 Conductivity :NMT 1.3 µ s/cm
4.	Heat distribution study for Manufacturing Vessel & Connected Loop	Description : A Clear colorless liquid PH : Between 5.0 to 7.0 Bacterial endotoxin :NMT 0.25 EU/ml Conductivity :NMT 1.3 µ s/cm
5.	Heat distribution study for Manufacturing Vessel & Connected Loop	Description : A Clear colorless liquid PH : Between 5.0 to 7.0 Bacterial endotoxin :NMT 0.25 EU/ml Conductivity :NMT 1.3 µ s/cm
6.	Heat Distribution Study For Holding Vessel & Connected Loop	Description : A Clear colorless liquid PH : Between 5.0 to 7.0 Bacterial endotoxin :NMT 0.25 EU/ml Conductivity :NMT 1.3 µ s/cm
7.	Biological challenge Study	Test unit that retains its purple color after sterilization indicates that sterilization parameters have been met.
8.	F_0 value Calculation	The calculated numerical Fo value of SIP cycle should be more than the biological indicator value, which is exposed in SIP validation.

13.0 REFERENCES:

- Validation Master 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 report 01 (Sterilization by Moist Heat).

14.0 DOCUMENTS TO BE ATTACHED:

- Calibration Certificates of Data Logger
- Raw data of Microbiological Analysis.
- Raw data of Performance Qualification

15.0 NON COMPLIANCE:

- In case of any Non-Compliance observed during performance qualification test, inform to head QA for required action.
- All the required action should be addressed in the report and justified

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 properties of product & 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 properties of product & prepare final conclusion.

18.0 ABBREVIATIONS:

%	:	Percent
No.	:	Number
Pvt.	:	Private
QA	:	Quality Assurance
QC	:	Quality Control
SIP	:	Sterilization in place
SOP	:	Standard operating procedure