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Prepared by	Date	Authorised By	Date

Performance Qualification Protocol (PQP) for **Steam/Air Cycle** in the Production Steam Steriliser (Autoclave), located in ……

PREPARED BY:

_____ _____
Validation Engineer Date

PROTOCOL APPROVAL:
This protocol has been approved by:

_____ _____
Microbiologist Date

_____ _____
Operations Manager Date

_____ _____
QA Manager Date

1 Scope and Protocol Objective

The Production Steam Steriliser (autoclave), located in ……. shall be used for sterilising aseptically-filled vials of selected products.

To qualify the performance of the Fedegari Steam Steriliser (Autoclave) as part of a change control qualification study (refer to CR-14- xxxx "Recommence Manufacture of xxxx Acid Injection 15 mg in 1 mL) This Performance Qualification shall be limited to demonstrating consistency and efficacy of the steam/air sterilisation cycle, using 1mL water filled into 2 mL vials. Equivalence for xxx Injection 15 mg in 1 mL product, which has been aseptically filled into 2 mL vials, shall be demonstrated in the subsequent Process Validation Study (see PQ protocol kkk).

This protocol has been prepared with reference to the following regulatory guidelines:

The Performance Qualification study (PQP kkk) for the autoclave equipment, included heat distribution studies for a porous load cycle only. This document shall include heat distribution studies for the steam/air sterilisation cycle, as part of the process development for the terminal sterilisation of filled vials.

The objective of this Performance Qualification, is to verify that the sterilising autoclave consistently provides the required sterility assurance, when operated under normal conditions, using standard minimum and maximum loading patterns and the specified settings:

The production cycle registered for Folic Acid product is 121.1 ^{o}C for fifteen (15) minutes, to provide a minimum F_o = 8 min.

The standard loading patterns shall be as follows:

Minimum load Six (6) trays 2 mL vials, 340 vials per tray, across two autoclave shelves
Maximum Load Nine (9) trays 2 mL vials, 340 vials per tray, across three autoclave shelves

A reduced cycle shall also be run, for the standard production load patterns, to demonstrate that sub-optimal conditions also yield an acceptable level of sterility assurance. This shall be achieved by changing the time-temperature combination for the standard production load patterns to 121.1 ^{o}C, for *ten* (10) minutes, which is 66% of the registered sterilising condition of 121.1^{o}C for 15 minutes.

2 Equipment Identification and Description

The Production Steam Steriliser is a multi-cycle, 210 L capacity, single-ended (*future:- double ended*) autoclave, manufactured by Fedegari, model FOB4S-TS (S/N aaaa). The installation and operation is described in the following supporting documents:

ED ED Commissioning – F3 Production Steam Steriliser (Fedegari FOB4S –TS Autoclave)
IQP for F3 Steam Steriliser (Autoclave)
IQR for F3 Steam Steriliser (Autoclave)
OQP "OQP for F3 Steam Steriliser (Autoclave)
OQR for Steam Steriliser (Autoclave)
PQP for F3 Steam Steriliser (Autoclave)
PQR for F3 Steam Steriliser (Autoclave)
PVP Terminal Sterilisation of xxxx Product

2.1 Definition of Terms - Engineering Specifications

The following terms have been used in this protocol and are explained below:

- **SAL:** Sterility Assurance Level

- **PNSU:** Probability of a non-sterile unit
- **D_T**: The time in minutes required for a one logarithm or 90% reduction of the population of micro-organisms used a biological indicator under specified lethal conditions at a reference temperature T.
- **F value:** measure of the microbiological inactivation capability of a heat sterilisation process
- **F_o value:** F value calculated using a reference temperature (T_{ref}) of 121.1°C, with a z value of 10°C and a D value of 1 minute
- **F_{phys}:** A term used to describe the delivered lethality, which is calculated using the actual physical parameters of the cycle against the reference temperature (T_{ref}).
- **Z value:** The number of degrees of temperature change necessary to change the D-value by a factor of 10

2.2 Responsibilities

Validation Staff shall prepare the Performance Qualification Protocol (PQP) document and train the Production Staff in its execution.

The Performance Qualification Protocol shall be approved prior to execution by:

- The Microbiologist
- The Operations Manager
- The Quality Assurance Manager

The Production Supervisor shall be responsible for conducting the test protocols. Validation Staff shall assemble data, review results and draw conclusions from the test protocols in order to prepare the Performance Qualification Report (PQR).

The PQ report shall be reviewed by:

- The Microbiologist
- The Operations Manager
- The Quality Assurance Manager

2.3 Other Relevant Documents

3 Process Description

Sterilisation shall be by the moist heat process, using saturated steam, where $F_0 > 8\ D_T$ (see below). A steam-air mixture is used to control chamber pressure and assist in pressure equalisation between chamber and vials, particularly during the cool-down phase.

In-process controls for the sterilisation phase of the cycle shall be temperature TE1 in the liquid product and sterilisation phase hold time. Temperature (TE 8 on the auxilliary heating device) and chamber pressure (TP01) are also required to control heating and forced cooling phases of the cycle.

For the purposes of the PQ, sterilisation phase temperature and hold time data shall be processed to demonstrate that the physical characteristics of the cycle in terms of accumulated lethality (F_{phys}) exceed the minimum cycle design criteria (F_o), where:

$$F_o > 8\ D_T \qquad \text{for } D_{Tref} = D_{121.1oC} = 1.0 \text{ minutes}$$

and

$$F_{phys} = \Delta t \sum 10^{(T-Tref)/z}$$

Reference: BP Appendix XVIII "Methods of Sterilisation" and registered particulars, where F_o > 8 min is required.

Biological Indicators shall be selected, to demonstrate the survival probability of a non-sterile unit (PNSU) ≤ 10^{-6} for both the normal production cycle ($F_0 \geq 15$) and a reduced cycle ($F_0 \geq 10$).

The selected cycle for normal operation is "P4 Steam Air Cycle", which has the following characteristics:

- Heating phase to 121.1 °C

- Sterilisation temperature 121.1 °C
- Sterilisation time 15 minutes
- Drying phase 10 minutes
- Forced cooling 30 minutes

The selected cycle for reduced cycle operation is a modified "P4 Steam Air Cycle", which has the following characteristics:

- Heating phase to 121.1 °C
- Sterilisation temperature 121.1 °C
- Sterilisation time 10 minutes
- Forced cooling 15 minutes

Refer to Appendices A and B for standard load components and loading patterns respectively. A standard load configuration shall be the combination of one set of load components, stacked in the specified loading pattern.

4 Performance Qualification Tests

Tests to be conducted and acceptance criteria are defined in the attached Performance Qualification Test Sheets.

Tests to be performed:

1 Test Instrument Calibration
2 Vacuum Leak Rate Test
3 Heat Distribution Test (empty chamber temperature mapping)
4 Heat Distribution Test (loaded chamber temperature mapping)
5 Heat Penetration studies for:
Production cycle standard loads (121.1 ^{o}C for 15 minutes, two consecutive cycles) and for "Reduced" cycle standard loads (121.1 ^{o}C for 10 minutes, one cycle)
6 Biological challenge testing for standard and reduced cycle loads

All attachments of raw data in the form of thermal printer records, shall include photo or printed copies.

Tests 4-6 Summary Table:

Load Description	Empty Chamber	Water Heat Distribution (probes outside vials, no BIs)		Water Heat Penetration (probes inside vials, with BIs)	
		Minimum load (6 trays)	Maximum load (9 trays)	Minimum load (6 trays)	Maximum load (9 trays)
Production Cycle (121.1°C, 15 min)	3	2	2	2	2
Reduced Cycle (121.1°C, 10 min)	0	0	0	1	1
Biological Indicators	No	No	No	Yes	Yes

Acceptance criteria for the overall program shall be based on the following:

1. Successful calibration data for all measuring instruments
2. Successful completion of all test functions listed in Section 4.

Protocol Report Reference:
The results of all test studies shall be included in the Performance Qualification Report (PQR): …. **"PQR for Steam/Air Cycle"**

The PQR shall include a summary report, with a list of all attachments to the protocol. Deviations from the Performance Qualification Protocol shall be listed in the report and filed electronically on copies of Form "Validation Deficiency Report" (VDR).

4.1 Rationale for Testing Schedule

Two consecutive standard production cycles shall be tested for each load configuration to demonstrate consistency of autoclave performance. The first cycle shall be run under "cold start" conditions, where it is the first heated run of the day (note Vacuum Leak Rate Testing may be run first, because it does not require the chamber to be heated).

One "reduced" cycle shall be tested for each load configuration, to demonstrate lethality > 10^{-10} under sub-optimal conditions. A reduced cycle of 121.1°C for 10 minutes has been selected, as this temperature-time combination delivers approximately half the lethality of the production cycle in terms of F_o design value:

121.1 °C for 15 minutes F_o = 15 minutes
121.1 °C for 10 minutes F_o = 10 minutes

In order to preserve raw data, the PQ report shall include copies of all raw test data, which has been recorded on thermal paper. All qualification test cycles shall be logged in the summary report.

As a minimum, biological indicators shall be placed in the first, "cold start" heat penetration standard load cycle and in the "reduced" qualification cycle. Biological indicators shall be presented in a liquid form, inside sealed glass ampoules, which are of a similar size to the filled product vials.

The Biological indicator shall have:

Population > 5 x 10^5 *Geobacillus stearothermophilus (ATCC 7953)* per ampoule,
with a "D" value of not less than 1.5 minutes and not more than 2.0 minutes.

A Vacuum Leak Rate Test shall be conducted prior to performance testing, with the temperature probes in place and thereafter on a daily basis, to confirm that the leakage rate remains within specification and that the autoclave chamber constitutes a sealed unit, during operation.

Performance Qualification Test Sheets

Test 1	Title of Test: Test Instrument Calibration

Objective
To Verify that the F3 oven and autoclave data recorder and temperature probes used for the qualification are successfully calibrated.

Test Method	Reference:
The data recorder, connected to 11 temperature probes, shall be temporarily installed during the qualification study, to provide and record data for thermal mapping and heat penetration studies.	TR vvvv "Extended Calibration Schedule"

Sampling and Recording Plan:

The oven and autoclave data recorder is calibrated at a minimum frequency of once per year (as recorded in TR pppp, before and after PQ study or within one month of execution of this protocol.

Test Equipment and Materials

Dry heat block and other test equipment supplied by calibration service contractor referenced to recognized (NATA) standards.

Acceptance Criteria
The temperature monitoring device shall be:

- Digital or analogue
- Accurate to +/- 1% over the scale range 50 °C to 150 °C
- Adjusted to +/- 0.5 °C at the sterilisation temperature (121.1 ^{o}C)

The temperature monitoring equipment shall meet the above calibration requirements before and after PQ study, *or* within one month of the qualification activity.

Test Results: Copies of calibration certification for protocol test equipment shall be included in the PQR.	Date:
Summary of results (analysis and conclusion) **Acceptance Criteria met: Pass/Fail**	
Analysed By: Validation Staff	
Reviewed By: Microbiologist	
Approved By: Operations Manager	
Approved By: QA Manager	

Test 2	Title of Test: Vacuum Leak Rate Test

Objective
To verify integrity of the chamber and door seals via pressure hold test under vacuum.

Test Method
Select and run a Vacuum Leak Rate Test cycle.

The Sterilising Autoclave has a "Validation Port" in the autoclave chamber. Remove the port cover and insert temperature probes via the stainless steel adapter provided. Perform Vacuum Leak Rate Testing after mounting and on removal of test equipment.

Reference:
SOP xxxx

Sampling and Recording Plan:
On a daily basis, for all working days of operation, and also including:

- Prior to and after fitting qualification probes
- Prior to and after qualification runs
- After removal of qualification probes

Test Equipment and Materials
The autoclave draws a vacuum. The controller measures and calculates the leak rate parameters automatically over a 16 minute pressure hold phase:

$$P = (P_3-P_2)$$

where
P_1 = approximately 15 kPa (absolute pressure) Note that ordinary atmospheric pressure = +101.3 kPa and therefore where absolute pressure measured < 101.3 kPa, a vacuum is described).
P_2 = equilibration pressure within the autoclave after a hold period of 10 minutes at P_1
P_3 = remaining pressure within the autoclave after a hold period of 16 minutes at P_2

Acceptance Criteria
The rate of pressure change shall not exceed 0.13 kPa/min over a 16 minute pressure hold phase, i.e.

$$(P_3-P_2) \leq 2.08 \text{ kPa}$$

Test Results: Attach copies of autoclave printer records to the PQR.	**Date:**
Summary of results (analysis and conclusion) **Acceptance Criteria met: Pass/Fail**	
Analysed By: Validation Staff	
Reviewed By: Microbiologist	
Approved By: Operations Manager	
Approved By: QA Manager	

Test 3	Title of Test: Heat Distribution Study (Empty Chamber Mapping)

Objective
Physical qualification of the Production sterilisation cycle to demonstrate that heat distribution within the empty chamber is consistent. Program 4 (Steam/air) shall be used as the standard sterilisation cycle.

Test Method
Fit the 11 thermocouple probes through the validation port in the autoclave door as described in Test Sheet 2 and check connections to the oven and autoclave data recorder. Close the door and perform a leak rate test cycle, as for Test Sheet 2.

Probe placement inside the chamber shall reflect the results from previous heat distribution studies (see Operational Qualification) for determination of hot and cold spots within the chamber. Record the position of each probe on a loading pattern diagram (see Appendix B).

The F3 oven and autoclave data recorder drain probe (AC1-B) shall be used to reference the temperature in the drain.

Select and run “Program 4” (steam/air cycle) whilst synchronising the F3 oven and autoclave data recorder start and finish times to collect all relevant data at 1 minute intervals. At least one cycle must be the first hot cycle run in the autoclave for that day (“cold start”).

Copy, sign and date, all thermal printer records and record the test method and results on TR 019H068. Process the raw data according to the procedure outlined in Appendix C.

Sampling and Recording Plan

Three runs for this first, heat distribution study (empty chamber mapping) on the standard Production cycle (Program 4).

Test Equipment, Materials and Calibrations	References
Refer to OI 003171 ‘Use of oven/autoclave data recorder”	OI xxxxx OI xxxxx

Test 3	Title of Test: Heat Distribution Study (Empty Chamber Mapping)

Acceptance Criteria:

1. Verify consistency of operation of sterilisation "Steam/air" cycle parameters:
   - Heating phase to 121.1 °C
   - Sterilisation temperature 121.1 °C
   - Sterilisation time 15 minutes
   - Drying phase 10 minutes
   - Forced cooling 15 minutes
2. Record the overall time for cycle and verify that heating time is consistent.
3. Leak rate tests remain within specification.
4. Stratification within the chamber is acceptable i.e. thermocouple probes measure within +/- 2^{o}C of each other.
5. All measured thermocouple temperatures show 121.1°C +3/-0°C and do not fluctuate by more than 1 ^{o}C during the sterilisation hold phase.
6. At least 10 thermocouple probes remain active during the study
7. Correlate autoclave temperature and pressure readings, during the sterilisation hold time, against saturated steam tables and record the results.
8. For information, calculate and record F_{phys} for each thermocouple probe, using:

$$F_{phys} = \Delta t \sum 10^{(To-Tref)/z}$$

Where:
Δt = 1 minute
T_o = thermocouple temperature K (°C)
T_{ref} = 121.1 K (°C)
Z = value specific to biological indicator used or 10 °C if not specified

Verify that the accumulated heat loads for each probe, as indicated by F_0, are consistent.

Test Results: Attach copies of marked-up probe location (loading diagrams), printer records, TR 019H068, F3 oven and autoclave charts and spreadsheet data.	**Date:**
Summary of results: (analysis and conclusion) **Acceptance Criteria Met: Pass /Fail**	
Analysed By: Validation Staff	
Reviewed By: Microbiologist	
Approved by: Operations Manager	
Approved by: QA Manager	

Test 4	Title of Test: Heat Distribution Study (Loaded Chamber Mapping)

Objective
Physical qualification of the Production sterilisation cycle to demonstrate that heat distribution within the loaded chamber is consistent and to locate the "cold spot" within the chamber.

Test Method
Load 6 trays of vials into the autoclave chamber, distributed across two shelves. Temperature probes shall be placed adjacent to vials and held in place with autoclave tape if necessary.

Probe placement inside the chamber shall reflect the results from the previous heat distribution study (Test 3) for determination of hot and cold spots within the chamber. Record the position of each probe on a loading pattern diagram (see Appendix B).

Seal the liquid load probe TE1 inside a product vial and position it at the location previously identified as the "cold spot" (Test 3). Similarly, for additional liquid probes TE3 and TE5.

The F3 oven and autoclave data recorder drain probe (AC1-B) shall be used to reference the temperature in the drain.

Select and run "Program 4", whilst synchronising the F3 oven and autoclave data recorder start and finish times to collect all relevant data at 1 minute intervals. The cycle must be the first hot cycle run in the autoclave for that day ("cold start").

Repeat the above cycle, leaving the coldest probes in place and rearranging the "hottest" probes, so that they are re-located, to new, previously unmapped and therefore potentially cold, locations.

Repeat for 9 trays of vials.

Copy, sign and date, all thermal printer records and record the test method and results on TR 019H068. Process the raw data according to the procedure outlined in Appendix C.

Sampling and Recording Plan

Two runs each for minimum and maximum loads, (Program 4).

Test Equipment, Materials and Calibrations	References
Refer to OI 003171 'Use of oven/autoclave data recorder"	OI 003121.1 OI 003171

Test 4	Title of Test: Heat Distribution Study (Loaded Chamber Mapping)

Acceptance Criteria:

1. Verify consistency of operation of sterilisation "Steam/air" cycle parameters:
   - Heating phase to 121.1 °C
   - Sterilisation temperature 121.1 °C
   - Sterilisation time 15 minutes
   - Drying phase 10 minutes
   - Forced cooling 30 minutes
2. Record the overall time for cycle and verify that heating time is consistent. Use the data to set upper and lower limits for heat-up time during the heat penetration study.
3. Leak rate tests remain within specification.
4. Stratification within the chamber is acceptable i.e. thermocouple probes measure within +/- 2^{o}C of each other.
5. All measured thermocouple temperatures show 121.1°C +3/-0°C and do not fluctuate by more than 1 ^{o}C during the sterilisation hold phase.
6. At least 10 thermocouple probes remain active during the study
7. Correlate autoclave temperature and pressure readings, during the sterilisation hold time, against saturated steam tables and record the results.
8. For information, calculate and record F_{phys} for each thermocouple probe, using:

   $$F_{phys} = \Delta t \sum 10^{(To-Tref)/z}$$

   Where:
   Δt = 1 minute
   T_o = thermocouple temperature K (°C)
   T_{ref} = 121.1 K (°C)
   Z = value specific to biological indicator used or 10 °C if not specified

   Verify that the accumulated heat loads for each probe, as indicated by F_0, are consistent.
9. Verify that the "cold-spot" location is constant, in two consecutive cycles, for minimum and maximum loads.

Test Results: Attach copies of marked-up probe location (loading diagrams), printer records, TR 019H068, F3 oven and autoclave charts and spreadsheet data.	**Date:**
Summary of results: (analysis and conclusion) **Acceptance Criteria Met: Pass /Fail**	
Analysed By: Validation Staff	
Reviewed By: Microbiologist	
Approved by: Operations Manager	
Approved by: QA Manager	

Test 5	Title of Test: Heat Penetration Study

Objective
Physical qualification of the sterilisation cycle to demonstrate that heat penetrates equipment load items effectively for the standard load configurations set out in this protocol. Program 4 (Steam/Air Cycle) shall be temporarily modified for the "reduced" qualification cycle.

Test Method
Refer to Appendix A for the standard load components.

Load the product inside the sterilising chamber, so that steam and air can flow around and condensate can drain freely from each item.

Seal temperature probes inside the product vials so that the probe is immersed in the liquid, without touching the sides. Refer to the photograph and sealing method described in Appendix D. Allow 24 hours for the sealant to cure before proceeding.

Position 11 temperature probes, sealed inside product vials, within the autoclave chamber to reflect the results from previous heat distribution studies for determination of hot and cold spots within the chamber.

Seal the liquid load probe TE1 inside a product vial and also position it at the location previously identified as the "cold spot" (Test 4). Similarly, for additional liquid probes TE3 and TE5.

The F3 oven and autoclave data recorder drain probe (AC1-B) shall be used to reference the temperature in the drain.

Place a Biological Indicator adjacent to each temperature probe location (See Test 6 "Biological challenge testing for standard loads").

Record the position of each item (probes and BIs) on a copy of the loading pattern diagram (see Appendix B).

For the first two runs, select and run the Production Steam/Air Cycle, whilst synchronising the data recorder start and finish times to collect all relevant data at 1 minute intervals. Select and run a reduced cycle program on the third run for each load pattern. ie 121.1 ^{o}C for **10** minutes.

All validation cycles, for each load pattern, shall include Biological Indicators (BIs) – see Test 6.

The first validation cycle run for each load pattern shall be the first hot cycle run in the autoclave for that day, to ensure it starts from a "cold" chamber and is worst case.

Reference

AS ISO 11134-2003 7.4.2

And SOP 003112

Test 5	Title of Test: Heat Penetration Study

Test Method *(continued)*
Open the door and prepare for the next test cycle by removing the used Biological Indicator adjacent to each temperature probe (See test schedule "Biological challenge testing for standard loads"). On completion of the third ("reduced") test cycle, remove the load and repeat the vacuum leak rate test cycle as for test schedule 2.

Re-instate production cycle conditions to the Steam/Air cycle.

Repeat for 9 trays of vials, laid across 3 autoclave shelves.

Copy all thermal printer records and record the test method and results on TR 019H068. Graphical presentation of data is by using secure software as described in OI 003173 "Operator Instructions for Data Review – F3 oven and autoclave data recorder". Use the "Review" software to download the raw data and process it on electronic spreadsheets, according to the procedure outlined in Appendix C.

Sampling and Recording Plan:

Include Biological Indicators (see Test 6). Record results on copies of TR 019H068 "F3 Production Steam Steriliser (Autoclave) Heat Penetration Results Sheet" for:

6 trays (minimum load)	2 x Production cycle (at least 1 "cold start")	1 x "reduced" cycle ("cold start")	1 x Vacuum Leak Rate Test
9 trays (maximum load)	2 x Production cycle (at least 1 "cold start")	1 x "reduced" cycle ("cold start")	1 x Vacuum Leak Rate Test

Test Equipment and Materials

Refer to SOP for operation of the data recorder SOP 003047 "SOP for Set-Up and Operation of Temperature recorder". Calibration certificates for the test equipment shall be included in the PQ report as described in Test Sheet 1.

Acceptance Criteria:

For minimum and maximum load patterns:

1. Verify consistency of operation of sterilisation production cycle parameters:
   - Heating phase to 121.1 °C
   - Sterilisation temperature 121.1 °C
   - Sterilisation time 15 minutes
   - Drying phase 10 minutes
   - Forced cooling 30 minutes

   Record the overall time for the cycle and verify that the load conditioning phase (heating), prior to sterilisation, operates consistently.

   Similarly, verify reduced cycle parameters:
   - Heating phase to 121.1 °C
   - Sterilisation temperature 121.1 °C
   - Sterilisation time 10 minutes
   - Drying phase 10 minutes
   - Forced cooling 30 minutes

2. Vacuum Leak Rate Tests remain within specification.

3. Stratification within the chamber is acceptable i.e. temperature probes measure within +/- 2 °C of each other.

Test 5	Title of Test: Heat Penetration Study

4. For all cycles, all measured temperature probe temperatures show 121.1 °C +3/-0 °C and do not fluctuate by more than 1 °C during the sterilisation hold phase.

5. At least 10 thermocouples stay active during the study.

6. Chemical indicators show a positive indication for threshold sterilisation temperature.

7. Correlate autoclave temperature and pressure readings, during the sterilisation hold time, against saturated steam tables and record the results.

8. Calculate and record F_{phys} for each temperature probe on at least one cycle per load configuration, using:

$$F_{phys} = \Delta t \sum 10(T_o - T_{ref})/z$$

Where:

Δt = 1 minute
T_o = temperature probe temperature K (°C)
T_{ref} = 121.1 K (°C)
z = value specific to biological indicator used or 10 °C if not specified

Test Results: Attach copies of loading diagrams, TR 019H068, raw data including printer records, F3 oven and autoclave data recorder charts, spreadsheets with formula checks to the PQR.	**Date:**
Summary of results (analysis and conclusion) **Acceptance Criteria met: Pass/Fail**	
Analysed By: Validation Staff	
Reviewed By: Microbiologist	
Approved By: Operations Manager	
Approved By: QA Manager	

Test 6	Title of Test: Biological challenge testing for standard loads

Objective
Biological qualification of the sterilisation cycle to demonstrate that the delivered lethality measured by the actual kill of micro-organisms on a Biological Indicator system results in a PNSU ≤ 10^{-6}.

Test Method	**Reference**
This test is run concurrently with Test 5 (i.e. temperature probes and Biological Indicators placed adjacently in the same loads).	AS ISO 11134-2003 7.4.2 d)

Guidelines for handling Biological Indicators (BIs):

- Collect the required number of BIs from the Microbiology Laboratory. Transfer the ampoules in a sealed bag to the facility.
- Handle the glass ampoules carefully. Keep spare gloves and sample jars close by in case of damage. If an ampoule is damaged, then transfer immediately to the sample jar and seal. Change gloves and notify Production, so that the area can be cleaned down accordingly, using a sporicidal sanitant; e.g. spray with 10% H_2O_2 in 70% ethanol solution and leave to soak for 60 minutes. Contaminated gloves, and samples shall be transferred to the Microbiology Laboratory for disposal.
- Treat all autoclaved BIs as potentially viable and handle as described above. Place in a sealed bag for transfer back to the Microbiology Laboratory.
- Verify that the number if BIs issued and the number returned from/to the Microbiology Laboratory are the same (100% accountability). Inform the Production Manager on duty immediately, of any discrepancies.

For each standard load configuration:

Place a Biological Indicator (BI) adjacent to each temperature probe location (see test schedule “heat penetration study”).

Record the position and tag of each BI.

Complete the sterilisation cycle (See test schedule “heat penetration study”)

Stop the heat penetration study after the first sterilisation cycle has completed (See test schedule “heat penetration study”). Open the door and prepare for the next test cycle by removing the used BI and placing a fresh BI adjacent to each temperature probe. Transfer the used BIs to the QC laboratory for incubation and testing.

Sampling and Recording Plan:

6 trays (minimum load)	2 x Production cycle (at least 1 “cold start”)	1 x “reduced" cycle (“cold start”)
9 trays (maximum load)	2 x Production cycle (at least 1 “cold start”)	1 x “reduced" cycle (“cold start”)

Test 6	Title of Test: Biological challenge testing for standard loads

Calculate the actual log reduction (N_F = actual PNSU) of micro-organisms using:

$$F_{BIO} = D_{121.1} (\text{Log } N_0 - \text{Log } N_F)$$

where:

$F_{BIO} = F_{phys}$ (*as found from heat penetration study*)
$D_{121.1}$ and N_0 (*from BI data*)

Test Equipment and Materials
See TR gggg "RMTR – M.org – Biological Indicator Ampoules (RM 6119)

QC shall quality-test and incubate a non-sterilised spore strip as a positive control, according to:
SOP 007J026 "Procedure for Determination of the Concentration of *Geobacillus stearothermophilus* (ATCC 7953) Spore Suspension and Strips used for Autoclave Validation".

Results shall be recorded on:
TR 019H054 "PQ for F3 Steam steriliser (Autoclave) Biological Indicator Result Sheet".

Acceptance Criteria
There shall be no growth of BIs, which have undergone the sterilising cycles.

There shall be positive growth of the control BIs, which have *not* undergone the sterilising cycles.

Bio-indicators shall comply with specification before use: Glass ampoules *G.stearothermophilus*, The Biological indicator shall have:
Population > 5 x 10^5 *Geobacillus stearothermophilus (ATCC 7953)* per ampoule,
with a "D" value of not less than 1.5 minutes and not more than 2.0 minutes.
Initial population shall be confirmed by QC.

PNSU calculations are for information only and shall be reviewed with reference to original background bio-burden data, where available.

Test Results: Attach copies of TR 019H054, TR 010X066 and Certificate of Analysis for the BI ampoules to the PQR.	**Date:**
Summary of results (analysis and conclusion) **Acceptance Criteria met: Pass/Fail**	
Analysed By: Validation Staff	
Reviewed By: Microbiologist	
Approved By: Operations Manager	
Approved By: QA Manager	

Appendix A: Standard Load Components for Qualification

Minimum Load

No. off	Description	Material	Mass single item (g)	Total mass (kg)
Total mass (kg)				

Maximum Load

No. off	Description	Material	Mass single item (kg)	Total mass (kg)
Total mass (kg)				

- *Designed to simulate Product xxx 15 mg in 1 mL product, aseptically filled into 2 mL glass vials, stoppered and capped.*

Appendix B: **Loading Pattern Diagrams**

Appendix C: Procedure for processing raw data from heat distribution/penetration studies

Appendix D: Procedure for sealing probes inside vials for heat penetration studies