Validation and Management of Heat Sterilization
(Autoclave and Dry Heat Oven)

Module Outcomes

On completion of this module the participant should be able to:

- List the essential cGMP requirements for sterilisation validation – specifically autoclaves and hot air sterilisers/dry hear ovens
- List the IQ, OQ and PQ requirements for heat sterilisation processes
- Differentiate between two sterilisation approaches (overkill and bioburden)
- Calculate and use an Fo for autoclave sterilisation validation
- Interpret a basic print-off for a sterilisation process.

Module Topics

- How does heat sterilization work
- Critical process parameters and metrics
- Developing a validation process / cycle
- Bioburden reduction vs. overkill cycles
- Content of protocols and reports

Useful References

- PIC/S Guide to Good Manufacturing Practices - PE 009 – 2014 – Annex 1
- FDA – Recommendations for Submitting Documentation for Sterilisation Process Validation, November 1994
- ANSI/AAMI/ISO 11134 – Sterilisation of HealthCare products – requirements for validation and routine control – Industrial moist heat sterilisation (1993)
- ISPE Good Automated Manufacturing Practices (GAMP)
- BP Appendix XVIII Methods of Sterilisation - Monograph for Biological Indicators
- ANSI/AAMI ST79:2006 – Comprehensive guide to steam sterilisation and sterility assurance in health care facilities
- AAMI TIR 13:1997 Principles of industrial moist heat sterilization

Useful References

- PDA Technical Monograph 1 – Validation of Steam Sterilisation Cycles 2007
- PDA Technical Report 3, (TR3) Validation of Dry Heat Processes Used for Sterilization and Depyrogenation (under revision)
- USP <1035 > Biological Indicators
- USP <1211> Sterilisation and Sterility Assurance of Compendial Articles

Define Sterile (IJ Pflug)

Sterile
Free from viable microorganisms.

Sterilisation
Any physical or chemical process which destroys all life forms, with special regard to microorganisms (including bacteria and sporogenous forms), and inactivates viruses. Therefore the terms "sterile" and "sterilization", in a strictly biological sense, describe the absence or destruction of all viable microorganisms. In other words, they are absolute terms: an object or system is either "sterile" or "not sterile".

The destruction of a microbial population subjected to a sterilization process follows a geometrical progression – to be 100% certain the article is sterile it would require infinite sterilisation.

Sterility Assurance Level (SAL)

For practical purposes the probability of finding a non-sterile unit (PNSU = Probability of Non Sterile Unit) must therefore be lower than 10^{-6}.

BP/ EP Monograph - XVIII

- Sterility is the absence of viable micro-organisms.
- **The sterility of a product cannot be guaranteed by testing;** it has to be assured by the application of a suitably validated production process.
- It is essential that the effect of the chosen sterilisation procedure on the product (including its final container or package) is investigated to ensure effectiveness and the integrity of the product and that the procedure is validated before being applied in practice
- Revalidation is carried out whenever major changes in the sterilisation procedure, including changes in the load, take place.

Industry Rules -Terminal Sterilisation (BP/EP)

- Wherever possible, a process in which the product is sterilised in its final container (terminal sterilisation) is chosen.
- If terminal sterilisation is not possible, filtration through a bacteria-retentive filter or aseptic processing is used;
- Wherever possible, appropriate additional treatment of the product (for example, heating of the product) in its final container is applied.
- In all cases, the container and closure are required to maintain the sterility of the product throughout its shelf-life.

Why Are Autoclaves Essential?

- Easiest way to sterilise large volumes of heat tolerant materials.
  - More effective than dry heat (lower temperature /shorter time
  - Not as messy as chemicals and more reliable
  - No need for radiation shielding etc.
- Once validated, simple indicators used to tell autoclaved and non autoclaved material apart – the temp/time/pressure trace is used to confirm sterilization occurred.
- Can deliver > 10^{12} sterility assurance

Heat Sterilisation Methods

- **Moist Heat (Steam)**
  - Air in autoclave chamber is displaced by saturated steam
  - Condensing water vapour acts as a conductor of heat

- **Dry Heat Oven or Tunnel**
  - Heated dry air is distributed throughout an oven or tunnel by convection or radiation

Common Types of Autoclaves

- **Production autoclave.**
  - Usually large
  - Loads one side (Grade C), unloads the other (Grade B)
  - Used to sterilize production equipment
  - May be used to terminally sterilize filled product (can have one opening)
  - If faulty, potential critical impact on sterile core or batch disposition

- **Microbiology Laboratory Autoclave**
  - May be large or small
  - Usually loads and unloads from same side - Sterilized items do not unload directly into production environment
  - Used to sterilize equipment as well as media. Also used to decontaminate materials before disposal

Definitions: D-Value, Z-Value and Fo

- **What is the D value?**
  - refers to **decimal reduction time** - The time required at a certain temperature to kill 90% (eg reduce population by log 1) of the organisms being studied. Thus after an organism is reduced by 1 D, only 10% of the original organisms remain. Dependant on microbe and initial numbers. Eg D value of 1.5 means it takes 1.5minutes to reduce 1 log (to 10%) @121oC. A Dvalue of 2.0 means more resistant while a Dvalue of 1min means less resistant.

- **What is a Z value?**
  - Refers to the temperature change required to produce a 1 log reduction in D value.

Definitions: D-Value, Z-Value and Fo

What is F_0?

- The number of minutes to kill a specified number of microbes with a Z value of 10^{o}C at a temp of 121.1^{o}C.
- Often confused with the time the chamber is held at elevated temperature and pressure and in practice is the same thing.
- Fos accumulate as the sterilisation cycle progresses – very little accumulation below 112^{o}C.

Overkill

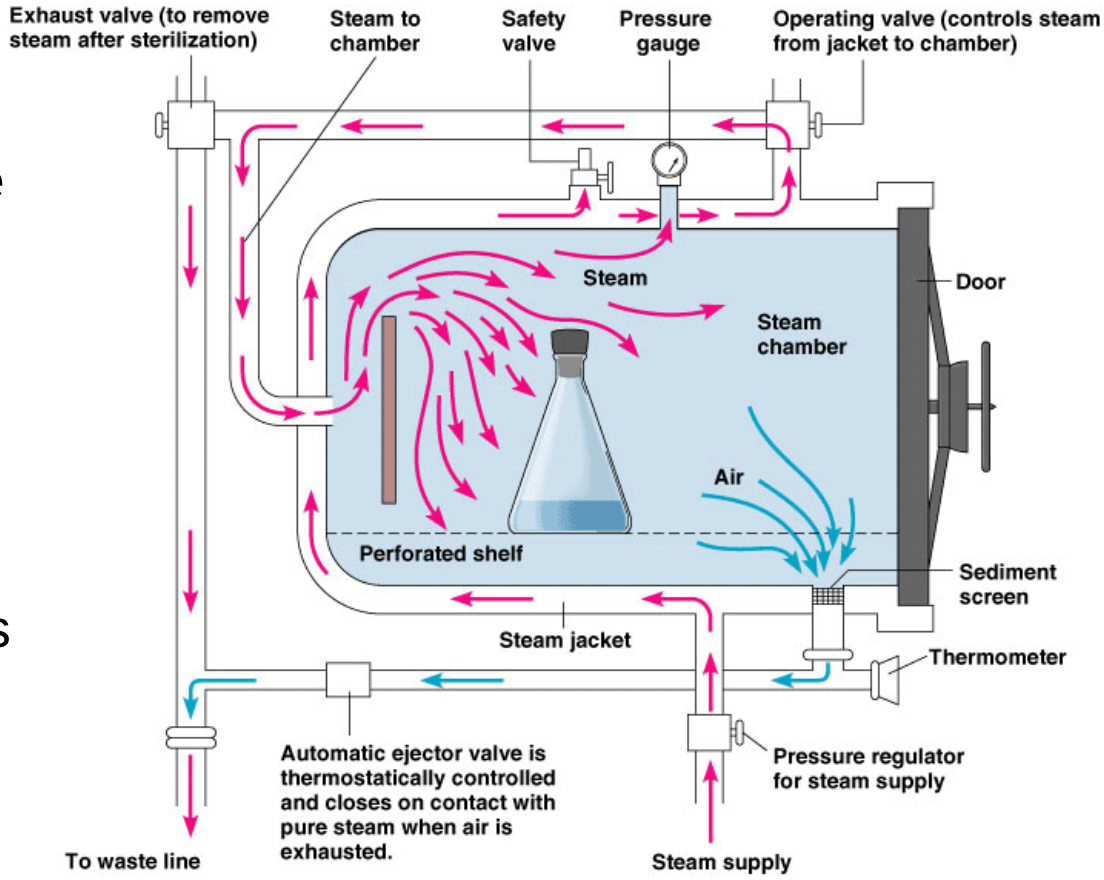
- Use many more microbes than would find on items typically autoclaved. Negates the need to test sample for bioload before running the cycle.
- Use a sterilisation time exceeding what is necessary to kill a large number of microbes. Negates the need to determine D value of microbe.
- Overkill is generally defined as a 12 log reduction in bioload

Autoclave Operating Mechanism

Steam enters the chamber jacket, passes through an operating valve and enters the rear of the chamber behind a baffle plate. It flows forward and down through the chamber and the load, exiting at the front bottom.

A pressure regulator maintains jacket and chamber pressure at a minimum of 15 psi, the pressure required for steam to reach 121ºC (250ºF).

Overpressure protection is provided by a safety valve.

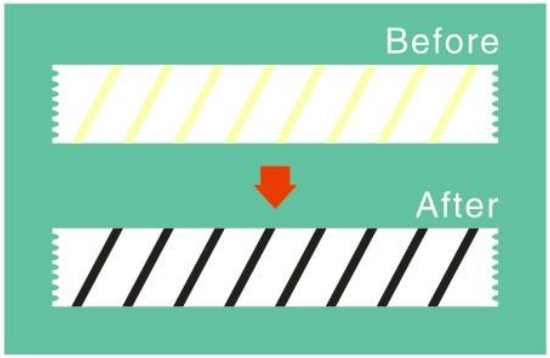


Monitoring of Sterilisation Processes

- **Biological measurements**
  - Required to demonstrate that sterilisation process was **effective**

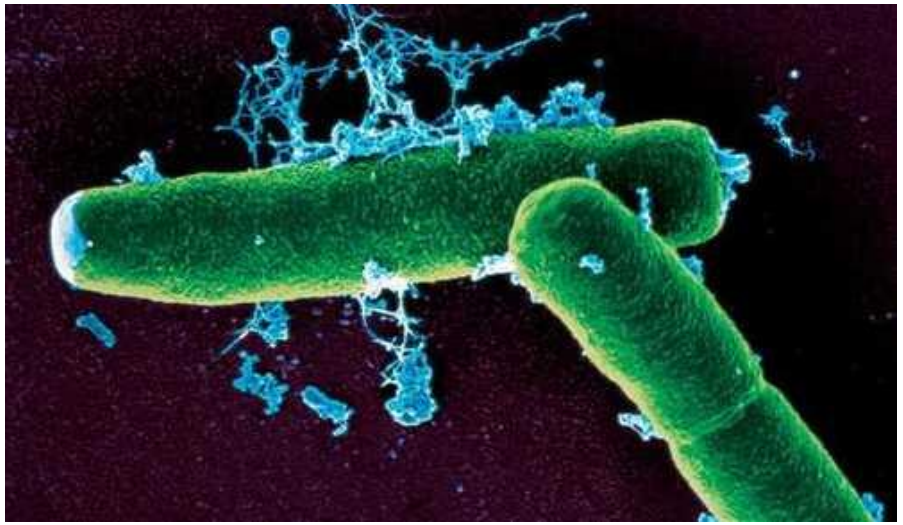
- **Physical measurements**
  - Time, temperature, pressure, vacuum.
  - Required to **calculate** sterility assurance levels (SAL)

- **Chemical measurements**
  - Autoclave tape or other indicators such as Bowie Dick



Hows Does An Autoclave Sterilize?

- Steam held at elevated temperature and pressure for time is used to transfer moist heat.
- The steam condenses on a surface and releases energy
- The energy splits open the cell wall.
- Heat acts to denature proteins, effectively killing all cells present.
- Effectiveness is reliant on saturated steam condensing



Thermal Monitors - Thermocouples (HSA Guidance)

- The number of thermal monitors used (≥10) and their location in the chamber should be described. A diagram is helpful.
- Accuracy of thermocouples should be ± 0.5^0C.
- Thermocouples should be calibrated before and after a validation experiment at two temperatures: 0^0C and 125^0C.
- Any thermocouple that senses temperature more than 0.5^0C away from the calibration temperature bath should be discarded. Stricter limits i.e., <0.5^0C, may be imposed according to the user's experience and expectations.
- Temperature recorders should be capable of printing temperature data in 0.1^0C increments.

Biological Indicators (BIs)

- A **characterized preparation** of a specific microorganism that provides a defined and stable resistance to a specific sterilization process.
- Typically **spore-forming** bacteria
- Used to:
  - Assist in the **PQ** of the sterilization equipment and
  - Assist in the **development and establishment** of a validated sterilization process for a particular article.
  - **Monitor** established sterilization cycles
  - **Periodically revalidate** sterilization processes
  - **Evaluate the capability** of processes used to decontaminate isolators or aseptic clean-room environments.

Examples of Biological Indicators

Sterilisation Method	Organism (Spore type)	Identification	No. Viable Organisms	D value
Steam	Bacillus stearothermophilus Clostridium sporogenes Bacillus subtilis spp	NCTC 10007 NCIB 8157 ATCC 7953 NCTC 8594 NCIB 8053 ATCC 7955	1.0×10^5 to 5.0×10^6 per unit	Typically 1.5 min to 2.5 min @ 121°C
Dry Heat	Bacillus subtilis	NCIB 8058 ATCC 9372	1.0×10^6 to 5.0×10^6 per unit	1min to 3 min @ 160°C Typically 1.9 min @ 160°C
Radiation	Bacillus pumilus (min. dose of 25kGy) Bacillus cereus (for higher dose levels)	NCTC 824 NCIB 8982 ATCC 14884 SSI C 1/1	$>10^7$ - 10^8 per indicator unit	~3 kGy (0.3 MRad)
Ethylene Oxide	Bacillus subtilis, variety Niger	NCTC 10073 ATCC 9372	1.0×10^6 to 5.0×10^7 per unit	2.5 min to 5.8 min @ ETO 600mg/l 60% RH and 54°C Typically 3.5
Filtration	Pseudomonas diminuta	ATCC 19146	recommend $\geq10^7$	NA

Example Dvalues of Organisms

AVERAGE VALUES OF D AND Z FOR SOME REPRESENTATIVE MICROORGANISMS Wallhauser 1980

Microorganism	D_{121}	z
Clostridium botulinum	0.2	10
Bacillus stearothermophilus	2.0	6
Bacillus subtilis	0.5	10
Bacillus megaterium	0.04	7
Bacillus cereus	0.007	10
Clostridium sporogenes	0.8 - 1.4	13
Clostridium histolyticum	0.01	10

Calculation of Fo

In mathematical terms, F_0 is expressed as follows:

$$F_0 = \Delta t \sum 10^{\frac{T-121}{z}}$$

where delta t = time interval between measurement of T
T = temperature of the sterilized product at time t
z = temperature coefficient, assumed to be equal to 10

If we assume a sterilization lasting 15 minutes, constantly at 121°C, we obtain:

$$F_0 = 15x10^{\frac{121x121}{10}} = 15x10 = 5x1 = 15\,\text{min}\,utes$$

indeed according to the definition of F_0

If we assume sterilization lasts 15 minutes, constantly at 111°C, we instead obtain:

$$F_0 = 15x10^{\frac{111x121}{10}} = 15x10^{\frac{-10}{10}} = 15x10 = 10^{-1}$$

$$F_0 = \frac{15}{10} = 1.5\,\text{min}\,utes$$

Therefore, a 15-minute sterilization at 111°C is equivalent, in terms of lethal effect, to 1.5 minutes at 121°C; this can be easily expected if z=10.

F_o Calculations – BP/EP

$F_o = D_{121}(\text{Log } N_o - \text{Log } N) = D_{121}\text{Log IF}$

D_{121} = D-value of the reference spores (5.1.2) at 121 °C,
N_0 = initial number of viable micro-organisms,
N = final number of viable micro-organisms,
IF = inactivation factor.

$IF = N_o/N = 10^{t/D}$

t = exposure time
D = D-value of micro-organism in the exposure conditions.

F_o Tables

Points to Note

1. 121.1 = Fo of 1min

2. Below around 112 very little accumulated Fos

3. Increase/decrease is exponential … slight changes have a big impact.

4. The F_0 value of a saturated steam sterilisation process is the lethality expressed in terms of the equivalent time in minutes at a temperature of 121 °C delivered by the process

TABLE OF LETHAL RATIOS

WHOLE °C	TEMPERATURES + TENTHS OF A°C									
	0.	1.	2.	3.	4.	5.	6.	7.	8.	9.
105	.024	.025	.026	.026	.027	.027	.028	.029	.029	.030
106	.031	.032	.032	.033	.034	.035	.035	.036	.037	.038
107	.039	.040	.041	.042	.043	.044	.045	.046	.047	.048
108	.049	.050	.051	.052	.054	.055	.056	.057	.059	.060
109	.062	.063	.064	.066	.067	.069	.071	.072	.074	.076
110	.077	.079	.081	.083	.085	.087	.089	.091	.093	.095
111	.097	.100	.102	.104	.107	.109	.112	.115	.117	.120
112	.123	.126	.128	.131	.135	.138	.141	.144	.148	.151
113	.154	.158	.162	.166	.169	.173	.177	.182	.186	.190
114	.194	.199	.204	.208	.213	.218	.223	.229	.234	.239
115	.245	.251	.256	.262	.268	.275	.281	.288	.294	.301
116	.308	.315	.323	.330	.338	.346	.354	.362	.371	.379
117	.338	.397	.406	.416	.426	.435	.446	.456	.467	.477
118	.489	.500	.512	.523	.536	.548	.561	.574	.587	.601
119	.615	.629	.644	.659	.674	.690	.706	.723	.739	.757
120	.774	.792	.881	.830	.849	.869	.889	.910	.931	.953
121	.975	.997	1.021	1.044	1.069	1.093	1.119	1.145	1.172	1.199
122	1.227	1.256	1.285	1.315	1.346	1.377	1.409	1.442	1.475	1.510
123	1.545	1.581	1.618	1.655	1.694	1.733	1.774	1.815	1.857	1.901
124	1.945	1.990	2.037	2.084	2.133	2.182	2.223	2.285	2.338	2.393
125	2.448	2.506	2.564	2.624	2.685	2.747	2.811	2.877	2.994	3.012
126	3.082	3.154	3.228	3.303	3.380	3.459	3.539	3.622	3.706	3.792
127	3.881	3.971	4.063	4.158	4.255	4.354	4.456	4.559	4.666	4.774
128	4.885	4.999	5.116	5.235	5.357	5.482	5.608	5.740	5.874	6.010
129	6.150	6.294	6.440	6.590	6.744	6.901	7.062	7.226	7.394	7.567
130	7.743	7.293	8.108	8.297	8.490	8.668	8.890	9.097	9.309	9.526

PNSU, SAL and Overkill

- Sterility assurance level (SAL) is the reciprocal of Probability of a Non-Sterile Unit (PNSU).
- The purpose of a BI challenge is to establish that the biological lethality is equivalent to the physically determined F_0, generally measured by thermocouples.
- **SAL = F_o / D_{value}**
  - With a D_{value} of 1.5min and a F_o of 18_{min} = we have an 12 log reduction. If we started with 10^6 we would end up with 10^{-6} which is the PNSU so we have an SAL of 10^{12}
- "Overkill" generally means that you develop a cycle that gives a complete kill of BIs with a N_o of 10^6 and then you double that cycle – otherwise can use a reduced cycle approach – Overkill is really over overkill and only sutiable for equipment.

Example Calculation of SAL

- Generally in sterilisation we are required to achieve an SAL of 10^6 (minimum) and often an additional 6 log reduction (overkill situation).

- For example if a material has a bioburden of 400cfu then to reduce the bioburden to 1 = log (400) = (2.60). This shows that only a 2.6 log reduction is needed to bring the population to 1 and therefore the total log reduction required for sterilisation with SAL of 10^6 = 2.6+6 = 8.60 – to achieve this we need a total sterilisation time at 121oC with a Dvalue of 2.0 = 2.0 x 8.6 = 17.2 min.

- For BI challenge, with a starting population of 10^6 and a Dvalue of 2.0, to reduce the population to 10^{-6} we need 2.0 x 12 logs = 24 minutes at 121oC to achieve overkill conditions.

Critical parameters needed for successful sterilization

- Article wrapping
- Chamber load pattern
- Air removal (steam displacement or vacuum)
- Moisture (saturated steam)
- Pressure / vacuum conditions
- Temperature
- Cycle Time and “Dwell” Time
- Contact with surfaces:
  - Packaging permeable to moist heat
  - Items designed to allow contact
  - Items designed to allow air removal

What Can Go Wrong ?

- Effective sterilization is dependant on:
  - initial bioload of incoming materials
  - Microbe resistance to heat (D_{value}) of that bioburden
  - Time the autoclave is held at a sterilizing temperature
  - Ability of steam to penetrate items being sterilized

Steam Penetration:

As steam is used to transfer heat, tightly wrapped items, or long tubing may not be properly penetrated. Would represent worse case for validation.

Air Pockets:

Trapped air creates localised dry heat conditions – reducing lethality rates

The Problem of Air

- Pockets of trapped air result in localized dry heat conditions which reduces the SAL.
- Autoclaves without vacuum are considered “non-GMP”
- Air removal relies on
  - Vacuum pre-pulsing the chamber before introduction of steam – generally 3 - 4 times
  - Careful consideration of the load pattern and contents
- Known issues with air removal:
  - Extended length of transfer tubing
  - Filters mis-orientated to trap air
  - Tank valves closed off to prevent removal
- Air inlet at end of the cycle must be sterilized via an air filter – filter must be periodically integrity tested.

Steam Supply Quality

- Expected to test steam quality regularly = WFI minus bioload.
- HTM-2010 (UK Standard) sets our requirements for steam quality wrt validation and monitoring
- HSA Guidance states “Steam quality must be tested periodically to ensure that:
  - moist heat (rather than dry-heat) sterilising conditions are achieved;
  - superheating does not occur;
  - wet loads are avoided;
  - non-condensable gases is below 3.5%; and
  - mineral and organic impurities (including bacteria and pyrogens) are below specified maximum levels.

The three basic steam quality tests are the superheat test, dryness value and non-condensable gas tests.

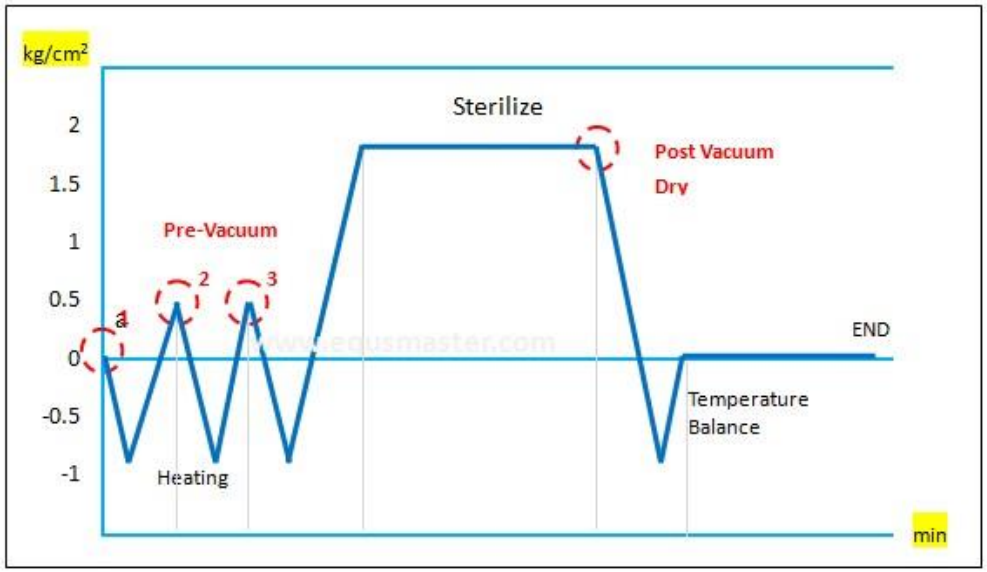
Saturation Temperatures and Pressures for Steam

Pressure – Temperature Relations in Autoclave
(Figure based on complete replacement of air by steam)

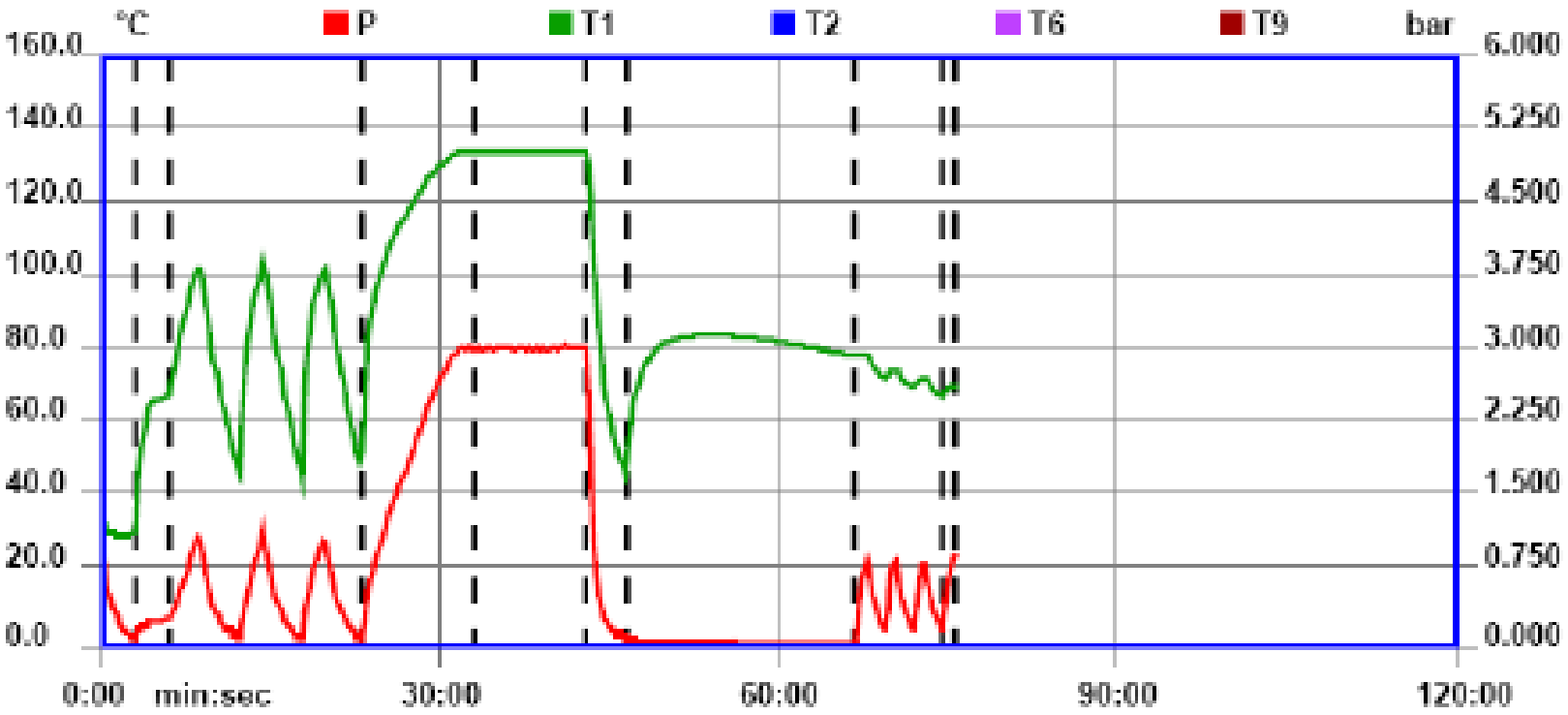
Pressure in (PSI)	Temperature °C	Temperature °F
5	109	228
10	115	240
15	121	250
20	126	259
25	130	267
30	135	275

Operating Characteristics of Steam Sterilisers

- Air Removal Options
  - ✗ **Gravity displacement:**
    - Steam enters and displaces the residual air through an open vent
  - ✔ **Vacuum air removal:**
    - Air is removed with a mechanical pump prior to dwell time.
- Pressure is needed to achieve high temperatures (steam)
- Must release pressure slowly for liquids (slow exhaust)
- Items must be allowed to dry before removal from chamber



Example time/temperature/pressure Print-off.



Sterilisation Cycle Development

- Two basic approaches are employed to develop sterilisation cycles for moist heat processes:
  - **Overkill,** used for equipment and for heat stable products, and,
  - **Probability of Survival (Bioburden Approach)**, used for heat sensitive products.
- Need to specify cycle conditions
  - Heat lability, or not, of the artlcles being sterilised
  - Pre-vac. conditions
  - Time/temperature and Fo requirements
  - Load patterns and orientations
  - Wrapping
  - Slow or fast exhaust

Cycle Development - Overkill Method

- Assumes all bioburden to be biological indicator species - worst case assumption. Requires a 12 log reduction of a resistant biological indicator with a known D-value of > 1 min.
- End point is SAL > 10^6 (In reality much higher)
- Consider a safety margin where the product demonstrates susceptibility for microbial growth and can handle extended heat exposure.
- Bioburden and resistance data are not required to determine the required F_0 values.
- Cycle parameters are chosen to ensure that the coldest point within the load receives an F_0 that will provide, at a minimum, the SAL level chosen for the cycle - typically $F_0 \geq 12$
- Overkill is always run with equipment loads

Cycle Development - Probability of Survival Method

- Used for semi heat labile product,
- The sterilisation process is validated to achieve the destruction of a pre-sterilisation bioburden to a level of at least 10^0, with a minimum safety factor of an additional six-log reduction (1×10^6) or
- SAL of 10^6,
- Requires D-value of bioburden to be measured and monitored.

Cycle Development - Demonstration of Sterility Assurance

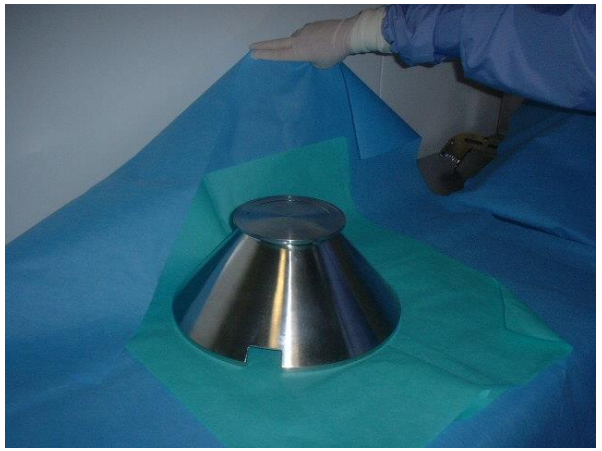
- For both approaches, must establish the cycle needed to provide the minimum F_0 values.
- Must do **heat distribution** and **heat penetration** studies to determine the amount of heat delivered to the slowest heating unit in each load.
- Validation studies must show that each unit receives the **minimum F_0** value to achieve the SAL.
- Must evaluate each load pattern:
  - Thermometrics
  - Lethality

Cycle Development - Demonstration of Sterility Assurance

- For lethality studies, use a defined resistant challenge organism such as **Geobacillus stearothermophilus** exposed to the product being validated.
- On establishment of the BI's resistance in a given product, provided the D-values of any potential bioburden or environmental isolates exhibits a lower D-value than the reference BI, it is safe to assume that the cycle will exhibit sufficient lethality overall.
- Problem is that it is very difficult to experimentally establish Dvalues so in practice this is not done.

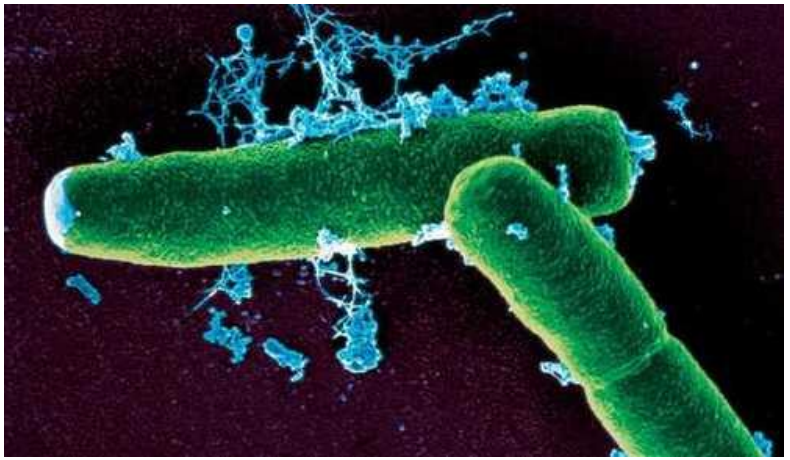
Wrapping Articles and Load Descriptions
(Must develop equipment wrapping program)

- Must completely seal the wrap
- Generally 2 - 3 sealed layers
- Overwrapped articles retain moisture
- Must include BI and T/C when validating artlcle
- Must specify load in autoclave
  - Number and type of artlcles
  - Specific location (diagram / photo)
  - **Load pattern must appear in operating procedure**



Steam Sterilizers and Validation

- Kill microbes with a very high degree of assurance even under worst case conditions
- Protect the contents of the load from deterioration or instability
- Can deliver more F_os for Equipment loads than for Product



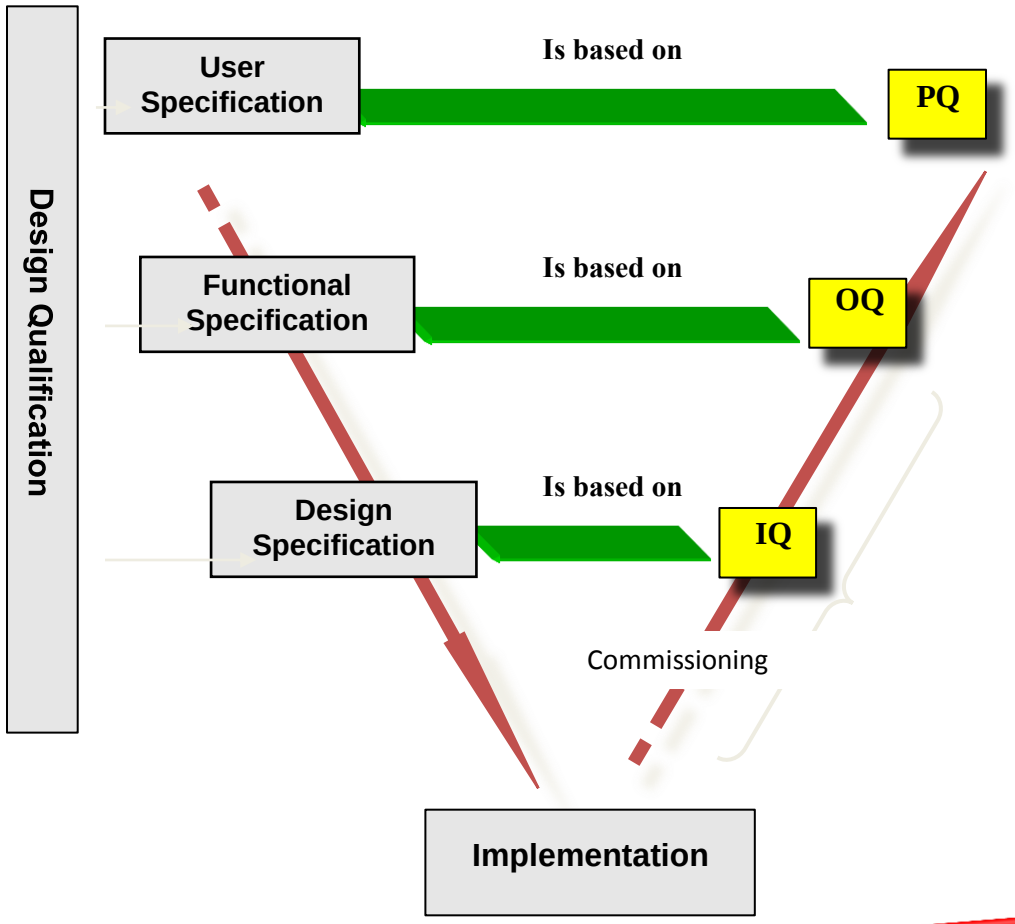
It's all about the bugs!

Validation Principles

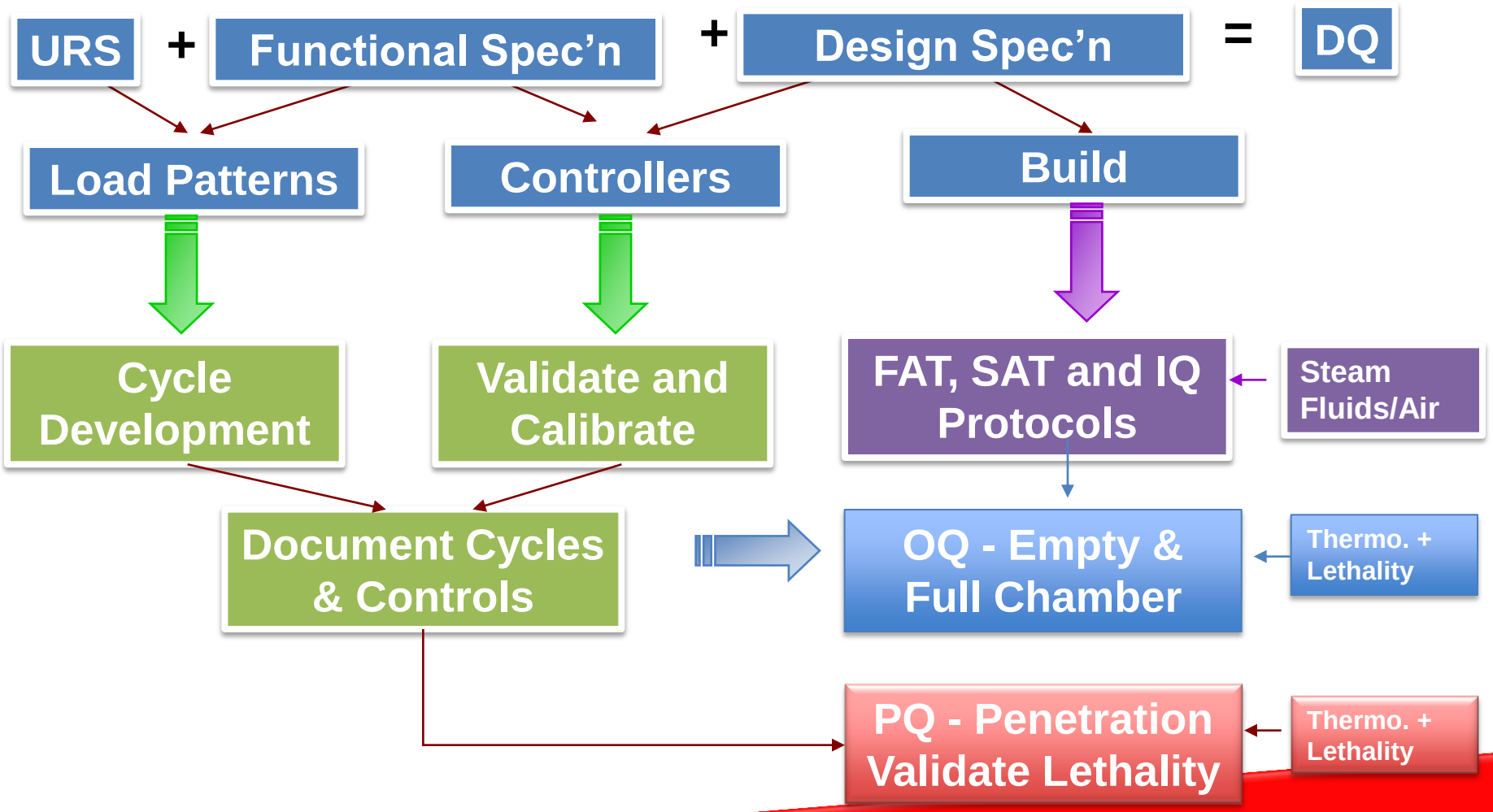
- The basic principles for validation of a heat sterilizing process are:
  - Must use BIs to demonstrate lethality
  - Must use thermometrics/ thermocouples
  - Cycle development and description of load patterns are pre-requisites
  - Can do time/temperature or Fo approach for control
  - Calibrate thermocouples both pre and again post
  - Must include **“worst case”** conditions
    - Maximum and minimum loads/ patterns
    - One run of reduced cycle time / temperature
    - Cold start for at least one of three runs per load pattern

Validation Approach and Sequences

- **DQ:** Has the item been specified correctly ?
- **IQ:** does equipment meet the URS requirements? Is everything that was on the box, in the box? Is the unit installed properly. Are support programs in place for ongoing operation of A/C?
- **OQ**: does the A/C operate properly? Does the unit hold temp and pressure correctly?
- **PQ**: validation of autoclave cycles and loading patterns – need to show sterilization.



Overview of Sterilisation Validation
(Scope of Works)



Validating Load Patterns
(Why are load patterns important?)

- Sterilization relies on steam penetration. Need to validate each set load patterns
- Very important to show what you put in an autoclave comes out sterile consistently
- Bis: When to use spore strips and when to use solutions
- **How to validate?**
  - 3x successful runs each loading pattern
  - Place BI with each item in worse case spot. Place thermocouple next to BI, but not touching item.
- How often to re qualify? – annually expected
- Loading patterns should be documented and adhered to.
  - Worse case validated – can use less but not more equipment

Pre-Qualification Activities - GMP DQ Considerations

- Materials of construction proposed and the quality of finish
- Clean-ability of the design;
- Air breaks on drain lines;
- Location of drains;
- Method by which the chamber maintains leak tight conditions to prevent back flow of non-sterilised air into the chamber;
- Interlocking of doors;
- The door type (swing or lift);
- A microbial retentive vent filter with provision for in-situ sterilization and integrity testing,
- Able to insert validation sensors through entry port
- Controller / HMI features – security and configuring / prints/downloads
- Alarm features
- Nominated cycles

Installation Qualification (IQ)

- Confirm item has been built according to design specifications
- Materials of construction are suitable for GMP standards.
- The vendor must provide evidence of a satisfactory completion Factory Acceptance Test (FAT) showing that the item meets fabrication, functional and preliminary performance standards prior to shipment.
- The item is installed in a safe manner and hooked up to the appropriately qualified services (water, steam, air) and drainage.
- The statutory documentation for the pressure vessel design, plumbing and electrical connections have been provided.
- Should do an empty chamber map.
- A typical acceptable range of temperature in the empty chamber is ±1oC when the chamber temperature is not less than 121oC

Autoclave - Installation Qualification
(Critical Services)

- Steam supply to the autoclave chamber is qualified as WFI grade or “clean” steam.
- Clean steam is produced using Water for Injection (WFI) and is tested to the relevant WFI pharmacopoeial requirements – except for bioburden.
- Need sampling ports to collect the steam
- The clean steam generator must be validated and have sufficient capacity to meet the peak loads.
- The autoclave has a sterilisable vent filter in place that is capable of being integrity tested.

Autoclave - Operational Qualification

- **Empty Chamber Thermal Mapping**
  - Verify the heat distribution pattern in an empty chamber
  - Repeat annually to re-confirm operation of autoclave
  - Conduct cold start and hot start

- **Controller Reliability**
  - Ensure each step in the PLC is in the correct sequence and is repeatable. Failure modes should include failure and restart of the critical services and include:
  - Electrical power loss,
  - Loss of equipment or instrument compressed air loss,
  - Service loss: jacket or pure steam, cooling water, vacuum,
  - Other critical service.

Operational Qualification – Control Systems

- **Control System Verification**:
  - Sterile Door Security,
  - Program Change/Alteration Security,
  - Cycle program Back Up and Recovery,
  - Calculation of F_0 Accuracy,
  - Independence of Controlling and Monitoring Thermocouples,
  - Accuracy of Printout Record.

- **Alarm and display indicators**,
  - Ensure these indicate the correct status of the autoclave for each cycle,

- **Door Interlock**
  - must work correctly not allowing access during the cycle,

- **Gasket Integrity/ Leak testing**
  - Verify positive/negative pressure seal of all door gaskets.
  - Bowie Dick Test to demonstrate air removal from chamber

Operational Qualification

- The operation of the autoclave shall be evaluated according to a written OQ protocol.
  - Empty chamber temperature distribution studies,
  - Full and minimum load chamber heat distribution studies**.
  - A minimum of three replicate cycles should be carried out for chamber heat distribution studies. An analysis of the data should identify:
    - The lowest temperature in the chamber (i.e. cold spot(s)) where a measurable temperature distribution exists,
    - Any movement of the “cold spot” between the repeats of the same cycle or between cycle types (i.e. empty, minimum and full loads).

**Could be done instead as part of PQ.

PQ of Autoclave

- PQ: validation of autoclave cycles and loading patterns.
- What SAL do you need?
  - Need to show a 10^6 or 10^{12} reduction of microbes.
- What is your starting bioload?
  - Spore strips have >10^6 CFU.
- What is the microbe's D value?
  - For *Geobacillus stearothermophilus*, this is around 1.5 – 2.0
- Must use physical, chemical and biological indicators (Bis).

PQ of Autoclave

- **Heat Distribution Study –** how does steam circulate around the contents ? Is it consistent ? Can be done with thermocouples only.

- **Heat Penetration Study –** how quickly does the heat penetrate the item or liquid.
  - Maximum Loads
  - Minimum Loads – what does this mean ?

- **Worst Case Conditions**
  - Reduced time and temperature
  - If overkill needed 50% of cycle to show >10^{-6} – production cycle is doubled to achieve 12 log reduction.

Performance Qualification
(Heat Penetration Studies)

- **Heat penetration studies** – carried out for each load configuration for each nominated cycle with the aim to:
  - Identify any cold spots within the load;
  - Measure the accumulated F_o for each challenge location within the nominated load.
- Microbiological challenge (lethality) studies carried out as part of heat penetration studies (reduced exposure).
- Product degradation (maximum exposure)
- Load “lag time” or come up determination – look for slowest to heat location
- BI is Geobacillus stearothermophilus with a certified D-value between 1.5 and 2.0 and a verified spore count of between $5x10^5$ and $5x10^6$,

Load Equilibration Time

- Equilibration time, that is, the time for the penetration thermocouples to show the same temperature as the chamber.
- Ideally equilibration time should be less than 15 seconds for chambers less than 800Litres and 30 seconds for larger chambers.
- If the equilibration time is exceeded it diagnoses:
  - Inadequate air removal OR
  - Inadequate steam penetration OR
  - Excessive non-condensable gases

Acceptance Criteria PQ

- The steriliser must meet current GMP Standards for Installation and Operation,
- The differential between the hottest and coldest thermocouple at any time during the dwell phase should not exceed 2^{o}C,
- Minimum of three acceptable consecutive sterilisation runs per load pattern – for a full (**maximum**) load and a **minimum** load pattern,
- The sterilisation hold time for the reference thermocouple(s) must not be less than the nominated cycle sterilisation hold time,
- The reference probe must be within - 0.5^{o}C to +0.5^{o}C of the nominated cycle conditions.

Acceptance Criteria PQ

- The general thermal profiles of the vacuum, heat-up and sterilisation hold phases for all thermocouples must be defined for each of the studies to provide a basis for the review of the autoclaves physical performance.
- Meets all minimum F_0 requirements for the nominated load conditions,
- All thermocouples should achieve a SAL value nominated for the cycle with a D-value of 1.0 of the BI in water. (If alternatives are used justification should be provided),
- All biological indicators (BIs) subjected to heat are:
  - rendered non-viable when incubated (i.e. there must be no growth from the recovered spore inoculum),
  - For a Reduced Cycle provide the cycle minimum SAL when calculated back to the full cycle time.
  - F_0 for a cycle with complete lethality and for a cycle with survivors.

Example Acceptance Criteria (Equipment Load)

- Four pulses of vacuum down to 25 kPa
- 3 positive pulses of steam to 160 kPa
- Sterilisation set-point temperature 124^{o}C for lowest T/C
- All T/Cs within range 124^{o}C -126^{o}C during dwell
- T/C does not fluctuate by > 1^{o}C during dwell
- Sterilisation dwell time 15 minutes
- Accumulate > 30 F_o
- All Bis show no growth
- Post sterilisation drying time 20 minutes
- Leak rate tests remain within specification
- At least 9 of 10 T/Cs remain within calibration

Final Validation Report

Table III: Accumulated F_0 value interpretation for a heat penetration study.

Function	Run No.	Thermocouple Number T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	T_{17}	T_{18}	T_{19}	Mean F_0 per Run	Std. Dev. per Run	Std. Error of the Mean	95% Confidence Interval		*t* Values across the Run
F_0	1	25.82	25.04	24.92	23.86	24.50	24.35	23.76	24.42	24.42	24.51	24.56	0.59	0.18	24.14*	24.98*	—
	2	23.95	24.81	24.01	23.50	24.20	23.91	24.25	24.80	24.90	25.12	24.35	0.53	0.16	23.97*	24.73*	0.4
	3	23.85	24.55	24.26	23.96	23.03	24.42	22.02	24.08	22.03	22.69	23.49	0.96	0.31	22.80*	24.17*	0.009
	4	24.68	26.05	26.13	26.14	26.05	26.43	25.65	26.32	25.15	26.06	25.87	0.55	0.17	25.47*	26.26*	0.00008
Mean of the four runs		24.58	25.11	24.83	24.36	24.45	24.78	23.92	24.91	24.13	24.60	24.57	1.08		24.23**	24.90**	
Standard deviation for the means		0.90	0.66	0.95	1.20	1.24	1.12	1.50	0.99	1.43	1.42						
t value		—	0.38	0.71	0.78	0.87	0.79	0.49	0.64	0.62	0.98						

* The confidence interval per run was calculated considering *t* for 0.05 (95%) confidence level = 2.262 (N-1 = 9 degrees of freedom).
** The confidence interval across runs was calculated considering the critical value for 95% level of confidence = 1.96.

Final Validation Report

- Ensure documentation has been completed and approved in line with the site quality procedures,
- Ensure an adequate training program has been performed to ensure operators manage the process consistently.
- Summarise all validation activities in a Validation Summary Report.
  - Report against the Validation Protocol.
  - Close out of all Deviations
  - Validation Certification.
  - Ensure system is under Change Control.

Annual Re-validation Example (Include the following tests)

1 Chamber leak rate test

2. Air removal and steam penetration test (Bowie Dick Test)

3. Heat distribution studies for empty chamber (1x)

4. Heat penetration studies for standard production loads:

    Load #1 Filling Components

    Load #2 Filling Machine Cap Components

    Load #3 Filling Machine Stopper Components

5. Biological challenge testing for standard loads

6. Steam condensate quality test

7. Planned preventative maintenance schedule, including instrument calibration

"Three consecutive cycles shall be tested for each load configuration to demonstrate consistency of autoclave performance".

Routine Monitoring of Autoclaves

- Sequential number runs and a running log
- Must double sign prints to verify cycle conditions met
- Record conditions met and any alarms activated
- Chamber Leak Rate Test (weekly)
- Physical indicator on each item in each load
- Bowie Dick Test (Optional)
- BIs are not routinely included in the cycle
- Reliance on controlling probe (directly correlated to the worst case (coldest) location for the validation probe
- For product loads usual ot probe a number of dummy vials in the load for added assurance.

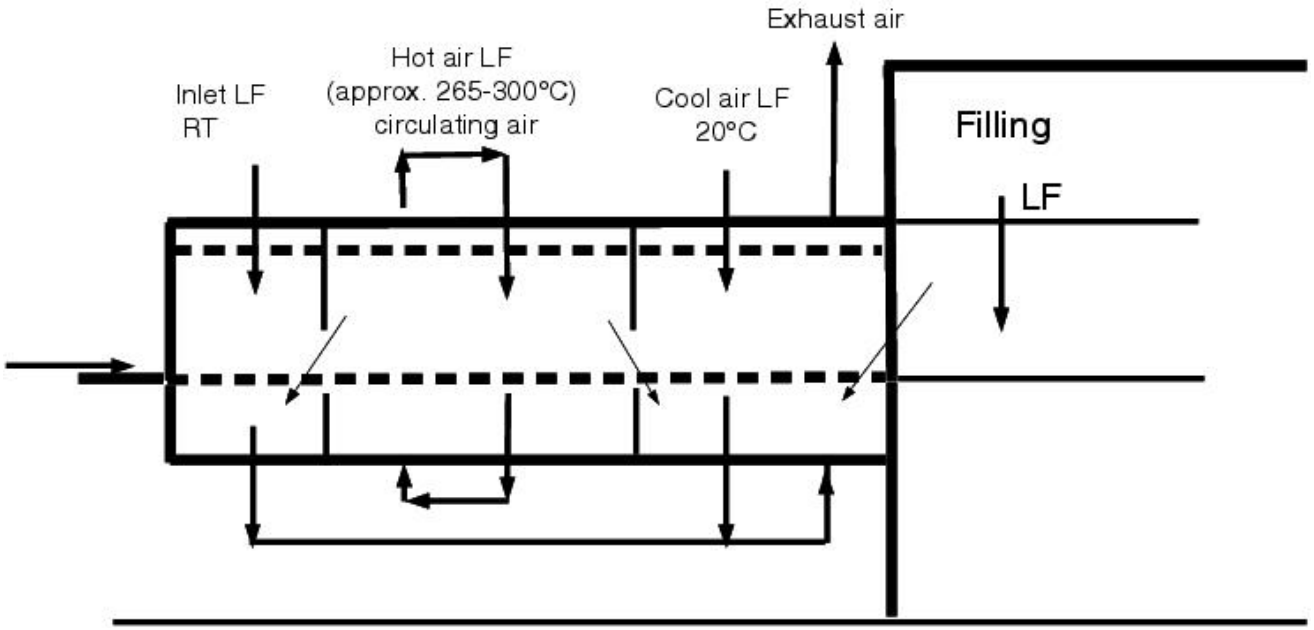
Auditor Considerations

What do GMP auditors look for in an audit

- Was re-validation conducted in time frame?
- Focus on PQ primarily but interest in IQ/OQ for newer autoclaves
- Coolest and warmest positions clearly stated in validation report?
- Preventative maintenance program, SOPS, leak rate test data ?
- Cycle time / Fo – is it sufficient for tested D values?
- Was validation equipment within calibration (pre and post use))
- Traces for validation and most recent cycles – consistency ?
- Are vacuum cycles used appropriately?
- Is anything thing not listed on the loading pattern present in the autoclave? Enough room for steam to circulate through chamber?
- Deviations from protocols. Are conclusions valid and justified?
- Can site demonstrate terminally sterilised product is stable?

Validation of Dry Heat Sterilisation Processes

Temperature and flow conditions in the sterilisation tunnel



BP/EP XVIII Monograph

- Minimum conditions of 160 °C for at least 2 hours for sterilisation.
  (Other combinations of time and temperature may be used provided that it has been satisfactorily demonstrated that the process chosen delivers an adequate and reproducible level of lethality when operated routinely within the established tolerances.)
- Dry heat sterilisation is carried out in an oven equipped with forced air circulation or other equipment specially designed for the purpose.
- The steriliser is loaded in such a way that a uniform temperature is achieved throughout the load. Knowledge of the temperature within the steriliser during the sterilisation procedure is usually obtained by means of temperature-sensing elements inserted into representative containers together with additional elements at the previously established coolest part of the loaded steriliser.
- The temperature throughout each cycle is suitably recorded.

Depyrogenation of Glassware

- Dry heat is used for depyrogenation purposes and results in complete destruction of micro-organisms
- It is accepted that validation of depyrogenation means also SALs much greater than 10^{-6}.
- Dry heat at temperatures greater than 220°C is frequently used for sterilisation and depyrogenation of glassware. In this case demonstration of a 3-log reduction in heat resistant endotoxin can be used as a replacement for biological indicators. (BP/EP)
- Spores of Bacillus subtilis (for example, var. niger ATCC 9372, NCIMB 8058 or CIP 77.18) are recommended as biological indicators.

Example of Depyrogenation Cycle Description

Cycle phase description	Set-point
Dehumidifying Rate:	6.0°C/min
Dehumidifying Time:	45 minutes
Dehumidifying Temperature:	120°C
Exposure Rate:	5.0°C/min
Exposure Time:	195 min
Exposure Temperature:	245°C
Cool Down Rate:	2.0°C/min
Cool Down Temperature:	50°C

Also need

- Load Pattern Description
- Location of T/Cs whroughout the chamber
- Cycle ranges for parameters

Installation Qualification

- Calibration of monitoring devices
- Preventative Maintenance program developed
- All filters are listed with the following information
  - identification
  - type
  - size
  - change frequency
  - air capacity
  - flow rate
  - integrity testing requirements
  - the air downstream from the filter should be tested for total and viable particulates to ensure the filters do not shed or leak particles

Operational Qualification Considerations

- PLC Reliability
- Blower Rotation - verify RPM and correct direction
- Heater Elements integral
- Air flow rate throughout the chamber
- HEPA filter installation integrity (inlet and exhaust) in clod condition
- Chamber non-viable particle monitoring - Grade A in cold condition
- Room Balance – chamber positive ot room at all times

Operational Qualification Considerations

- **For Ovens:**
  - Door interlocks
  - Gasket integrity
- **For Tunnels:**
  - Belt velocity and chart recorder speed calibrated
- Empty chamber heat distribution profile
  - Temperature profile – wall and chamber
  - Minimum of 3 studies
  - Record all critical process parameters

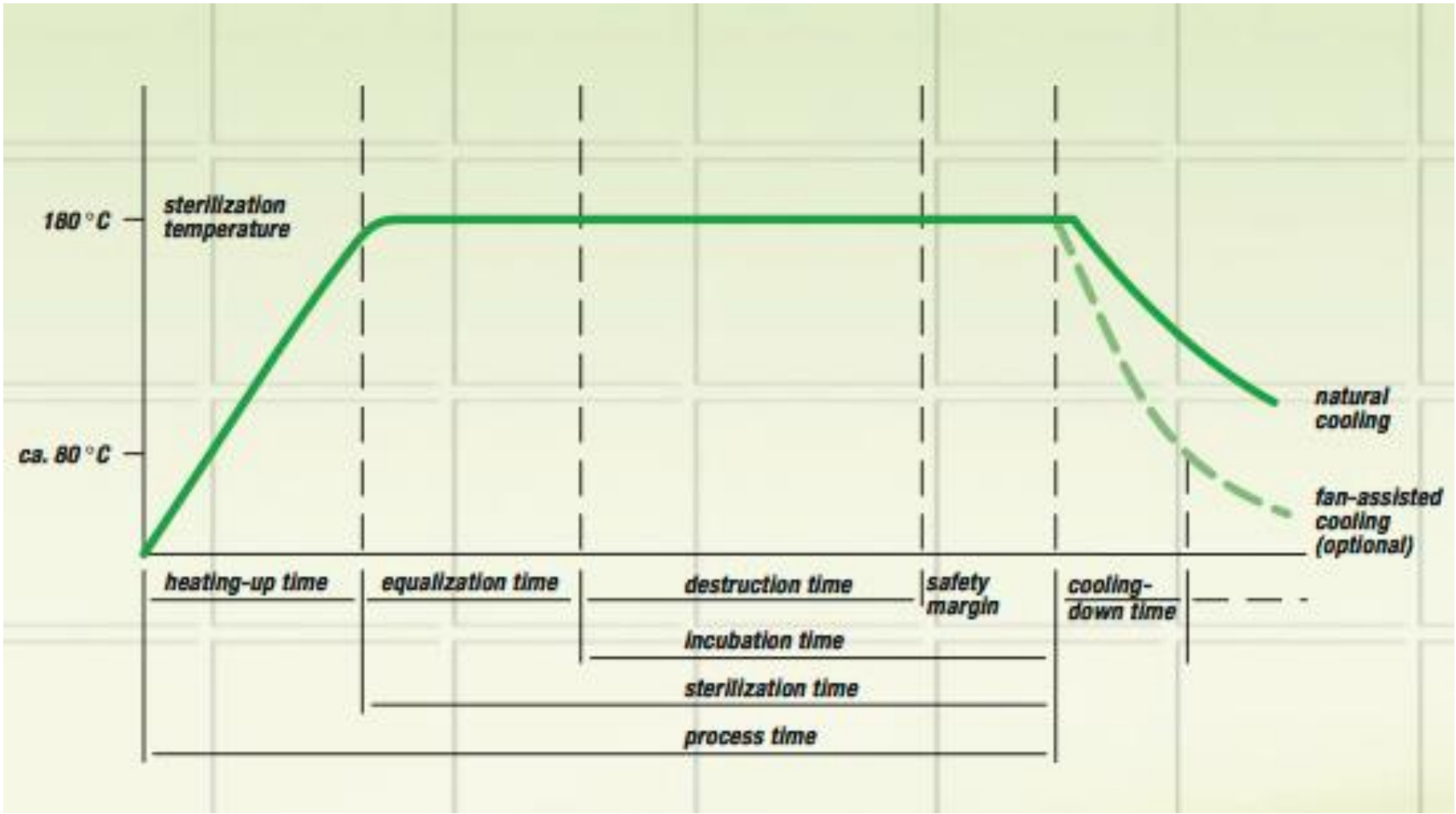
HAO Performance Qualification for Depyrogenation

- Expected to apply endotoxin the inside of glass vials
- Techniques and methods for recovering and testing endotoxin must be validated.
- should recover a minimum of 50% of applied endotoxin from glass surfaces.
- Recovery studies should be performed at the level of expected endotoxin.
- Need to challenge with >10,000 Endotoxin Units (EUs)
- Acceptance criteria is > 3 log reduction demonstrated on 3 consecutive runs for each load pattern.

Performance Qualification

- Loaded chamber heat distribution
- Loaded chamber heat penetration (min and max load patterns)
- Biovalidation
  - Sterilisation cycles only
- Depyrogenation verification
  - Endotoxin challenge studies must indicate at least a 3-log reduction for all locations for all runs

Example HAO Control Probe Print



USP <1211>
Dry-Heat Sterilization/Depyrogenation

- A dry-heat sterilization/depyrogenation system is supplied with heated, HEPA filtered air, distributed uniformly throughout the unit by convection or radiation and employing a blower system with devices for sensing, monitoring, and controlling all critical parameters.

- A typical acceptable range in temperature in the empty chamber is ±15oC when the unit is operating at not less than 250 .

Example Acceptance Criteria for HAO

Cycle Conditions

Must meet the nominated ranges of the cycle conditions

Thermometrics

- All thermocouple locations shall indicate temperatures continuously in excess of 220oC for a period of at least 2 hours 15 minutes, during the exposure phase of the cycle.
- The timing of the exposure phase of the cycle starts from the slowest to heat thermocouple reaching 220 oC and finishes with the fastest to cool thermocouple falling below 220 oC.

“Pyrometrics” - > 3 log reduction

Maintaining the Validated State
(Annual Routine Re-validation)

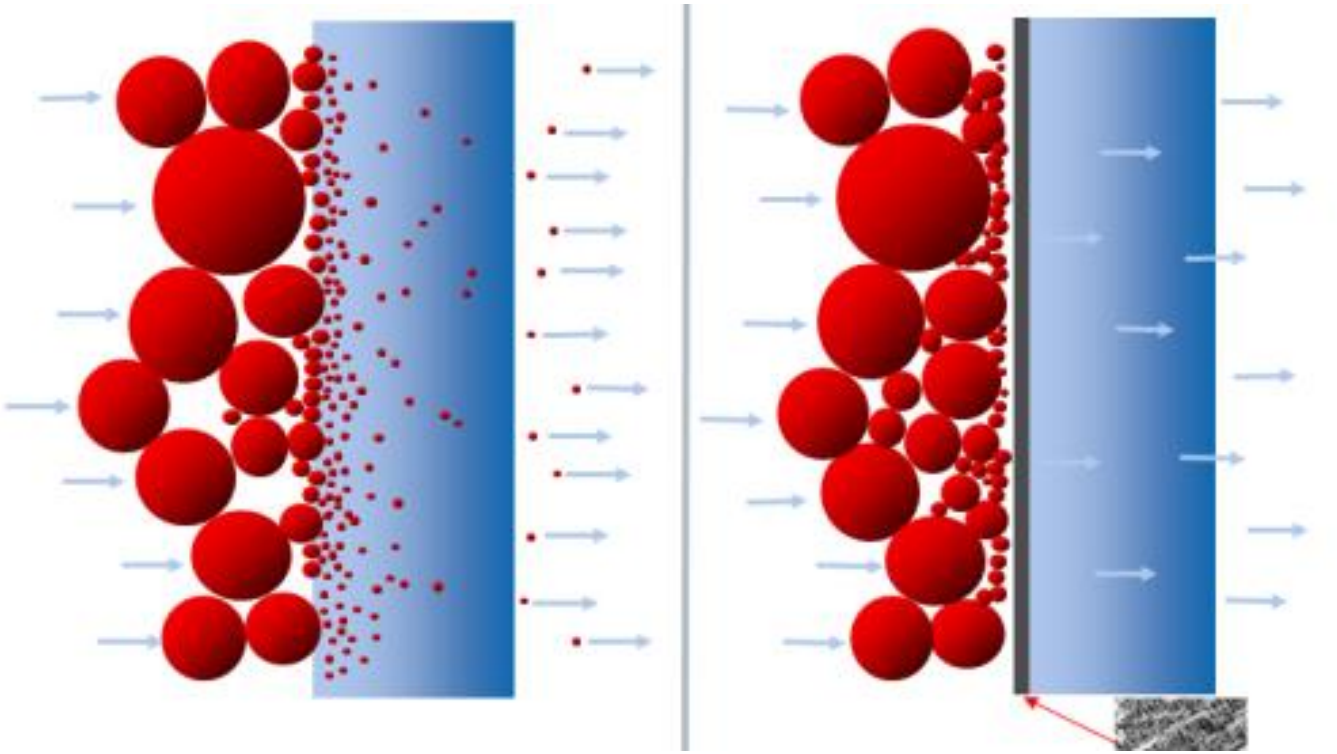
- Routine requalification program containing at least:
  - **Annual requalification** of the sterilisation process (for example, heat distribution on representative load(s), determination of min. F_0 values), at least annually.
  - **Preventive maintenance program** giving the scheduled maintenance measures required, SOP's for their performance, responsibilities, requirements for documentation.
  - **Change control** procedure specifying under what circumstances a re-validation is needed e.g repairs.

Sterile Filtration Basics

Useful References

- PIC/S Guide to Good Manufacturing Practices - PE 009 – 2014 – Annex 1
- PDA Technical Report No.26: Sterilizing Filtration of Liquids
- PDA Technical Report No.40: Sterilising Filtration of Gases
- FDA Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice 2004)

Filter Types



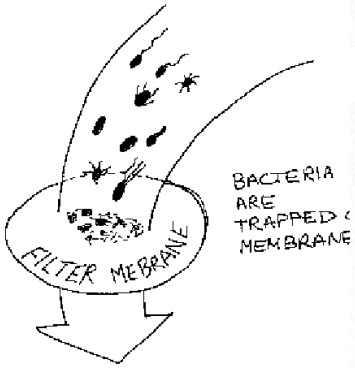
Depth Filter

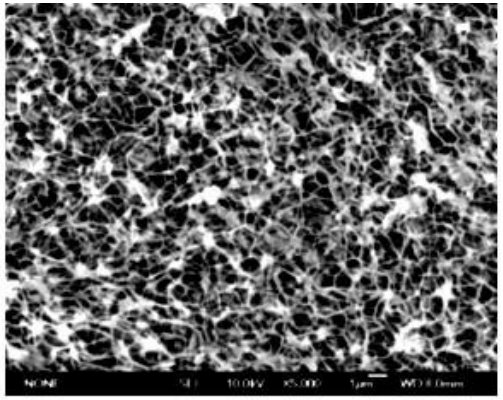
Membrane Filter

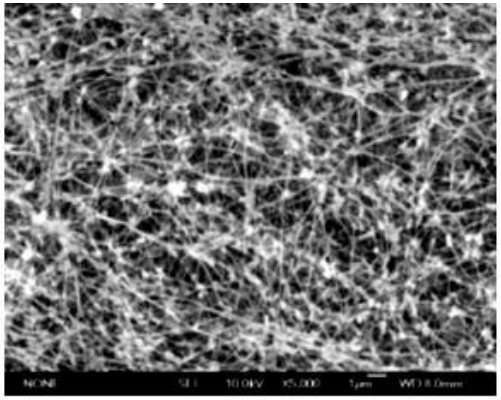
Membrane Filters

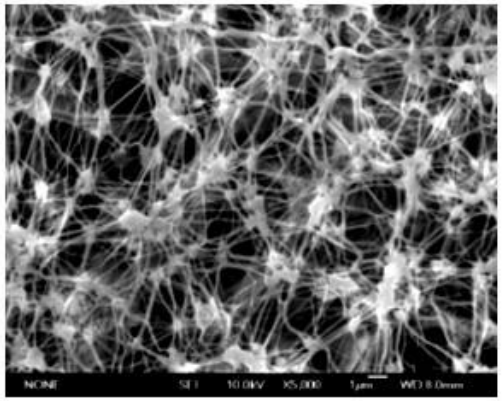
- Thin polymer films that have many microscopic pores which can be of different pore sizes (0.1, 0.22, 0.45 etc)
- Retain microorganisms by sieving, entrapment or adsorption (or a combination thereof) e.g.
  - Size exclusion (combination of sieving and entrapment); is very reliable
  - Size of filter pores required to screen out:
    - Yeast 0.45 -1.2 µm
    - Bacteria 0.2 µm
    - Viruses and mycoplasmas 0.01-0.1µm
- Membrane filtration is usually employed for heat-sensitive products;
- Most are hydrophobic in nature

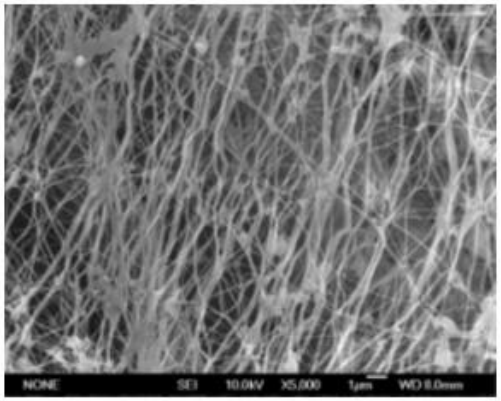
Examples of Membrane Filters











Filter Selection and System Design Criteria

- Retention Capability
- Integrity Testing
- Filtration Rate and throughput
- Materials of construction
  - Hydrophobicity
  - Durability
  - Toxicity
  - Leachables / Extractables
  - Particle Shedding
  - Gas/Filter Compatibility
- Water Blockage
- Design Consideration for Condensation Control

Applications

- Sterilising (Membrane/Cartridge/Disc) filters are used in pharmaceutical manufacture for:
  - Bulk Product Filtration
  - Steam Sterilisation in place (SIP)
  - Gas Filters
  - Vent Filters

- Other (Depth) filters are used for:
  - Clarifying bulk product
  - Reducing bioburden and filtering viruses (nano-filtration)
  - Reducing endotoxin (positively charged filters)

General Principles from PIC/S GMP- Annex 1

- Filtration alone is not considered sufficient when sterilisation in the final container is possible.
- If product cannot be sterilised in the final container, solutions or liquids can be filtered:
  - Through a filter of nominal pore size of 0.22 micron or less
  - Into a previously sterilised container
- Such filters can remove most bacteria and moulds but NOT all viruses or mycoplasmas
- Consideration should be given to complementing the filtration process with some degree of heat treatment

General Principles
PIC/S GMP- Annex 1

- For products which do not undergo terminal sterilisation, a second further filtration (double filtration) is recommended:**
  - immediately prior to filling
  - as close as possible to the filling point

- Fibre shedding characteristics should be minimal

** This is also an FDA recommendation

General Principles
PIC/S GMP- Annex 1

- Filter integrity should be verified **before and immediately after** use by:
  - Bubble point, or
  - Diffusive flow or
  - Pressure hold test

- The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter should be determined during validation and any significant differences from this during routine manufacturing should be noted and investigated.
- Integrity of critical gas and air vent filters should be confirmed after use.
- Integrity of other filters at appropriate intervals.

General Principles
PIC/S GMP- Annex 1

- The same liquid filter should not be used for more than one working day unless such use has been validated.

- The filter should not affect the product by removal of ingredients from it or by release of substances into it**

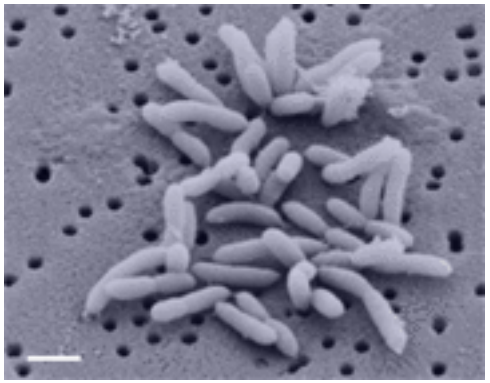
** *this often requires leachables and exractables studies to verify the suitability of a filter under conditions of use.*

Filtration Time Limits

- Time limits should include, for example, the period between the start of bulk product compounding and its sterilization, filtration processes …etc.
- The time limits established for the various production phases should be supported by data.
- Bioburden and endotoxin load should be assesse when establishing time limits for stages such as the formulation processing stage.
- The total time for product filtration should be limited to an established maximum to prevent microorganisms from penetrating the filter.
- Such a time limit should also prevent a significant increase in upstream bioburden and endotoxin load.

Filtration Efficacy

- A sterilizing grade filter should be validated to reproducibly remove viable microorganisms from the process stream, producing a sterile effluent.
- Currently, such filters usually have a rated pore size of 0.2 µm or smaller.
- Use of redundant sterilizing filters should be considered in many cases.
- Validation should include microbiological challenges. The microorganism *Brevundimonas diminuta* (ATCC 19146) is generally used
- A challenge concentration of at least 10^7 organisms per cm^2 of effective filtration area



Pre- Filtration Bioburden Requirements

- "Since the effectiveness of the filtration process is also influenced by the microbial burden of the solution to be filtered, the **determination of the microbiological quality** of solutions prior to filtration is an important aspect of the validation of the filtration process in addition to the establishment of the other parameters of filtration procedure, such as pressures, flow rates, and filter unit characteristics." *USP*

- Universally accepted that pre-sterilisation bioburdens are monitored – consensus limit is < 10cfu/100mL

What we know about filtration

- Products can alter the size of micro-organisms
- Osmotic pressure, pH can change organism size
- Large incident bioburden can cause grow-through
- Filters have limited number of retentive pores – once pores are saturated can get “breakthrough”
- Industry evidence of very small micro-organisms
- Experiences of penetration of very small bacteria through 2 in series 0.2micron filters

GMP Records

- Must record the integrity testing of filters in the batch record – filtration is generally a critical step – generally a printout verified by dated signature.
- The limits should be included in the record
- The limits should reflect the validation reports
- If there are initial failures this must be recorded as a deviation – even if resolved.
- Integrity testing devices must be qualified

Filter Validation Studies

Supplier Responsibility

- Show correlation between integrity test result and P. diminuta reduction
- Provide instructions, specifications and limits for test
- Determine bubble point of product compared to water

User responsibility

- Prove sensitivity of test in situ
- Perform test in accordance with test specifications
- Record integrity test results
- Provide product samples for bubble point ratio determination

- Require a protocol – supplied by vendor and approved by client
- Methodology: ASTM F838-83 standards or comparable
- Must use product to do the microbial challenge
- Once conditions established in the laboratory same conditions used in use
- Integrity limits established and verified after each use.

The following table (adapted from Carleton & Agalloco[1]) lists the elements that comprise a sterile filtration validation study.

[1] Validation of Pharmaceutical Processes Sterile Products 2nd Ed Carleton & Agalloco

Validation Element	Filter Manufacturer	Filter User
Filter Reproducibility	Validation	Qualify Manufacturer
Sterilisation	Provide Recommendation	Operate within recommendation
		Validation
Integrity Test	Provide procedures and limits	Follow manufacturer's procedures
	Correlate test with bacterial retention	Validation
		Perform integrity ratio work if wetting with product
Operation	Provide Limits for operating, temperature, pressure	Establish operating parameters within the limits provided
Bacterial Retention	Validation	Provide product/process details
		Review and Authorise Report
Extractables	Validation	Provide product/process details
		Establish and document acceptance criteria
		Review and Authorise report
Compatibility	Provide information on materials	Document compatibility
		Perform Studies
Adsorption / binding	Provide information regarding known issues	Perform Studies
Particulates	Provide data for removal	Verify limits are achieved
Fibers	Meet non-fibre releasing claim (21CFR 210.3 b(6))	Preflush filters according to recommendations
Endotoxins	Perform analysis	Verify low endotoxins from filters
Toxicity	Perform testing (Class VI plastics, cytotoxicity) and provide results	Obtain results and reports.
		Review and document conclusions

In Conclusion

- These guys are you best friends

CBE

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