Sterilization - validation, qualification requirements

Dawn Tavalsky

Sterilization - Overview

Objectives
- Discuss definition of “Sterile”
- Briefly describe sterilization methods
- Describe approaches to be used for the validation of a sterilization process using Moist Heat as an example
- Describe requirements for routine monitoring and control of sterilization
- Review issues that are specific to other sterilization processes
Sterile Products - Overview

• Certain pharmaceutical products must be sterile
  – injections, ophthalmic preparations, irrigations solutions, haemodialysis solutions

• Two categories of sterile products
  – those that can be sterilised in final container (terminally sterilised)
  – those that cannot be terminally sterilised and must be aseptically prepared

Sterilization - Overview

What is the definition of “sterile”?

• Free from microorganisms
  In practice no such absolute statement regarding absence of microorganisms can be proven

• Defined as the probability of 1 in a million of a container being contaminated \((10^{-6})\)

• This referred to as the Sterility Assurance Level (SAL)

• Organisms are killed in an exponential fashion
Definition of “Sterile”

Resistance of an organism is referred as its “D-value”

D-value - Time (or dose) required to reduce the population of organisms by 1 log (or 90%)
Definition of “Sterile”

• A sterilization process must deliver a Sterility Assurance Level (SAL) of 1 in a million (10^-6)
• It is not possible to measure “10^-6”
• The required SAL can be achieved by applying a process that will reduce the number of organisms to zero and then apply a safety factor that will deliver an extra 6 log reduction

Definition of “Sterile”

Example
• For an initial bioburden of 10^2 the sterilization process will need to achieve an 8 log reduction in viable organisms
• This will require 8 times the D-value (e.g. if the organism has a D value of 2 minutes then 8 x 2 = 16 minutes will be required to achieve an 8 log reduction and an SAL of 10^-6) (Point Z)
Sterilization - Overview

Commonly used methods of sterilization
- Moist Heat
- Dry Heat
- Gas (Ethylene oxide)
- Radiation (Gamma or Electron)
- Filtration
- Others - UV, Steam and formaldehyde, hydrogen peroxide

Moist Heat

- Saturated steam
- Common cycles:
  - 121°C for 15 minutes
  - 134°C for 3 minutes
  - Other cycles of lower temperature and longer time may be used (e.g. 115°C for 30 minutes)
- Used for sterilization of:
  - terminal sterilization of aqueous injections, ophthalmic preparations, irrigation & haemodialysis solutions, equipment used in aseptic processing
Moist Heat

- not suitable for non-aqueous/dry preparations
- preferred method of sterilization

Dry Heat

- Lethality due to oxidative processes
- Higher temperatures and longer exposure times required
- Typical cycles:
  - 160°C for 120 minutes
  - 170°C for 60 minutes
  - 180°C for 30 minutes
  - tunnels used for the sterilisation of glass vials may use much higher temperatures (300°) for a much shorter period
Dry Heat

- Used for:
  - glassware and product containers used in aseptic manufacture, non aqueous thermostable powders and liquids (oils)
- also used for depyrogenation of glassware (≥250°C)
  - (Pyrogens - substances found in cell wall of some bacteria which can cause fever when introduced into the body)

Ethylene Oxide Gas

- Either pure or in mixtures with other inert gases
- Requires presence of moisture
- Complex process
- Typical cycles:
  - 1-24 hours
  - 25-1200 mg/L gas
  - 25-65°C
  - 30-85% relative humidity
Ethylene Oxide

- Used for:
  - heat labile product containers
  - surface sterilization of powders
- Adequate aeration to reduce toxic residues

Radiation

- Gamma rays generated by Cobalt 60 or Caesium 137 radionuclides; or
- Accelerated electrons from an electron generator
- 25 kilograys (kGy) usual dose
  - dose dependent on bioburden (resistance of organisms not predictable)
- process must be properly validated
- used for:
  - dry pharmaceutical products
  - heat labile product containers
- can cause unacceptable changes
Filtration

- Removes organisms from liquids and gasses
- 0.2 - 0.22 micron for sterilization
- composed of cellulose esters or other polymeric materials
- filter material must be compatible with liquid being filtered
- used for bulk liquids, gasses and vent filters

Validation - Overview

- Selection of sterilization process must be appropriate for product
  - terminal sterilization is the method of choice
  - moist heat (autoclaving) is the most common process used for terminal sterilization
  - product must not be affected by heat
  - container/closure integrity must be established
  - items being sterilised must contain water (if sealed) or material must allow for removal of air and penetration of steam for steam (moist heat) sterilization
Validation - Protocol

Requirements for Moist Heat Sterilization

Other processes follow similar requirements

• Validation protocol should include the following details for each sterilization process
  – process objectives in terms of product type, container/closure system, SAL required
  – specifications for time, temperature, pressure and loading pattern
  – description of all equipment and support systems in terms of type, model, capacity and operating range

Validation - Protocol

Moist Heat continued:

– performance characteristics of all equipment e.g. pressure gauges, valves, alarm systems, timers, steam flow rates/pressures, cooling water flow rates, cycle controller functions, door closure gasketing and air break systems and filters
– methodology for monitoring performance of equipment and the process and labatory testing methodology
– personnel responsible for all stages and final evaluation (should have experience and necessary training and be authorized)
Validation - Calibration

• Laboratory testing should be performed by a competent laboratory, methodology should be documented

• All instruments must be calibrated e.g.
  – temperature recorders and sensors
  – thermocouples
  – pressure sensors for jacket and chamber
  – timers
  – conductivity monitors for cooling water
  – flow metres for water/steam
  – water level indicators when cooling water is used
  – thermometers including those for thermocouple reference, chamber monitoring and laboratory testing

Validation - Calibration

• Indicators should be calibrated
  – physical and chemical indicators should be tested to demonstrate acceptable response to time and temperature
  – biological indicators should be tested for count and time/temperature exposure response
    • for commercial indicators - test certificate with count and D-value and exposure response should be available. Results acceptable if verified “in house” periodically.
    • In house indicators must be fully characterized (D-value, identification) and appropriate for sterilization process

All indicators should be appropriately stored and within expiry
Validation - Cycle Development

• Concept of $F_0$
  – Lethality factor equivalent to time at 121°C
    • 1 minute at 121°C is equivalent to $F_0$ of 1.
    • Lethality can accumulate during heat up and cool down phases

Typical temperature profile of a heat sterilization process

What would be the $F_0$ of a cycle at 121°C for 15 minutes?

Validation - Cycle Development

• $F_0$ is calculated using the following equation:

$$F_0 = \Delta t \Sigma 10^{(T-121)/Z}$$

where:

• “$\Delta t$” is the time interval between measurements of temperature (T)
• “T” is the temperature of sterilised product at time (t)
• “Z” is a temperature coefficient which measures the number of degrees required to change the D-value of an organism by 1 log
Validation - Cycle Development

• The minimum $F_o$ required by a sterilization process is related to the resistance of the bioburden (D-value)

$$F_o = D_{121} (\log A - \log B)$$

where:

• "$D_{121}$" is equal to the time at 121°C to reduce the population of the most resistant organism in each product container by 90% (or 1 log)
• "A" is the number of microorganisms per container
• "B" is the maximum acceptable probability of survival (Sterility Assurance Level, 10^-6)

Validation - Cycle Development

• Two approaches to sterilization
  – Overkill
  – Probability of survival

• Overkill approach used when the product can withstand excessive heat treatment without adverse effects
  – Cycle should deliver an $F_o$ of at least 12
    • This will achieve a 12 log reduction of microorganisms with a D-value of 1 minute
    (Assuming each product unit contains $10^6$ organisms a 12 log reduction will result in $10^4$ organisms per unit or probability of survival (SAL) of 1 in a million)
    (Normal bioburden is usually much lower and the organisms normally much less resistant than this)
**Validation - Cycle Development**

**Biological Indicators**

- device consisting of a known number of microorganisms, of a known resistance to a particular sterilization process in or on a carrier and enclosed in a protective package.
  - Organisms are in the form of endospores (not vegetative state) as these are most resistant to sterilization

<table>
<thead>
<tr>
<th>Organism</th>
<th>D-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Geobacillus stearothermophilus</em> (most common)</td>
<td>1 - 2.8</td>
</tr>
<tr>
<td><em>Bacillus coagulans</em></td>
<td>0.3</td>
</tr>
<tr>
<td><em>Clostridium sporogenes</em></td>
<td>0.8 – 1.4</td>
</tr>
<tr>
<td><em>Bacillus atropheus</em></td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Validation - Cycle Development**

- Spore Strips - a narrow strip of fibrous paper impregnated with a bacterial spore suspension contained in a glassine envelope
- Spore Dots - Circular pieces of fibrous paper impregnated with the spore suspension
- Spore Suspensions - pure spore suspension of the desired challenge organism which can be inoculated onto the surface of a material
- Self contained units containing spore strips or suspensions and the media in which they are to be incubated (simple/convenient to use)
Validation - Cycle Development

- Probability of Survival approach used used for heat labile products

The process is validated to achieve a destruction of the presterilization bioburden to a level of 10⁰ (Point Y), with a minimum safety factor of an additional six-log reduction (Point Z)

Determination of the minimum $F_o$ required is based on the bioburden and its heat resistance

What $F_o$ would be required for a bioburden of 10² (100) if D-value was 1?

Validation of Sterilization

Basic Principles

- Installation Qualification (IQ)
  - Ensuring equipment is installed as per manufacturer’s specification

- Operation Qualification (OQ)
  - Ensuring equipment, critical control equipment and instrumentation are capable of operating within required parameters

- Performance Qualification (PQ)
  - Demonstrating that sterilizing conditions are achieved in all parts of sterilization load
    - Physical and microbiological
Validation - Equipment

Installation Qualification
• Ensuring equipment is installed as per manufacturer’s specification
  – considerations for new and existing equipment
  – specifications for the type of autoclave, construction materials, power supplies and support services, alarm and monitoring systems with tolerances and accuracy requirements
  – for existing equipment documented evidence that the equipment can meet process specifications

Validation - Equipment

Operational Qualification
• Ensuring equipment, critical control equipment and instrumentation are capable of operating within required parameters
• Three or more test runs which demonstrate
  – controls, alarms, monitoring devices and operation indicators function
  – chamber pressure integrity is maintained
  – chamber vacuum is maintained (if applicable)
  – written procedures accurately reflect equipment operation
  – pre-set operation parameters are attained for each run
Validation - Performance

Performance Qualification

• Demonstrating that sterilizing conditions are achieved in all parts of sterilization load
• Physical and microbiological

Physical

• Heat distribution studies on empty chamber
  – maximum and minimum cycle times and temperatures
  – to identify heat distribution patterns including slowest heating points
  – multiple temperature sensing devices should be used (thermocouples)
  – location of devices should be documented and ensure that heat distribution is uniform

Performance Qualification - Physical (2)

• Heat distribution of maximum and minimum chamber load configurations
  – multiple thermocouples throughout chamber (not inside product containers) to determine effect of load configuration on temperature distribution
  – temperature distribution for all loads using all container sizes used in production should be tested
  – position of thermocouples should be documented
  – Slowest to heat/cold spots in each run should be documented, including the drain
  – repeat runs should be performed to check variability
  – temperature distribution profile for each chamber load configuration should be documented
Validation - Performance

Performance Qualification - Physical (3)

• Heat penetration studies to detect the maximum and minimum temperature within all loads
  - all parts of each load must be on contact with steam
  - need to determine lowest and highest temperature locations and slowest and fastest to heat locations (measured inside product containers)
  - need to consider all variables such as container size, design, material, viscosity of solution and fill volume.
    Container with maximum fill volume and slowest to heat solution should be used
  - maximum and minimum load configurations for each sterilization cycle using routine cycle parameters

Validation - Performance

Performance Qualification - Physical (4)

• Heat penetration (2)
  - May be necessary for container mapping for larger volumes - cold spot then used for penetration studies
  - Need to consider effects of packaging e.g. overwrapping
  - Three runs performed once cold spots have been identified to demonstrate reproducibility
Validation - Performance

Performance Qualification - Microbiological

• Biological challenge studies
  – used when Probability of Survival approach is used
  – may not be necessary when cycle is > 121°C for 15 minutes (except US and Australia)
  – biological indicators (BI) containing spores of *Geobacillus stearothermophilus* are most commonly used (considered “worst case”). BIs containing other organisms may be used
  – performance studies based on product bioburden require a considerable amount of work
  – indicators should be placed throughout the load, adjacent to thermocouples, at “cold spots” and slowest to heat locations (identified during heat penetration studies)
  – any growth is unacceptable unless processing errors demonstrated

Validation - Performance

• Validation report must demonstrate requirements in Validation protocol have been met, any deviations must be justified

• Requalification must be repeated on an annual basis (usually one run is acceptable)

• Any changes or modifications must be evaluated
  – may just require requalification
  – any changes to loading patterns, new container/closure systems or cycle parameters require full validation
Routine Production

Issues considered for routine production

• Manufacturing environment should be controlled

• Procedures in place to minimize the pre-sterilization bioburden
  – bioburden limits specified (although not so important when “overkill” cycle used)

• Time between filling and sterilization should be specified

• Integrity of container/closure system should be periodically verified

• Periodic leak testing of chamber (if vacuum is part of cycle)

Routine Production

• Cooling water should be sterile

• Differentiation between sterilized and not-yet sterilized product
  – Physical separation (double ended autoclave)
  – Labelling and use of visual indicators (e.g. autoclave tape)

• Periodic testing of containers to verify integrity of container/closure system

• Quality of steam should be defined and periodically tested for contaminants
Routine Production

- Each sterilization cycle must be monitored
  - temperature, time and pressure recorded
  - temperature recorder independent from cycle controller
  - second independent temperature recorder
  - drain temperature should be recorded
  - chemical and biological indicators (if applicable)

- Sterilisation cycle records should form part of batch records

Other Sterilization Processes

Sterilization using other processes should follow a similar approach as that described for moist heat
- Validation protocol
- Equipment calibration
- Determining the process that will deliver the desired SAL (10^-6)
- IQ, OQ, PQ
- Requirements for routine monitoring and control
Other Sterilization Processes

Dry Heat

- Should have air circulation in the chamber
- Positive pressure in the chamber to prevent entry of non-sterile air
- HEPA filtered air supplied
- Biological indicators containing *Bacillus atropheus* (if used)
  - removal of endotoxin is usually sufficient
- When removing pyrogens need to validate process using challenge tests

Other Sterilization Processes

Radiation

- Usually performed by contracting service (need to ensure validation status, responsibilities)
- Based on bioburden of product being sterilised
  - Biological indicators may be used as additional control but may not be as resistant as naturally occurring bioburden
- Method defined in International Standard ISO 11137
Other Sterilization Processes

Radiation (2)
- Correct dose of radiation (~25 kGy) received by all products (measured with dosimeters)
  - quantitative measurement
  - number, location, within calibration time-limit
- Radiation sensitive colour discs applied to packaging
- procedures to distinguish irradiated and non-irradiated materials
- Variation in density of packaging should be addressed during validation

Other Sterilization Processes

Gasses and Fumigants
- e.g. ethylene oxide, hydrogen peroxide vapour
- Only when no other method is suitable
- Must demonstrate that process does not adversely affect product
- Packaging must be able to permit ingress of gas and humidity
- Ensure product load is adequately heated and humidified prior to sterilization (called “conditionning”)
  - need to take into account validation performed in summer or winter
Other Sterilization Processes

Gasses and Fumigants (2)
- Temperature distribution is acceptable
- Concentration of sterilant gas is sufficient
- Use of biological indicators is important (Bacillus atropheus)
- Half cycles
  - If cycle of half normal time destroys biological indicators (10⁶ organisms), double time will achieve SAL of 10⁻⁶
- Aeration
  - Ventilated conditions
  - Defined limits of residuals
  - Process included in validation
- Safety and toxicity issues considered

Useful Publications

- ISO/EN 17665 - Sterilization of health care products - Moist Heat (Parts 1 and 2)
- ISO/EN 11135 - Sterilization of health care products - Ethylene Oxide (Parts 1 and 2)
- ISO/EN 11137 - Sterilization of health care products - Radiation (Parts 1,2 and 3)
Questions?