Sterilization - validation, qualification requirements

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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

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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

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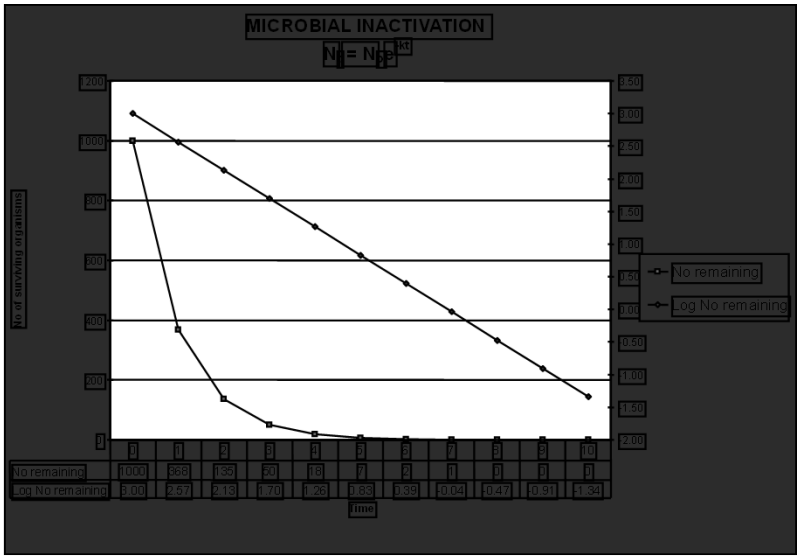
Definition of “Sterile”



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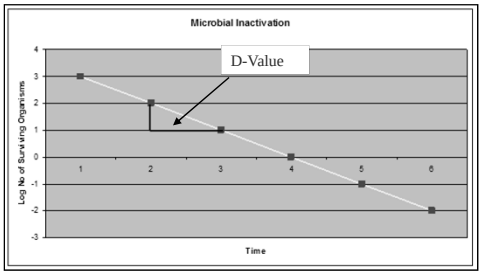
Definition of “Sterile”

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%)

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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

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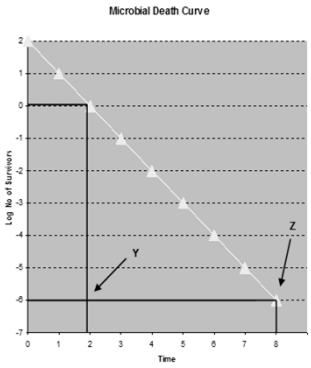
Definition of "Sterile"

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)



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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**

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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**

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Moist Heat

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

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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

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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)**

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Ethylene Oxide Gas

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

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Ethylene Oxide

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

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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

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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

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Validation - Overview

- Selection of sterilzation 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

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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**

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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)**

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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

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Validation - Calibration

- Indicators should be calibrated
  - physical and chemcial 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

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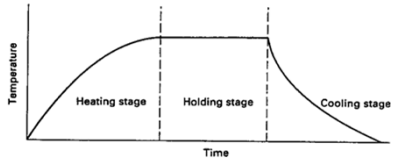
Validation - Cycle Development

- **Concept of F_o**
  - **Lethality factor equivalent to time at 121°C**
    - **1 minute at 121°C is equivalent to F_o of 1.**
    - **Lethality can accumulate during heat up and cool down phases**



Typical temperature profile of a heat sterilization process

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

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Validation - Cycle Development

- **F_o is calculated using the following equation:**

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

  where:
  - **"Δt" 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**

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Validation - Cycle Development

- **The minimum F_o required by a sterilzation process is related to the resistance of the bioburden (D-value)**

  $F_o = D_{121}$ (LogA - 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 microoganisms per container**
  - **"B" is the maximum acceptable probability of survival (Sterility Assurance Level , 10^{-6})**

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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^{-6} 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)

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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**

Organism	D-Value
Geobacillus stearothermophilus (most common)	1 - 2.8
Bacillus coagulans	0.3
Clostridium sporogenes	0.8 – 1.4
Bacillus atropheus	0.5

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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 inocluated 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)

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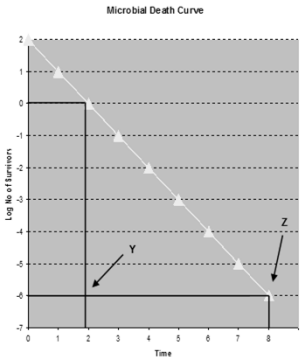
Validation - Cycle Development

- **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 10^0 (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 10^2 (100) if D-value was 1?

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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 Qualifcation (PQ)**
  - **Demonstrating that sterilizing conditions are achieved in all parts of sterilization load**
  - **Physical and microbiological**

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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**

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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**

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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
  - mulitple temperature sensing devices should be used (thermocouples)
  - location of devices should be documented and ensure that heat distribution is uniform

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Validation - Performance

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, inlcuding the drain
  - repeat runs should be performed to check variability
  - temperature distribution profile for each chamber load configuration should be documented

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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**

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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**

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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 demonsrated**

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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**

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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)**

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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**

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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 biololgical indicators (if applicable)
- Sterilisation cycle records should form part of batch records

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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

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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**

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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 occuring bioburden**
- **Method defined in International Standard ISO 11137**

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Other Sterilization Processes

Radiaton (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

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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

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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 (10^6 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**

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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)**
- ***"Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and ongoing Control.* PDA Technical Report No. 1 Revised 2007**

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Questions?

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