Terminal Sterilization

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Disclosure Statement

- Dr. Ross Caputo has no relevant financial relationship(s) with ineligible companies to disclose.

  and

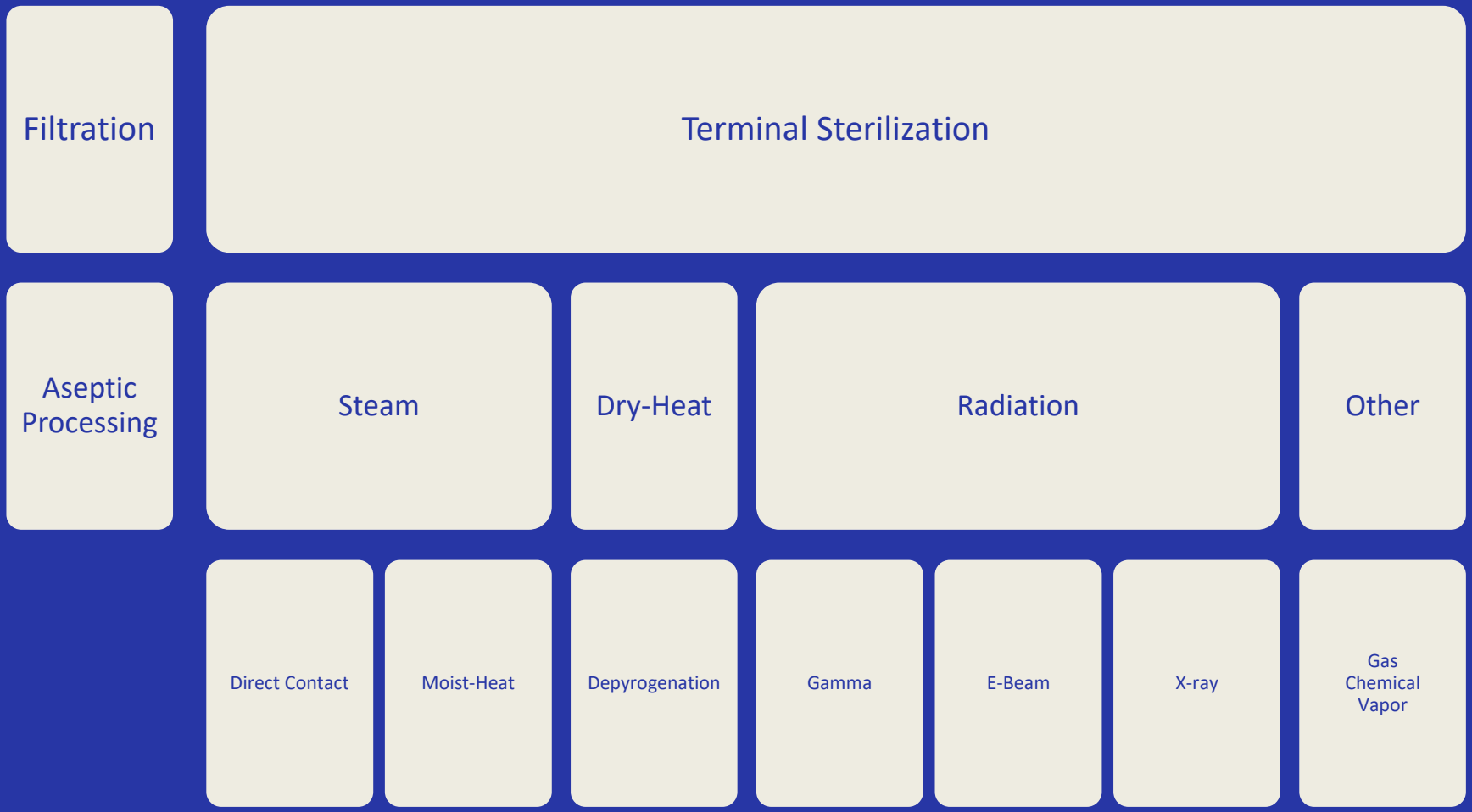
- None of the planners for this activity have relevant financial relationships with ineligible companies to disclose.

Learning Objectives

At the completion of this activity, the participant will be able to:

1. Describe the parameters of terminal sterilization using steam and radiation sterilization.
2. Explain the requirement for validation, 10^{-6} PNSU (probability of a nonsterile unit).
3. Define PNSU based upon bioburden.

Types of Sterilization



Types of Sterilization

Sterilization by Filtration

- A process whereby a solution is sterilized via filtration through bacterial retention. The product is subsequently aseptically filled in a controlled environment.

Terminal Sterilization (Preferred)

- *"A process whereby product is sterilized within its sterile barrier system."*

Method of Sterilization

- Factors to consider
  - Nature of drug product and components
    - Physicochemical properties
    - Stability
    - Dosage form
  - Container-closure system

Common Applications

Terminal Sterilization (Preferred)

- Steam
  - Porous/hard goods
  - Aqueous CSPs in final CCS
- Dry-heat
  - Nonaqueous CSPs
  - Not compatible with steam
- Radiation
  - Raw material
  - Implant/pellets
  - Medical devices

Filtration

- Solutions or packaging incompatible with terminal sterilization

Filter Sterilization/Aseptic Processing

- Time limit
  - 6 hours
- Filter requirements
  - Sterile, non-pyrogenic
  - Nominal pore size ≤ 0.22 µm
  - Pharmaceutical grade
  - Low-shedding
  - Retain 10^7 *Brevundimonas diminuta*
- Bubble point
  - Water vs alcohol
- Single filter
  - Pre-filter

- Considerations
  - Volume capacity
  - Compatibility
  - Conditions
    - Pressure, temperature, flow rate
  - Process Validation
    - Media-fill test
  - Filter Validation
    - Bacterial challenge test
    - Physicochemical compatibility
    - Product-specific bubble point

Insanitary Conditions

Using a filter for the purposes of product sterilization that is not certified as both sterilizing-grade and pharmaceutical-grade, or using a sterilizing-grade and pharmaceutical-grade filter in a manner that is not adequate to accomplish sterilization (e.g., using it in excess of its capacity or when it is clogged)

Using a filter in drug production whose integrity is compromised; failing to conduct post-use filter-integrity testing on filters used to sterilize products

Using a particle-shedding filter in any stage of sterile drug production

Using parameters for sterilization (e.g., temperature, pressure, and time) that are not lethal to resistant microorganisms

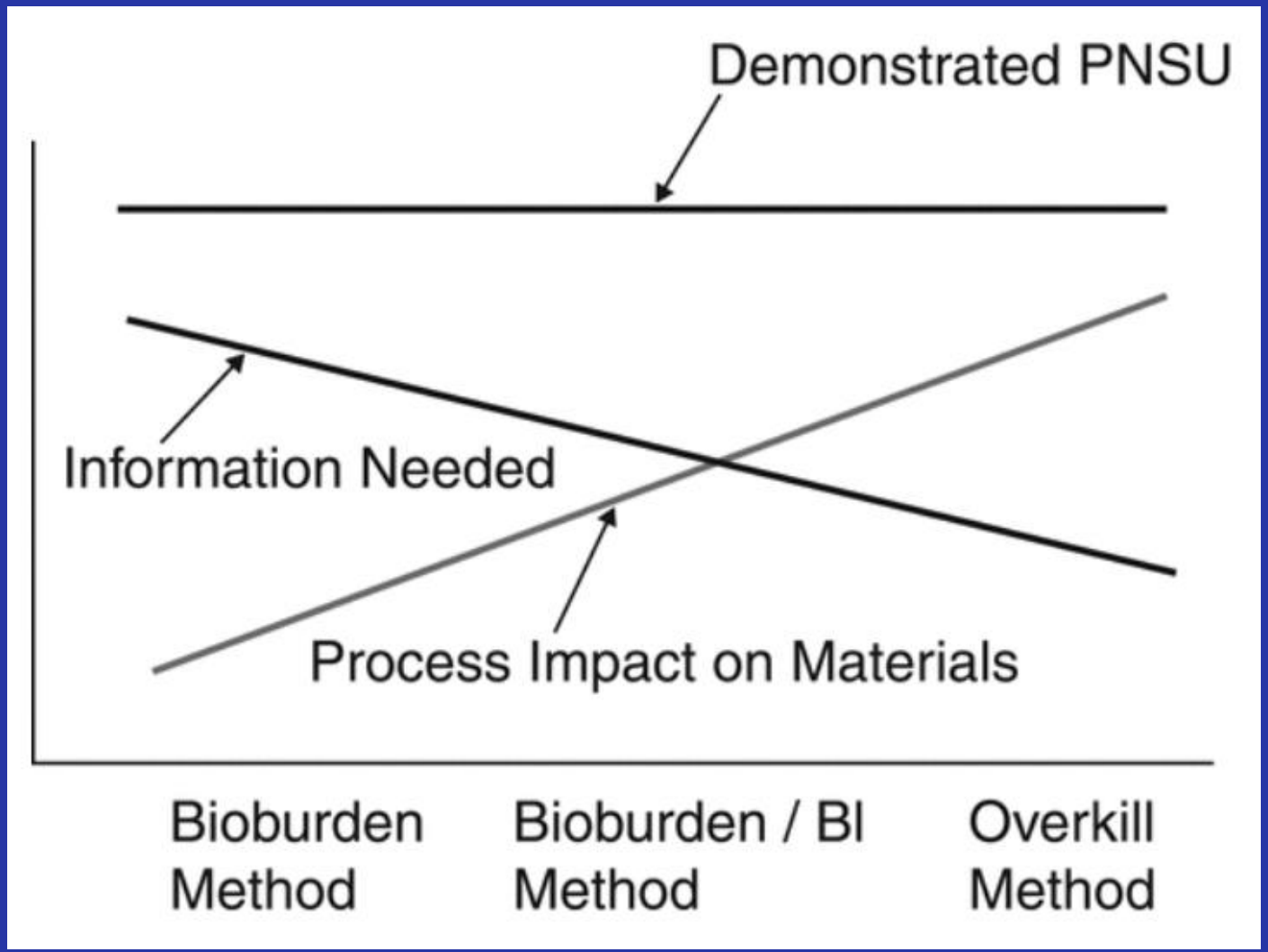
Terminal Sterilization Specifics

Sterility Assurance Level (SAL)

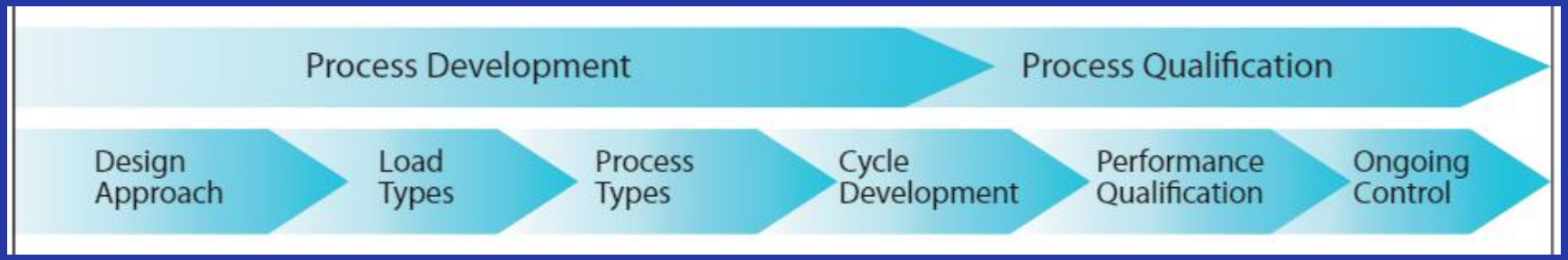
- Probability of a nonsterile unit (PNSU)
  - Probability that ≤ 1 unit in 1 million is nonsterile
- Lethality (F_0)
  - Measure of sterilization effectiveness

$$F_0 = \int_{t1}^{t2} 10^{\left(\frac{T-121}{10}\right)} dt = \sum_{t1}^{t2} 10^{\left(\frac{T-121}{10}\right)} \Delta t$$

Sterilization Approach



Sterilization Science



General Cycle Development

1. Determine load type
   - Liquid (moist heat)
   - Porous/hard goods (direct contact)
   - Nonaqueous CSP (dry-heat)
2. Determine cycle parameters
   - Time, temperature, F-value
3. Define load and perform mapping studies
   - Minimum and maximum configuration
   - Thermocouple and BI location based on mapping

Temperature Mapping

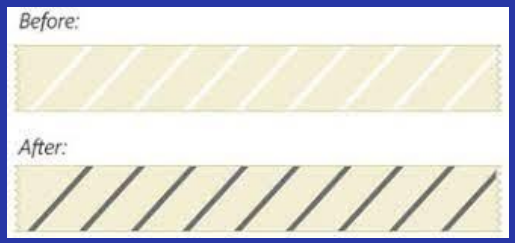
- Thermocouple location(s)
  - Geometric pattern throughout loading zone
- Evaluate worst-case locations
  - Slowest-to-heat
  - Cold spot (lowest lethality or temperature)
  - Hot spot

Load Configuration

Indicators and Integrators

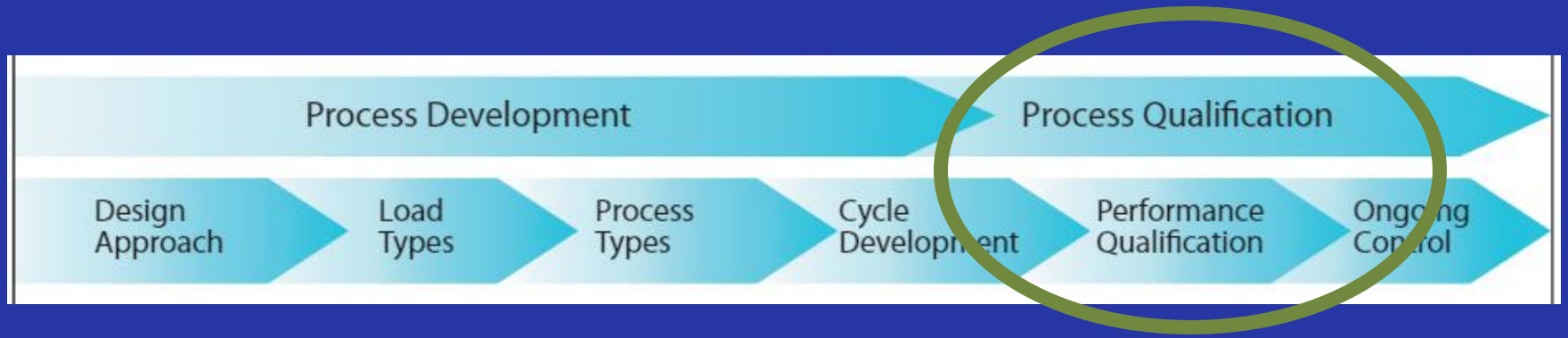
Biological Indicators

Sterilization Indicators/Integrators



<1229> Sterilization of Compendial Articles

*"Sterility can be accomplished only by the use of a **validated** sterilization process under appropriate current good manufacturing practices and cannot be demonstrated by reliance on sterility testing alone."*


Process Development
Process Qualification
Design Approach
Load Types
Process Types
Cycle Development
Performance Qualification
Ongoing Control

Validation Approach

- Performance process qualification
  - Physical
    - Temperature distribution
    - Heat penetration
    - F-value
  - Biological indicator challenge
    - Direct contact or overkill method: *Geobacillus stearothermophilus*
    - Moist-heat: *Clostridium sporogenes* and *Bacillus subtilis*
    - Dry-heat: *Bacillus atrophaeus*
      - Depyrogenation: endotoxin challenge vials
- Acceptance Criteria
  - Minimum and maximum exposure time, temperature, pressure, lethality, and variation
  - Successful BI challenge
  - Triplicate

USP <797> and Steam Sterilization

- *"The description of steam sterilization conditions and duration for specific CSPs shall be included in written documentation in the compounding facility. The effectiveness of steam sterilization shall be verified using appropriate Bis of Bacillus stearothermophilus (see Biological Indicators for Sterilization <1229.5>) and other confirmation methods such as temperature-sensing devices (see Sterility Assurance <1211> and Sterility Tests <71>)."*

Autoclave Sterilization

Steam Sterilization by Direct Contact

- Applications
  - Hard goods
  - Porous loads
- Typical cycle
  - 121° at 15 psi
  - 20-60 minute cycle
    - 12 minute exposure time

Moist-Heat of Aqueous Liquids

- Applications
  - Aqueous liquids (solutions, suspensions, emulsions) in final container-closure system
- Cycle
  - Product-specific

Autoclave Cycle Development

1. Determine load type
   - Liquid (moist-heat)
   - Porous/hard goods (direct contact)
2. Determine cycle parameters
   - Time, temperature, lethality (F_0)

$$F_0 = \int_{t1}^{t2} 10^{\left(\frac{T-121}{10}\right)} dt = \sum_{t1}^{t2} 10^{\left(\frac{T-121}{10}\right)} \Delta t$$

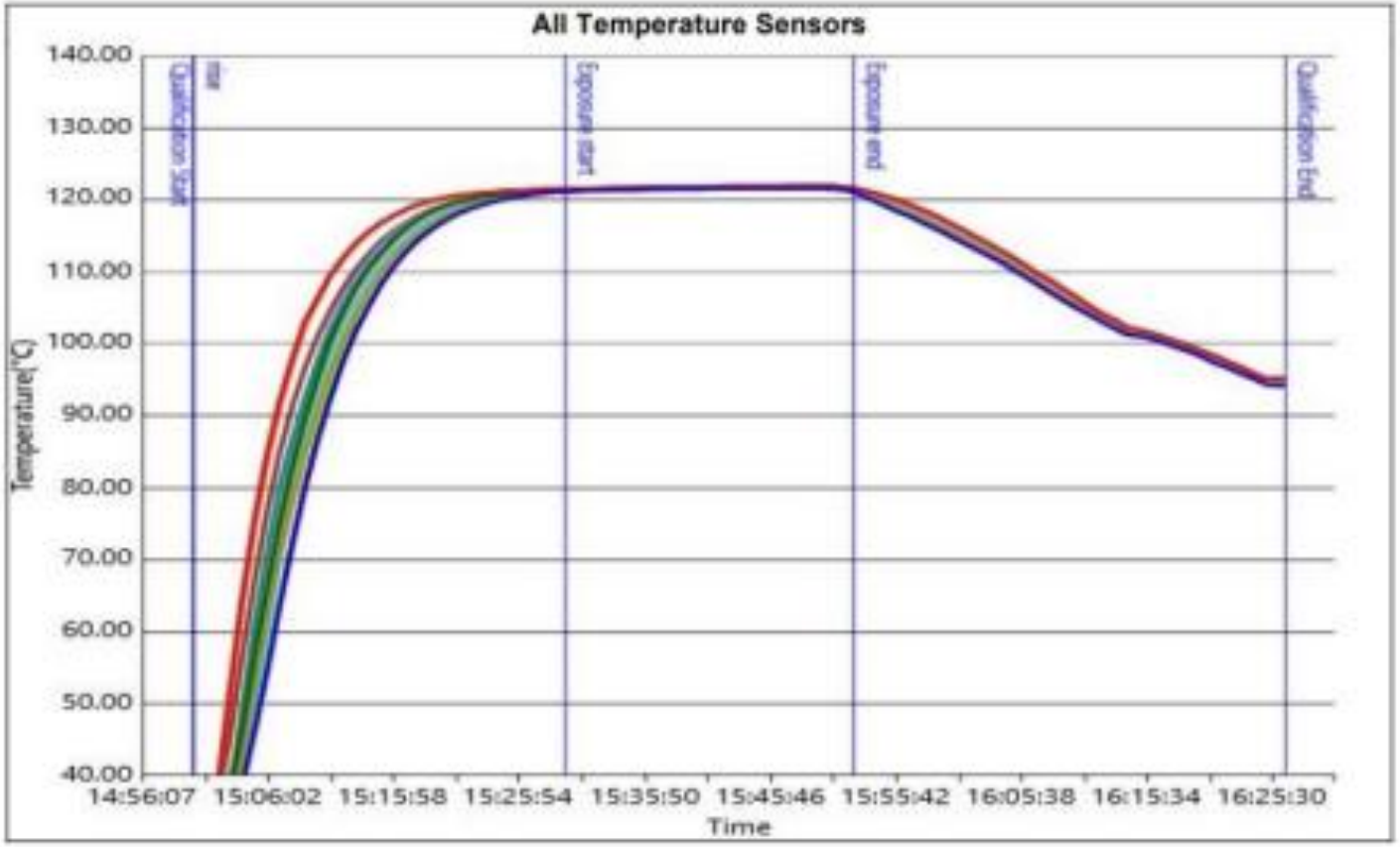
3. Define load and perform mapping studies
   - Minimum and maximum configuration
   - Thermocouple and BI location based on mapping

Autoclave Cycle Validation

- Define
  - Cycle parameters
    - Duration, temperature, and pressure
  - Load pattern
- Develop protocol
- Process cycle in triplicate
  - Monitor
    - Temperature distribution, heat penetration, time, pressure, lethality (F0)
  - Biological indicator challenge
    - *Geobacillus stearothermophilus*
- Evaluate against acceptance criteria
  - Temperature distribution
  - Heat penetration
  - Biological indicator
  - Stability, as applicable
- Document and summarize results

Autoclave Cycle Validation

$$F_0 = 10^{\left[\frac{T-121}{10}\right]}\Delta t$$



USP <797> and Dry-Heat Sterilization

- *"The description of the dry heat depyrogenation cycle and duration for specific load items shall be included in written documentation in the compounding facility."*
- *"The effectiveness of the dry heat depyrogenation cycle shall be verified using endotoxin challenge vials (ECVs)."*
- *The bacterial endotoxin test should be performed on the ECVs to verify the cycle is capable of achieving a 3-log reduction."*

Endotoxin - Oven Validation	
Endotoxin Control Vial 1 (Numerical Value):	170000 EU/mL
Vial 1 Numerical Value :	<1 EU/mL
Vial 1 Log Reduction:	5.230

Dry-Heat Sterilization

Product-Specific

- Applications
  - Nonaqueous products
- Exposure temperature

$$F_H = \int_{t1}^{t2} 10^{\left(\frac{T-170}{20}\right)} dt = \sum_{t1}^{t2} 10^{\left(\frac{T-170}{20}\right)} \Delta t$$

- Biological indicator
  - *Bacillus atrophaeus*

Depyrogenation

- Applications
  - Glassware, metal, instruments, containers, and chemicals
- 250°C for **30 minutes** or equivalent
  - F value ≥ 30

$$F_D = \int_{t1}^{t2} 10^{\left(\frac{T-250}{50}\right)} dt = \sum_{t1}^{t2} 10^{\left(\frac{T-250}{50}\right)} \Delta t$$

- Endotoxin challenge vials
  - ≥ 3-log reduction relative to control

Dry-Heat Cycle Validation

- Define
  - Cycle parameters
    - Time and temperature
  - Load configuration
    - Empty/minimum
    - Maximum
  - Preparation
- Challenge at worst-case location
  - Dry-heat resistant organism (*Bacillus atrophaeus*)
    - Depyrogenation – endotoxin challenge vials (ECVs)
      - Acceptance criteria
        - » ≥ 3-log reduction to control
- Execute
  - Protocol or SOP
    - Requalification requirements
  - Monitor temperature
- Evaluate
  - Temperature
    - $F_{D/H}$ (accumulated destruction)
  - Exposure time
  - ECV log reduction

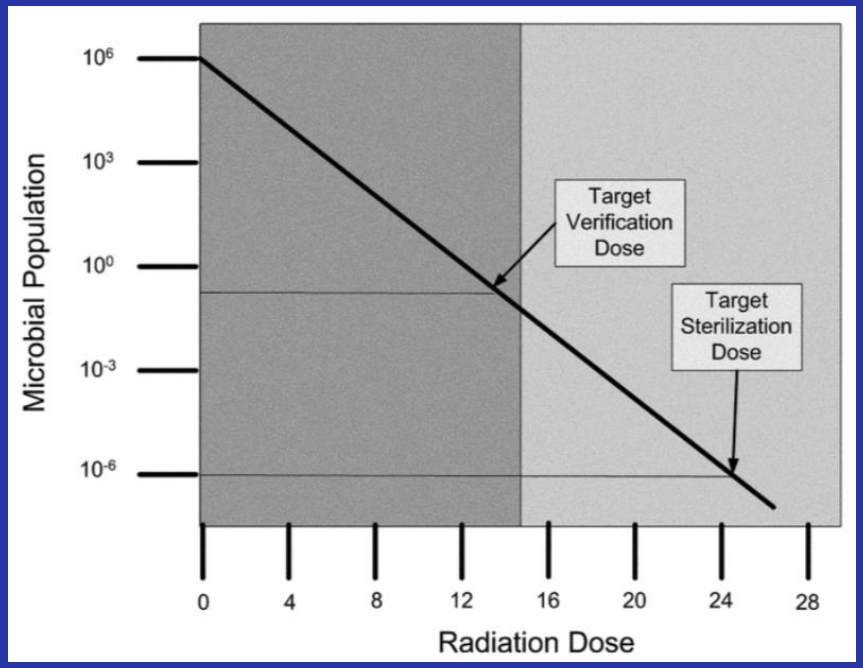
Radiation Sterilization

- Applications
  - Medical devices
  - Implants (pellets)
  - Raw materials
- Advantages
  - Simplicity
  - Reproducibility
  - Efficiency
    - Radiation dose precisely measured
    - Parametric release
- Types
  - Gamma
  - X-Ray
  - E-Beam
    - Dose mapping required
- Factors to consider
  - Bioburden
  - Radiation dose (kGy)
- Process control
  - Dosimeter
  - Periodic dose audit

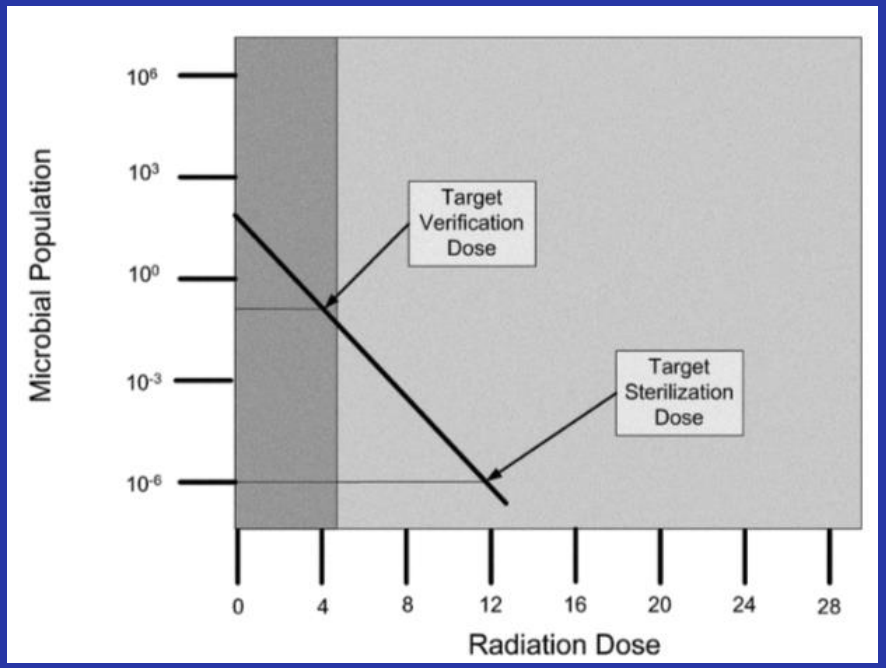
Radiation Sterilization Validation

- Define
  - Radiation dose
    - Minimum and maximum
    - Must not adversely affect product quality
  - Method (ISO)
    - Method 1, 2A, 2B, **VD_{max}**
      - Bioburden ranges
  - Dose mapping with dosimeters
    - Empty chamber
    - Load dose
      - Minimum and maximum dose location
- **NO** biological indicator challenge
  - Physical and dosimetric measurements more reliable, reproducible, and robust
- Execute
- Evaluate
  - Dosimetry

Dose Setting – VD_{max}



Higher bioburden population, higher resistance to radiation sterilization.



Lower bioburden population, lower resistance to radiation sterilization.

Routine Terminal Sterilization

- Document
  - Load information
    - Load/lot number and date
  - Cycle information
    - Parameters and monitoring
  - Biological indicator/integrator information
    - Lot, expiration, process time, result
  - Equipment
    - Calibrated and qualified

Ongoing Process Control

- Routine monitoring
  - Exposure time, temperature, pressure, ± lethality, ± dosimeter, load information
- Change control
- Personnel training
- Calibration and preventive maintenance
- Requalification
  - NLT annually

Equipment

- *"It is necessary that equipment, apparatus, and devices used to compound CSPs be* ***consistently capable of operating properly and within acceptable tolerance limits. Written procedures*** *outlining required equipment calibration, annual maintenance, monitoring for proper function, and controlled procedures for use of the equipment and specified time frames for these activities are established and followed.* ***Routine maintenance and frequencies shall be outlined in these SOPs****."*

USP <797>

Proposed Revisions

Beyond-Use Dating

- BUD dependent on method of sterilization and processing
  - Aseptic process
  - Terminal sterilization

Category 2

Category 2 [c]				
Preparation Characteristics		**Storage Conditions**		
Compounding Method	**Sterility Tested and Passed**	**Controlled Room Temperature 20° to 25°C**	**Refrigerator 2° to 8°C**	**Freezer -25° to -10°C**
Aseptically Processed	**No**	≥ 1 nonsterile ingredient(s): 1 day	≥ 1 nonsterile ingredient(s): 4 days	≥ 1 nonsterile ingredient(s): 45 days
		All sterile ingredients: 4 days	All sterile ingredients: 10 days	All sterile ingredients: 45 days
	Yes	30 days	45 days	60 days
Terminally Sterilized	**No**	14 days	28 days	45 days
	Yes	45 days	60 days	90 days

[c] Category 2 CSPs must be prepared in a cleanroom suite.

Category 3

Category 3 [d, e, f]			
Preparation Characteristic	**Storage Conditions**		
Compounding Method	**Controlled Room Temperature 20° to 25°C**	**Refrigerator 2° to 8°C**	**Freezer -25° to -10°C**
Aseptically processed, sterility tested, and passing all applicable tests for Category 3 CSPs	60 days	90 days	120 days
Terminally sterilized, sterility tested, and passing all applicable tests for Category 3 CSPs	90 days	120 days	180 days

[d] Category 3 CSPs must be prepared in a cleanroom suite and must meet additional requirements including demonstrating garbing and hand hygiene competencies, garbing requirements, and increased frequencies of environmental monitoring and sporicidal disinfectant application.

[e] BUDs must be supported by stability studies performed utilizing a stability-indicating analytical method in accordance with USP <1225> Validation of Compendial Procedures, for the exact formulation tested, in its final container-closure system, and include USP <788> Particulate Matter in Injections or USP <789> Subvisible Particulate Matter in Ophthalmic Solutions, USP <51> Antimicrobial Effectiveness Testing for multiple-dose CSPs, and USP <1207> Package Integrity Evaluation – Sterile Products, as applicable.

[f] Release testing for sterility testing and bacterial endotoxin testing must be performed in accordance with USP <71> Sterility Tests (or an alternative, noninferior, validated method) and USP <85> Bacterial Endotoxins, as applicable.

…and don’t forget…

- Document! Document! Document!
- Standard Operating Procedures (SOPs)
  - Sterilization and depyrogenation requirements
    - Parameters, qualification, and process control
  - Equipment
    - Operation, maintenance, calibration, and qualification requirements

References

1. USP <797> Pharmaceutical Compounding – Sterile Preparations
2. USP <1211> Sterility Assurance
3. USP <1228> Depyrogenation
4. USP <1228.1> Dry-Heat Depyrogenation
5. USP <1229> Sterilization of Compendial Articles
6. USP <1229.1> Steam Sterilization by Direct Contact
7. USP <1229.2> Moist Heat Sterilization of Aqueous Liquids
8. USP <1229.8> Dry-Heat Sterilization
9. USP <1229.10> Radiation Sterilization

Need More Information?

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