USP’s Revised Content on Sterilization & Sterility Assurance

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Disclaimer

This presentation draws on official chapters & in-process drafts on sterilization processes within the United States Pharmacopeia.

The interpretations and emphasis placed on subjects within this presentation are the author’s personal opinion and not official USP positions.

The draft and final chapters to be issued by USP in Pharmacopeial Forum on these subjects (publication is already underway) may differ from this presentation.
Who’s on the Micro Expert Committee?

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- Anthony Cundell, Ph.D., Independent consultant
- Dennis Guilfoyle, Ph.D., J&J
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- Horacio Pappa, Ph.D, Director
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What Was Wrong?

- The practice of sterilization had descended into rote repetition of wrong headed expectations.
- USP’s sterilization content was outdated, and lacked content in many important areas.
- Newer sterilization methods were not addressed.
- The sterilization related content lacked cohesiveness with supportive materials.
- The existing materials were not usable outside industrial settings.

Where is USP Headed?
<1211> The Revision

- Started Here: Sterilization at a more basic level: more instruction, less standardization
  - Individual chapters on each sterilization method: allows for more specific detail, less confusion and easier revision.
  - Separated gas & vapor sterilization; dry heat sterilization & depyrogenation; parts and terminal by steam; none of these are really the same process.
  - New chapters on liquid chemical sterilization: no prior information.
  - Aseptic processing as a separate chapter: not strictly a sterilization subject, needs better connection to other chapters.
  - Updated references throughout. Newer definitions for sterilization validation models. Clarify the role of the biological indicator. Clarify PNSU, SAL and risk to patient.
  - Integrate new chapters with existing BI & CI content.
  - Move BI monographs out of “official chapters”.
  - Allow for future development of other content as needed content.
  - Depyrogenation treated independently of sterilization.

- Going Here: Separation of both Sterilization, Depyrogenation and Sterility Assurance content.

Basic Premise to the New Content

- A balance must be achieved between the need to maintain a safe, stable and efficacious product while providing sufficient lethality to attain a minimum level of sterility assurance.
The Forgotten Objective

- Achieving sterility (aka minimum PNSU) is only half of what must be accomplished.
- In order to use the materials after the process their essential quality attributes have to be maintained.
- We can most definitely have too much of a good thing. If in the effort to kill microorganisms, we do lasting physical or chemical damage to the items being sterilized we have accomplished nothing of value.

Consequences of Over-processing

- Reduced potency
- Increased degradation
- Increase in extractables / leachables
- Increase in particles – visible & sub-visible
- Loss / weakening of package integrity
- Appearance changes
- Changes in physical properties
- Limited growth promotion (lab media)
- And most important of all – using an aseptic process instead of a terminal sterilization process.
Old & New Structure Overview

- <1211> Sterilization & Sterility Assurance of Compendial Articles split into three pieces:
  - <1211> General Concepts for Sterility Assurance
    - Aseptic Processing, Environmental Monitoring, Sterility Testing, sterility assurance related content, etc.
  - <1229> General Concepts for Sterilization
    - Sterilization processes, BI, CI’s, other sterilization related content
  - <1228> General Concepts for Depyrogenation
    - Depyrogenation processes, EI’s, related content

- Work on <1211> section was deferred because the sterilization & depyrogenation chapter revisions were urgently needed.

The Project Plan

- Revise and expand the entire content, separating it into smaller units for ease of development, review, approval and roll out.
- All current content would remain in place and be removed when the replacement sub-chapters are official.
- Sterilization process specific content has been given priority, but supportive content revision is also underway.
- Many of the new <1229> chapters are already official.
Sterilization Microbiology 101:

What you absolutely must know in only 6 slides!

D - Value

Microbial Death Curve

Population

Time

1 log

D value
The D-Value

- The D-value is the time required to reduce a population of microorganisms by one log or a 90% reduction in count.
- A D-value is meaningful only if referenced to specified lethal conditions.
- Thermal process D-values should always be referenced to a temperature, without that reference they have no meaning, i.e., moist heat $D_{121.1^\circ C}$ or dry heat $D_{170^\circ C}$.
- For D-values in gases / liquids the agent concentration, RH and temperature must be indicated, i.e., $D_{900}$ PPM, 75% RH, $30^\circ C$.

What's the Primary Objective?

- A minimum PNSU of $10^{-6}$ is required.
- That means that in routine operation the possibility for a surviving bioburden microorganism must be less than 1 in 1,000,000.
  - 1 non-sterile unit in 1,000,000 units or
  - 1 chance in a 1,000,000 that a single unit is non-sterile
- It has little to do with the biological indicator, and even less to do with the BI population.
Process Objective

Biological Indicator Death Curve

Bioburden Death Curve

Here

Not Here

Calculation of PNSU (SAL)

\[ \log N_u = -\frac{F}{D} + \log N_0 \]

where:

- \( N_u \) = SAL / PNSU
- \( D \) = D-value of the natural bioburden
- \( F \) = F-value (lethality) of the process
- \( N_0 \) = bioburden population
Calculation of PNSU (SAL)

$$\log N_u = \frac{-F}{D} + \log N_0$$

The bioindicator and physical measurements confirm this
The bioburden defines these

What’s Complete?
Provided an overview and introduces common elements related to all sterilization methods. Includes:
- Establishing & Justifying Sterilization Processes
- D-value and Microbial Resistance
- Biological & Physical Data
- Sterilization Indicators & Integrators
- Selection of an Appropriate Method
- Routine Process Management
The Main Point

“It is generally accepted that sterilized articles or devices purporting to be sterile attain a $10^{-6}$ microbial survivor probability, i.e., assurance of less than 1 chance in 1 million that viable bioburden microorganisms are present in the sterilized article or dosage form.”

The Process Objective
Steam Sterilization Split

- Separated prior sub-chapter into:
  - Steam Sterilization by Direct Contact <1229.1>
  - Steam Sterilization of Aqueous Liquids <1229.2>
- This allows for separate treatment of what are really different processes.
- Separates processes where over-processing is not a concern from those where it is.
- In theory parts sterilization has no upper limit, while terminal / liquid sterilization is bounded both above and below the desired process.
Parts vs. Liquid Sterilization

Product Quality Attributes
Non-issue - Overkill Method

Non-Sterile
Sterile

Direct Heat to Surface
Indirect heat via container wall

Heat Input

Non-Sterile Stable
Sterile Stable
Non-Sterile
Sterile

Non-Issue - Overkill Method
An issue – BB/BI Method

Overkill Sterilization

Complete destruction of the BI at this time point

Result in overkill of the bioburden to the PNSU where these line intersect

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Steam Sterilization of Aqueous Liquids

The method of choice for liquid parenteral products, similar processes for media and process intermediates.

“Where the overkill approach can be utilized for terminal sterilization of sealed liquid containers, it is the preferred approach.”

“a dual set of requirements is established for nearly every important processing parameter. Sterilization time-temperature or F₀ conditions will include both lower (sterility related) and upper (stability related) limits to simultaneously assure safety and efficacy of the processed materials.”
Steam Sterilization of Aqueous Liquids

- Terminal sterilization of products
  - High/Low F₀, variety of Bl’s, Overkill & BB/Bl method
- Media for laboratory usage
  - High/Low F₀, Overkill & BB/Bl method, Bl usage? - self indicating?
- Intermediates / process aides
  - High/Low F₀, Bl usage, Overkill & BB/Bl method
- Laboratory and production bio-waste
  - Low F₀, *G. stearothermophilus*, Overkill method, condensate collection / kill

Probability of a Non-Sterile Unit (PNSU)

\[
\log N_u = \frac{-F}{D} + \log N_0
\]

Where

\( N_u \) = Probability of a Non-Sterile Unit

\( D \) = D-value of the natural bioburden

\( F \) = F-value of the process

\( N_0 \) = Bioburden population per container

<table>
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<tr>
<th>Validation</th>
<th>Routine Usage</th>
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<tbody>
<tr>
<td>( F_0 = 8.0 ) minutes</td>
<td>( F_0 = 8.0 ) minutes</td>
</tr>
<tr>
<td>( D_{121} ) of Bl = 0.5 minutes</td>
<td>( D_{121} ) of bioburden = 0.005 minutes</td>
</tr>
<tr>
<td>( N_0 ) of Bl = ( 10^6 )</td>
<td>( N_0 ) of bioburden = 100 (or ( 10^2 ))</td>
</tr>
<tr>
<td>PNSU for Bl = ( 10^{-10} )</td>
<td>PNSU for Bioburden = ( 10^{-1.598} )</td>
</tr>
</tbody>
</table>
1229.3
Monitoring of Bioburden

Official in 2S to USP 36 - December 2013

Note: results should be reported on an individual container basis regardless of the volume or weight sampled.
Sterilizing Filtration

- Sterilizing filtration is a retentive process, not a destructive one.
- Physical removal of microorganisms depends on the upstream bioburden, the properties of the solution, the filtration conditions and the filter itself.
- Can be validated to consistently yield solutions that are sterile as defined in <1229>.
<1229.4> Sterilization by Filtration

Definition and description of “sterilizing-grade filter”
Retention mechanisms and factors affecting retention
- Nature of “pores” and microorganisms
- Composition and structure of filter matrix
- Composition of filtered solution
- Filtration conditions
Filter efficacy
- Log-reduction value

<1229.4> Sterilization by Filtration

Validation
Integrity test principles and methods
- Bubble point
- Diffusive flow
- Pressure hold
Pre- and post-filtration and sterilization integrity testing
Requirement for pre-filtration bioburden control
Troubleshooting common filtration problems
1229.7
Gaseous Sterilization

Official in USP 37 – May 2014

<1229.7> Gaseous Sterilization

- Applicable to single phase gaseous processes only.
  - Condensation will not occur.
    - Ethylene oxide – model for all systems
    - Chlorine dioxide
    - Ozone
    - Nitrogen dioxide

- Two validation approaches defined
  - Traditional half-cycle method
  - Bracketing method – variations in concentration, relative humidity and temperature. More efficient & more scientific as well.
Gas, Liquid & Vapors - D-Values

- A D-value is only meaningful if referenced to specified lethal conditions. For example wet or dry heat D-values should always be referenced to a temperature, without that reference they have no meaning, i.e., $D_{121.1\,^\circ C}$ or $D_{170\,^\circ C}$.

- For D-values in gases / liquids the agent concentration, RH and temperature must be indicated, i.e., $D_{900\,\text{PPM}, 75\%\,\text{RH}, 30\,^\circ C}$.

- D-values cannot be accurately determined for vapors.

1229.6
Liquid Chemical Sterilization

Official in 2S to USP 37 – December 2014
<1229.6> Liquid Sterilization

- Chemical Sterilants in aqueous solutions
  - Aldehydes – CH₂O, CH₃CHO, etc.
  - Acids – HNO₃, H₂SO₄, peracetic, etc.
  - Bases – NaOH, KOH, etc.
  - Oxygenating compounds – H₂O₂, O₃, ClO₂, etc.
  - Halides – NaOCl, Cl₂, etc.

- Must include an aseptic post-cycle quench step to stop process prior to adverse material impact.

- Validation methods nearly identical to gas sterilization
  - Half cycle method (maybe, but has limited value)
  - Bracketing method – vary concentration of agent, temperature. Moisture always present. Agitation is generally needed.

1229.8

Dry Heat Sterilization

Official in USP 37 – May 2014
<1229.8> Dry Heat Sterilization

- Distinction made between dry heat sterilization and depyrogenation due to process differences.
- Dry heat sterilization:
  - Is almost always performed in batch ovens.
  - Uses a biological indicator *B. atrophaeus*.
  - Is usually in the 160-180°C temperature range.
  - A reasonable mathematical correlation between physical data and microbial effect exists.
- Physical requirements are less definitive than for steam processes, but still apply.
- Dry heat depyrogenation will be addressed as a part of chapter <1228> Depyrogenation.

1229.10 Radiation Sterilization

Official in USP 37 – May 2014
Radiation Sterilization

“The prevalent radiation usage is either gamma rays or electron beams. Other methods utilize x-rays, microwaves and visible light. The impact of radiation on materials can be substantial and is a major consideration in the selection of radiation as a processing method.”

“Radiation sterilization is unique in that the basis of control ... is the absorbed radiation dose, which can be precisely measured. Dose setting and dose substantiation procedures are used to validate the radiation dose required to achieve sterility assurance level.”

The use of BI’s in radiation sterilization is not necessary:

- Non-spore-formers have been identified as more resistant than *B. pumilus*.
- Dose measurement is accurate and has been closely correlated to microbial destruction.

The dose setting methods of AAMI/ISO are well established and easily adapted to pharmaceutical applications. $V_{D_{\text{max}}}$ has been utilized for terminal sterilization of several pharmaceutical preparations.
VD_{MAX} - Example

1229.11
Vapor Sterilization

Official in 1S USP 37 – August 2015
Gas/Liquid vs. Vapor Sterilization

- Gases are more penetrating, liquids more uniform, and each is less subject to variations in temperature and relative humidity than a vapor.
- Vapors have different concentrations in each phase. When a vapor has 2 condensable components it is even more difficult to predict conditions anywhere.

<1229.11> Vapor Sterilization

- The kill rates in the gas and liquid phase are different reflecting the different agent concentrations and moisture levels present in each phase.
- The conditions within an vapor system are unlikely to be uniform because the agent supply is at a higher temperature than the chamber.
- The conditions at any location may change during the course of the process.
- Reproducible kill is possible, because the agent is lethal in both phases, it’s just a more complex process than the other sterilization methods.
Vapor Sterilization

Two validation approaches can be utilized, with the only supportive evidence from microbial destruction.
- Traditional half-cycle method
- Bracketing method

Linearity of destruction is not assured as the conditions are not homogeneous.

The efficacy of the agent used should assure sterilization, however predictability is difficult.

An empirical approach is recommended.

Bi-Phasic Kill Possibilities

The difference in kill rates is unknown.
Bi-Phasic Death Curves

- Gas Phase Death Curve
- Liquid Phase Death Curve
- Composite Death Curve

Bracketing Approach

- Validation Cycles
- Routine Process
- Worst case sterilization cycle
- "worse case" sterilization cycle
What's In-Process?

1229.5
Biological Indicators for Sterilization

To be Official USP 39 1S, August 2016
What is USP doing with BI’s?

- The BI content is predominantly in subchapter <1229.5>
- Shift the thinking towards the use of the BI as a process measurement tool, rather than the process target.
  - Require D-value & population be known for the purposes of control, but without the current arbitrary values.
- Provide clearer guidance on how they are to be used for validation & control of sterilization processes.

Biological Indicators

- They are process measurement tools, much like the thermocouple.
- **Their destruction in the PQ need NOT be mandatory.**
- They should be placed in the load in difficult to penetrate locations.
- They come in many formats: strips, coupons, threads, wire, & suspension.
- Their resistance to the process must be known.
Where will USP’s BI content be?

<table>
<thead>
<tr>
<th>Monographs</th>
<th>&lt;55&gt; Biological Indicators—Resistance Performance Tests</th>
<th>&lt;1035&gt; Biological Indicators For Sterilization</th>
<th>&lt;1229.5&gt; Sterilization Of Compendial Items</th>
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<td>6 Individual Monographs</td>
<td>Total Viable Spore Count</td>
<td>Types of Bioindicators</td>
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<td>General Description</td>
<td>D-value Determination Methods</td>
<td>Use for In-process Validation</td>
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<td>Labeling</td>
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<td>Identification</td>
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<td>D-value</td>
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<td>Survival &amp; Kill Window</td>
<td>Some Portions Moved</td>
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<td>Total Viable Spore Count</td>
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<tr>
<td>Disposal</td>
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</table>

What You Won’t See

- Required D-values for any sterilization process as the process should be established for kill of the expected bioburden to a minimum PNSU of 1x10^-6. That can be readily accomplished with a variable, but well characterized BI.
- D-value requirements for processes where the specific conditions of kill are undetermined (i.e., vapor sterilization).
55
Biological Indicators – Resistance Performance Tests

Draft in Pharmacopeial Forum – March 2015

1229.9
Physicochemical Integrators and Indicators for Sterilization

To be Official USP 39 1S, August 2016
1229.9 Indicators & Integrators

- An extensive revision of existing chapter <1209> Sterilization - Chemical & Physicochemical Indicators and Integrators.
- Will link with ISO 11400 Sterilization of health care products -- Chemical indicators -- Part 1: General requirements which provides comprehensive treatment of their use.

1229.12 New Sterilization Methods

To be Official USP 39 1S, August 2016
1229.12 - Novel Methods

- A working draft has been developed.
- Will outline USP recommendations for the investigation, use and control of a novel sterilization method.

1229.13 Sterilization-in-Place

To be Official USP 39 2S, December 2016
1229.13 - Sterilization in Place

This chapter includes design & operational content on sterilization in place using steam, superheated water, dry heat, gases and liquids for process equipment that cannot be placed inside a chamber sterilizer as described elsewhere in <1229>.

1229.14 Sterilization Cycle Development

Draft expected PF 42(5); September 2016
1229.14 – Sterilization Cycle Development

This chapter is anticipated to have 2 main components:

- How to select an appropriate sterilization method
- How to define a sterilization cycle for the more common methods

1229.15 Sterilizing Filtration of Gases

In early stages of development
1229.15
Sterilizing Filtration of Gases

- Gases used in preparation of products
  - Inert gases in container / vessel headspace
  - Gases used for product manufacture / movement
  - Tank and process equipment vent filters
- Only content unique to use for gases will be included. Much of the official content in 1229.4 Sterilizing Filtration of Liquids applies without change.

<1228> Depyrogenation
### <1228> Depyrogenation

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<td>Depyrogenation</td>
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<td>Depyrogenation by Filtration</td>
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<td>1228.4</td>
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<td>1228.7</td>
<td>Other Endotoxin Reduction Methods</td>
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### <1211> Sterility Assurance
<1211> Sterility Assurance

The chapter revision will embrace the non-sterilization aspects of sterility assurance.
The content will be drawn from multiple USP sources with edits / additions as needed.
Content expected to embrace: Aseptic Processing, Environmental Monitoring, Sterility Testing, Isolators & RABS, & other general sterility assurance related content.
This activity is just beginning; the first drafts likely won’t appear until early 2017.
Many **THANKS** for **YOUR** Attention

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