Biological indicators and biological validation

Maria Luisa Bernuzzi, Fedegari group

Index

Regulatory references

United States Pharmacopeia 41

European Pharmacopeia 9.2

AAMI/ISO 11138 - Sterilization of health care products – Biological Indicators

- *11138-1* – General
- *11138-2* – EtO
- *11138-3* – Moist Heat
- *11138-4* – Dry Heat
- *11138-5* – Low-temperature Steam and Formaldehyde

AAMI/ISO 14161 – Sterilization of health care products – Biological indicators – Guidance for the selection, use and interpretation of results

AAMI/ISO 18472 – Sterilization of health care products – Biological and chemical indicator – test equipment

What is a Biological Indicator?

«It is a well-characterized preparation of a specific microorganism that has know resistance to a specific sterilization process.»

USP 41, General Chapter 1229.5

What is a Biological Indicator?

	ISO 11138 -3 (2017)	EP 9.2	USP 41
Strain	Geobacillus stearothermophilus	Geobacillus stearothermophilus (ATCC 7953, 12980, NCTC 10007, CIP52,81, NCIMB 8157)	Geobacillus stearothermophilus (ATCC 12980 or ATCC 7953)
			Clostridium sporogenes (ATCC 7955)
			B. atrophaeus (ATCC 9372)
			Bacillus subtilis (ATCC 5230)
Population	$\geq 1{,}0 \times 10^5$		
D_{121} value	≥ 1,5 min	1,5 min to 4,5 min	
z	≥ 6°C		

Biological Indicators: purpose

Biological indicators are designed to show by the survival of test microorganisms whether specified sterilization conditions have been attained.

The absence of growth of a test microorganism after exposure to a sterilization process demonstrates that a specified level of microbiological inactivation has been delivered.

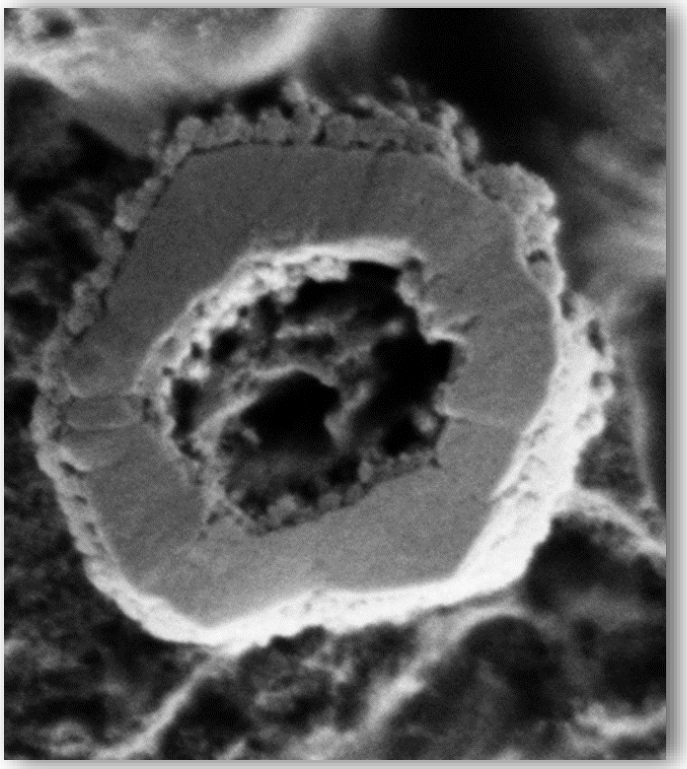
Survival of a test microorganism subjected to a sterilization process indicates that the process has failed.

Biological Indicators: purpose

The physical method of F_0 value calculation provides an estimate of the conditions to which the biological indicator is subject, however it cannot predict the full effect of moisture on the biological indicator.

Biological indicators may be used to give a microbiological correspondance to the physical parameters assessed.

What are Biological Indicators?



Microorganisms widely recognized as suitable for BIs are spore - forming bacteria because more resistant than normal microflora.

Types of BIs

There are at least three types of Bis

Types of BIs

Spore added to a carrier (a disk or strip of filter paper, glass, plastic or other material) and packed

Types of BIs

Carriers and primary packaging

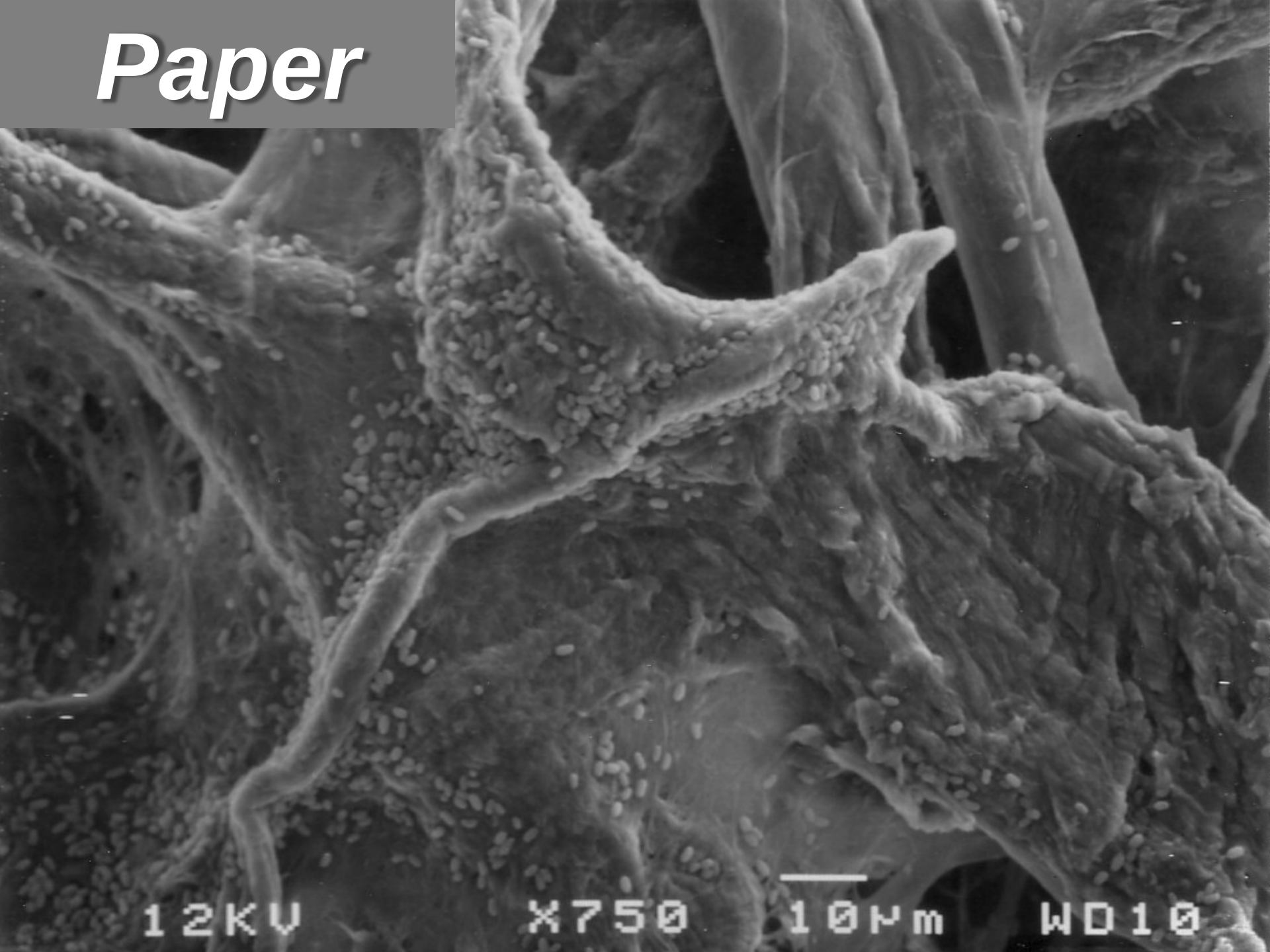
- no chemical/microbial contamination
- no degraded by the sterilization process
- they should minimize the loss of the original inoculum during transport, handling and shelf life storage

Types of BIs

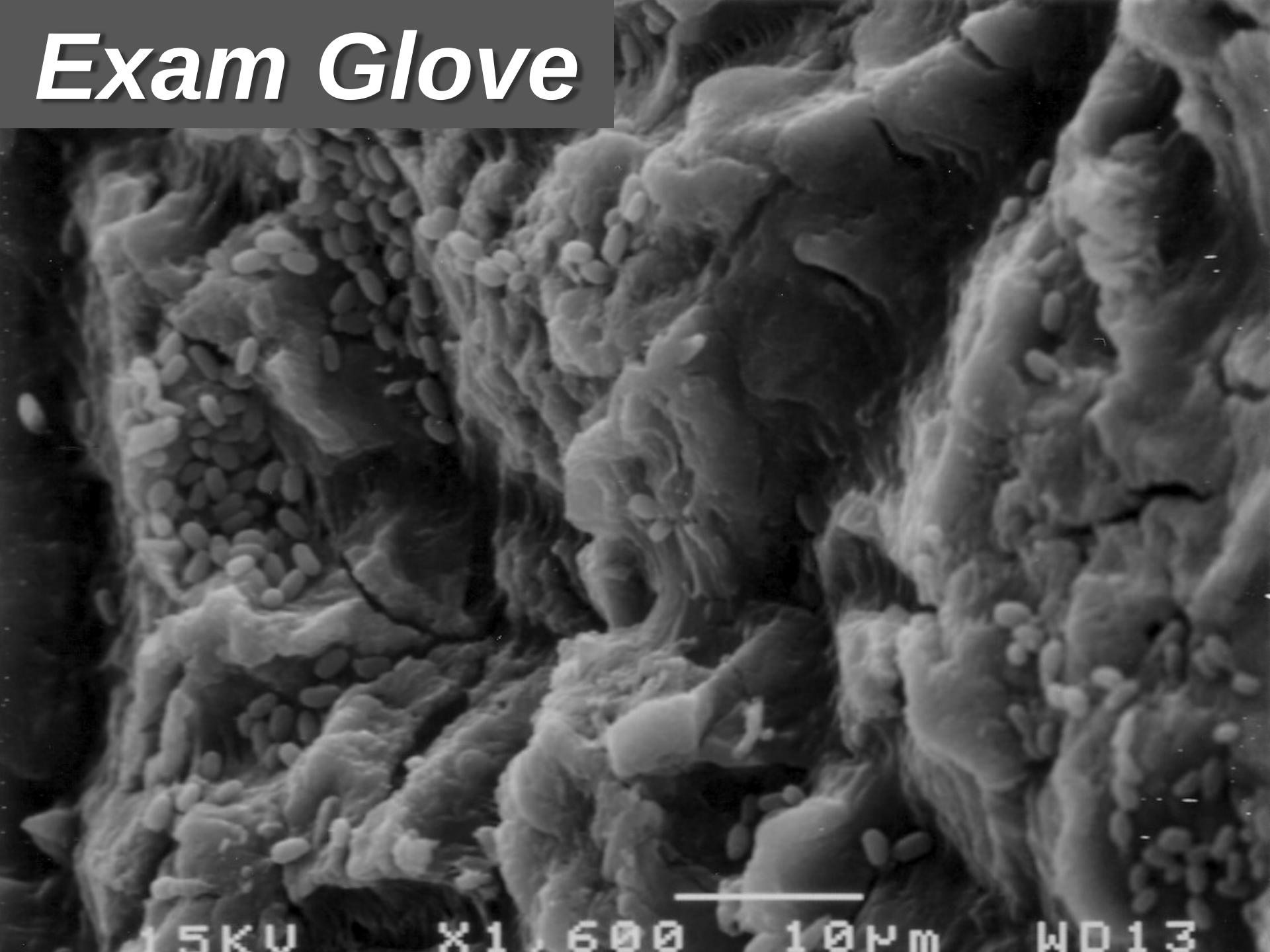
Carriers and primary packaging

Must not retain residual sterilizing agent such that it could hinder outgrowth of low numbers of surviving spores.

Paper



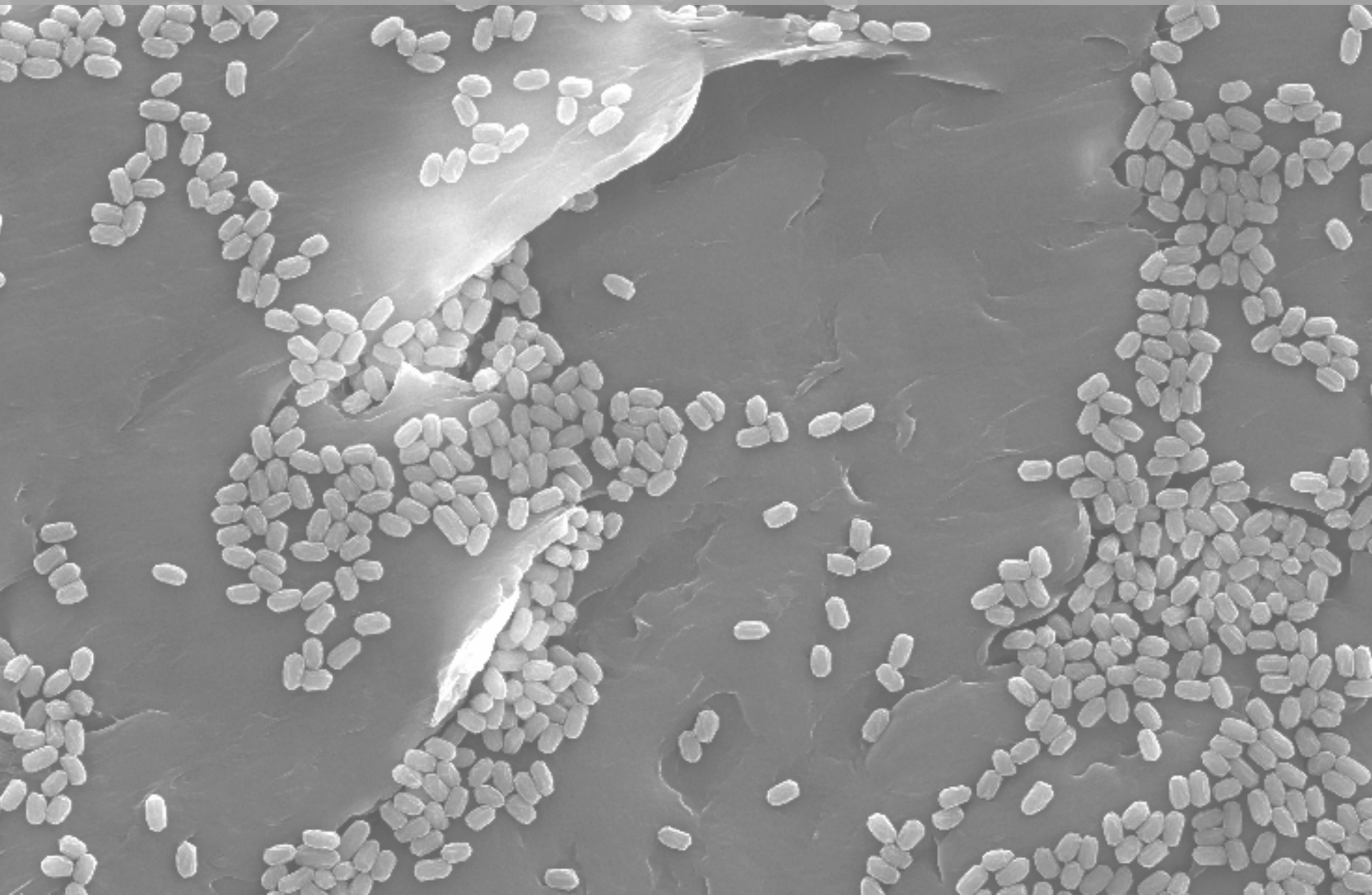
Exam Glove



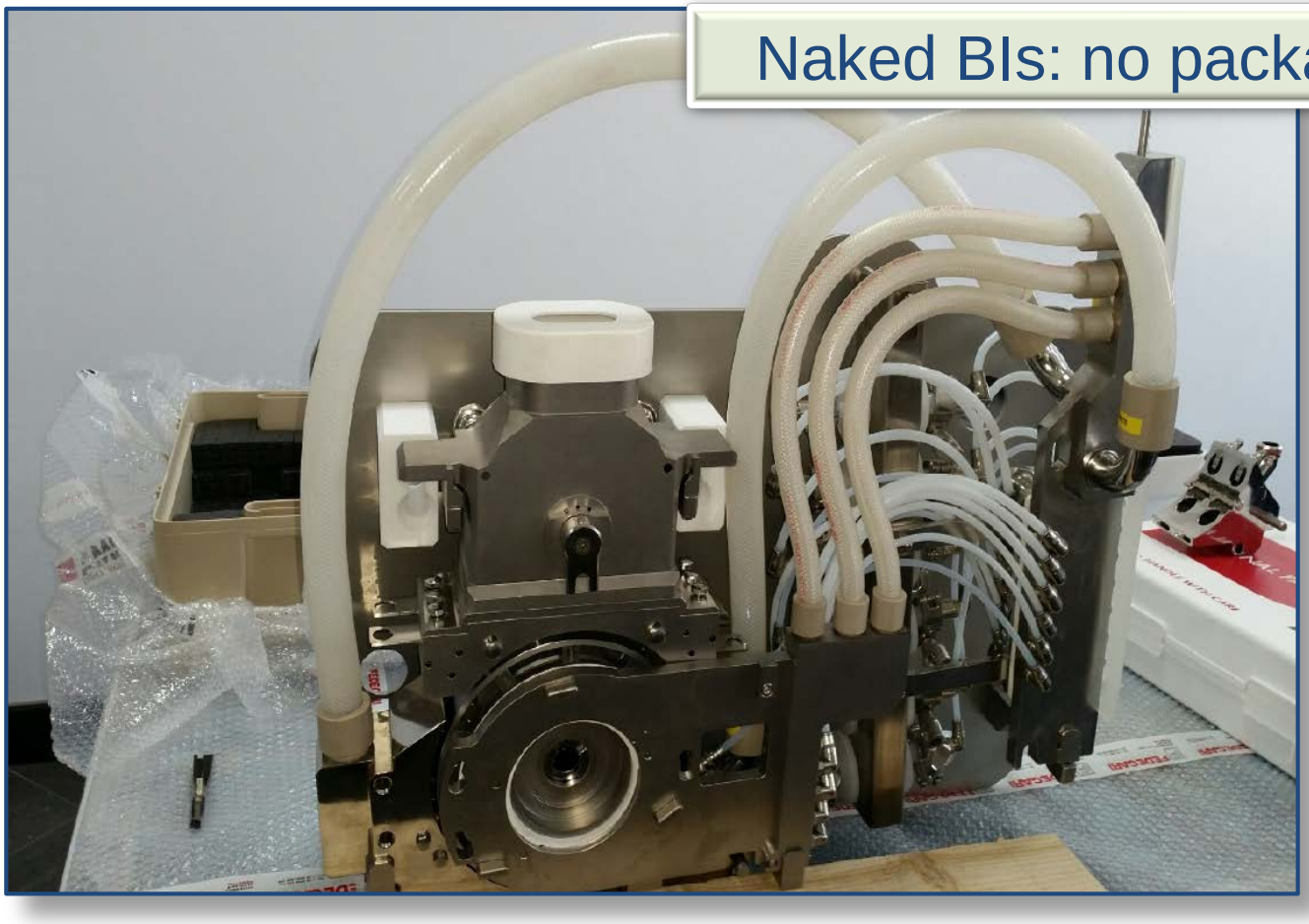
Stainless Steel

Fiberglass

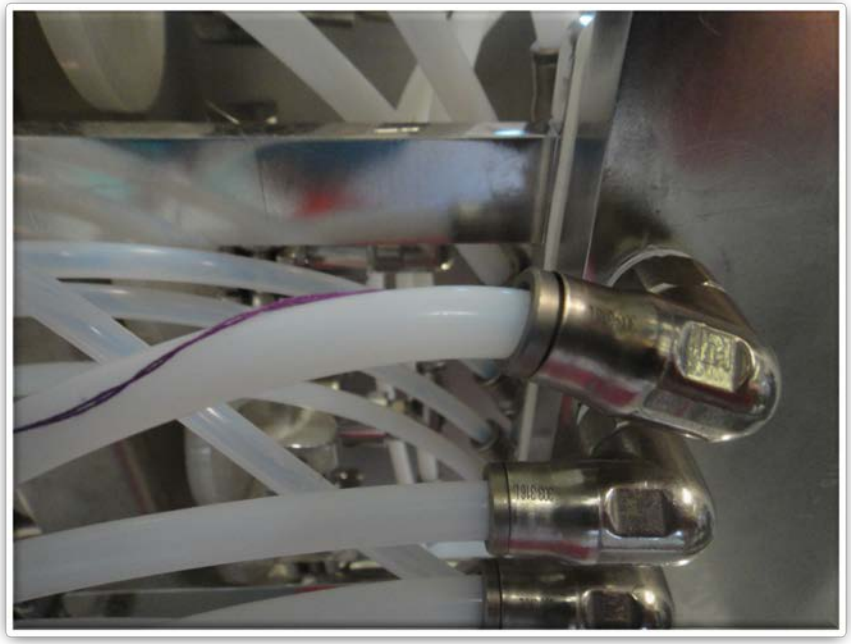
Laminated Aluminum Foil

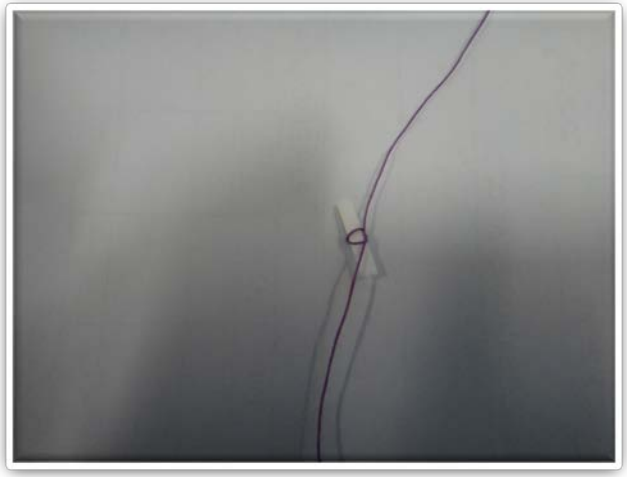


Types of BIs



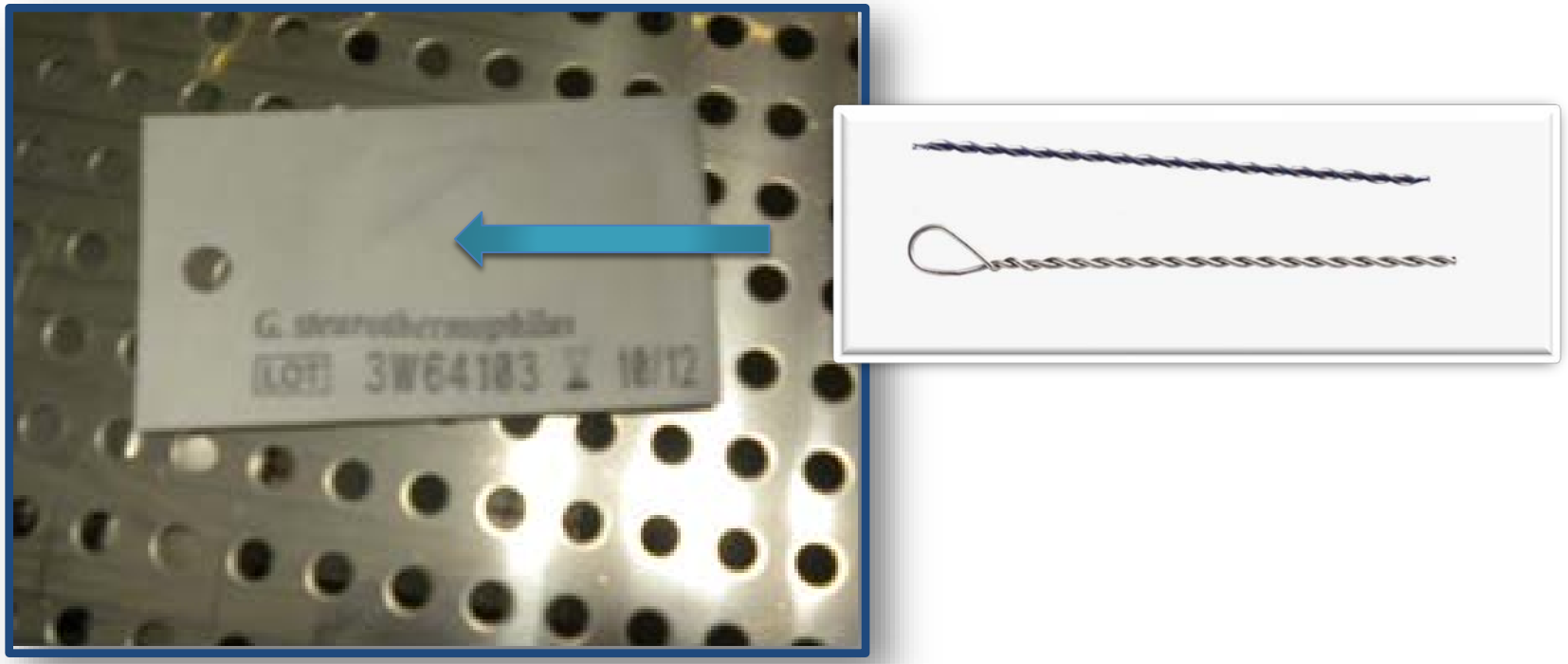
Types of BIs





Types of BIs

Types of BIs



Types of BIs

Spore suspension that is inoculated on or into representative units of the product to be sterilized

Application: sterilization of vials closed with rubber stoppers, plungers of syringes…

Types of BIs

SPORE SUSPENSIONS INOCULATED ON A SURFACE


STOPPERS

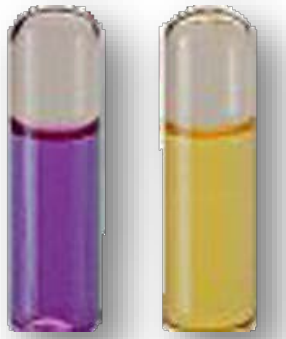
Types of BIs

- Spore supensions with a known D-value should be used to inoculate the actual or simulated product.
- In the case of liquid inoculated products, its advisable to determine the D-value of the biological indicator microorganism in the specific liquid product.

Types of BIs

3

Self-contained indicators



Types of BIs

Sealed system that includes the growth medium for recovery of process exposed BI microorganisms.

SOLID

If the self contained is a paper strip or a disk in a package that includes a culture medium, the package design should be penetrable by the sterilizing agent.

Types of BIs

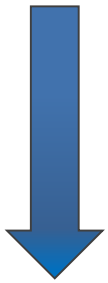
After the sterilization cycle the spores disc or strip is immersed in the culture medium by manipulation which allows contact with the culture medium

Types of BIs

Self- contained biological indicators may also consist of a spore suspension in its own medium; they often contain a dye which indicates positive or negative results after the incubation period.

Types of BIs

The entire system provides resistance to the sterilization process.



The D-value should be characterized for the system and not only for the strip in the self contained unit.

Types of BIs

The user should establish in-house acceptance standards for BIs and consider rejection in the event the BI does not meet the established in-house performance standards.

	USP 41 General Chapter 1229	EP 9.2 Chapter 5.1.2
BI USER'S RESPONSIBILITY	• Suitability for use must be established • Should obtain a certificate of analysis • Resistance of BI need not be reconfirmed when used according to manufacturer's directions • When the BIs are not used according to manufacturer's directions the following BI's characteristics need to be reconfirmed - Resistance - Identification - Purity - Population - Packaging and Storage - Expiration Data - Disposal • For the custom-made BI, the user must determine - D-value - population	• Suitability for use must be established • Should obtain a certificate of analysis • Resistance of BI need not be reconfirmed when used according to manufacturer's directions • When the BIs are not used according to manufacturer's directions the following BI's characteristics need to be reconfirmed - Resistance - Identification - Purity - Viable count • For the custom-made BI, the user must determine - D-value - z-value • If the BI's manufacturer can't be audited, the resistance of BI shall be independently verified

MESASTRIP

BIOLOGICAL INDICATOR

For Industrial Use Only

CERTIFICATE OF ANALYSIS

Reorder No.: S2X10/6

Geobacillus stearothermophilus 7953(1)

For: Steam sterilization

Culture: Soybean casein digest broth.

Purity: No evidence of contaminants using standard plate count techniques.

Lot No.: CGST-290

Manufacture Date: 2015 April 13

Expiration: 2017 April 13

Heat Shocked Population: 2.1 x 10^6 Spores/Unit

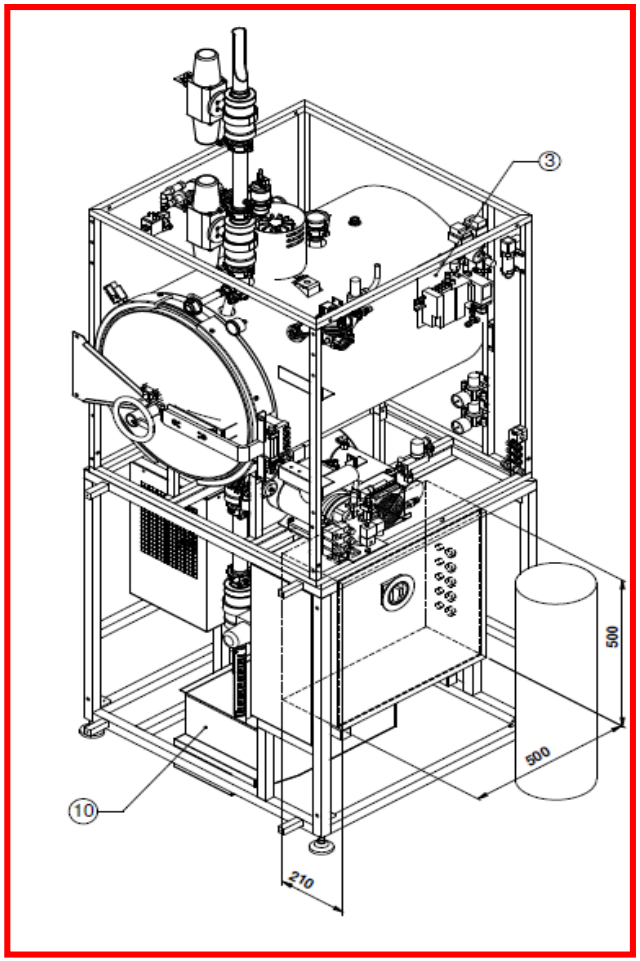
Carrier Size: 2 x 10mm

Assayed Resistance:

Temperature	D-value(2)	Survival(3)	Kill(3)	
121°C	2.3	9.95	23.78	min.

Z-value: 8.1°C

D value determination



BIER

The user may consider conducting a D value determination

D value determination

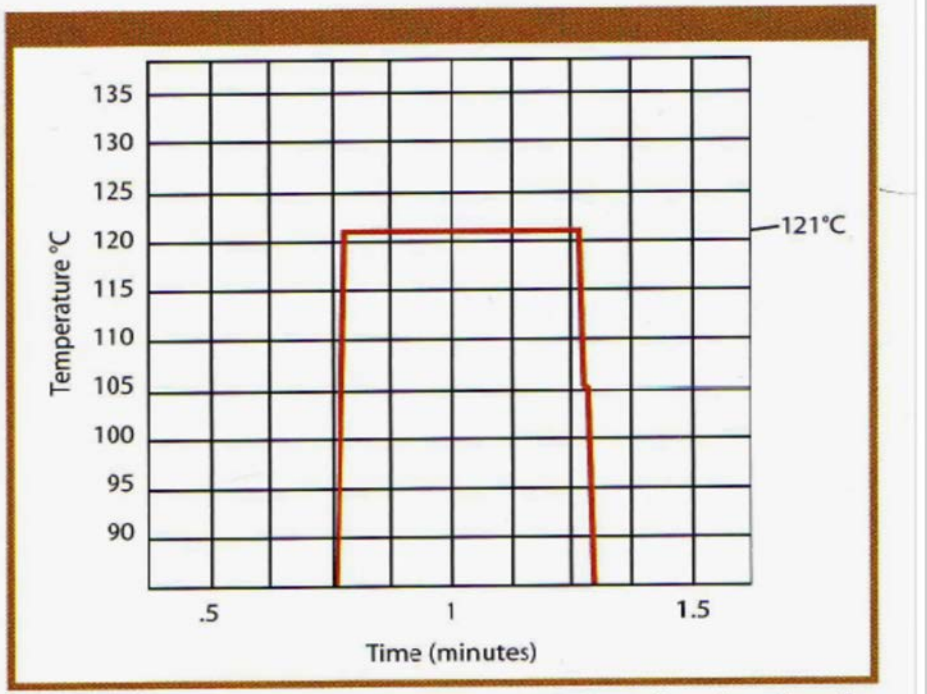


Figure 1: The BIER vessel's square-wave system. Samples placed in the BIER vessel are taken from ambient conditions, brought to the sterilizing condition, and returned to the ambient conditions.

Square wave profile

What are you sterilizing?

Is it a solid load or a liquid one?

What are you sterilizing?

Blood bags

What are you sterilizing?

Customer's choice: self-contained BI. They were inserted into the empty bag.

Is it the right choice?

What are you sterilizing?

Culture medium

Culture media sterilization

The effects of the sterilization method and conditions on the media should be validated by sterility and growth-promotion testing of the media. In addition, if sterilized by moist heat, the autoclave cycle should be validated to ensure proper heat distribution for selected loads and volumes. Typically, manufacturers recommend using an autoclave cycle of 121° for 15 minutes using a validated autoclave. These conditions apply to time at temperature of the media. As container size and the load configuration of the autoclave will influence the rate of heating, longer cycles may be required for larger loads. However, the sterilization time will be dependent on the media volume and autoclave load. Sterilization cycles in which the autoclave is slow to come up to temperature may result in overheating of the media. Therefore, care must be taken to validate a sterilization cycle, balancing the need for **sterile media against the tendency of the media to degrade under excessive heating.**

USP 41 General chapter ⟨1117⟩ MICROBIOLOGICAL BEST LABORATORY PRACTICES

Choice of the right BI

Customer’s choice:

Choice of the right BI

AFTER INCUBATION

PANTONE®
13-0755
Primrose Yellow

SUPPOSED

PANTONE
UNIVERSE
266 C

OBTAINED

PANTONE®
16-1144
Oak Buff

BIs supplier's

At the end of the sterilization cycle….
Why the biological indicators changed their color?

"The media turning brown during a long cycle is normal. All liquid media is susceptible to thermal degradation which will change the color of the media. What occurs is that the sugars in the media will caramelize and change the color of the media. The color of a thermally insulted liquid BI can range from light purple to grey to light brown to dark brown but generally the longer the cycle the more discolored the media will become. If your cycle provides enough thermal insult to degrade the color of the media, it is best to use a negative control to have a comparison as to what a negative result from your cycle should look like. The purpose of the negative control which contains no spores is to process them in the same cycle with the regular ampoules containing spores and incubate both until the reads are taken. At the end of the incubation period, the negative control is then compared to the MagnaAmp which contained spores."

MesaLabs

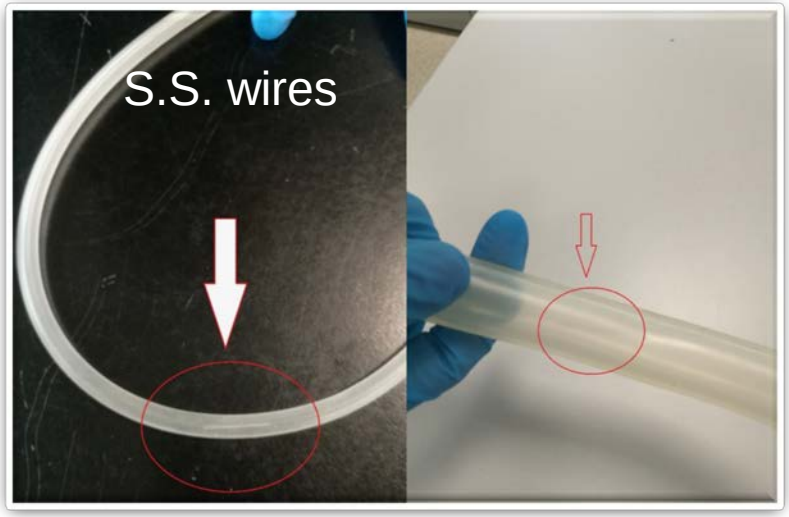
A case study

- Plastic bioreactor to be sterilized fully assembled
- 1 liter of water contained

A case study

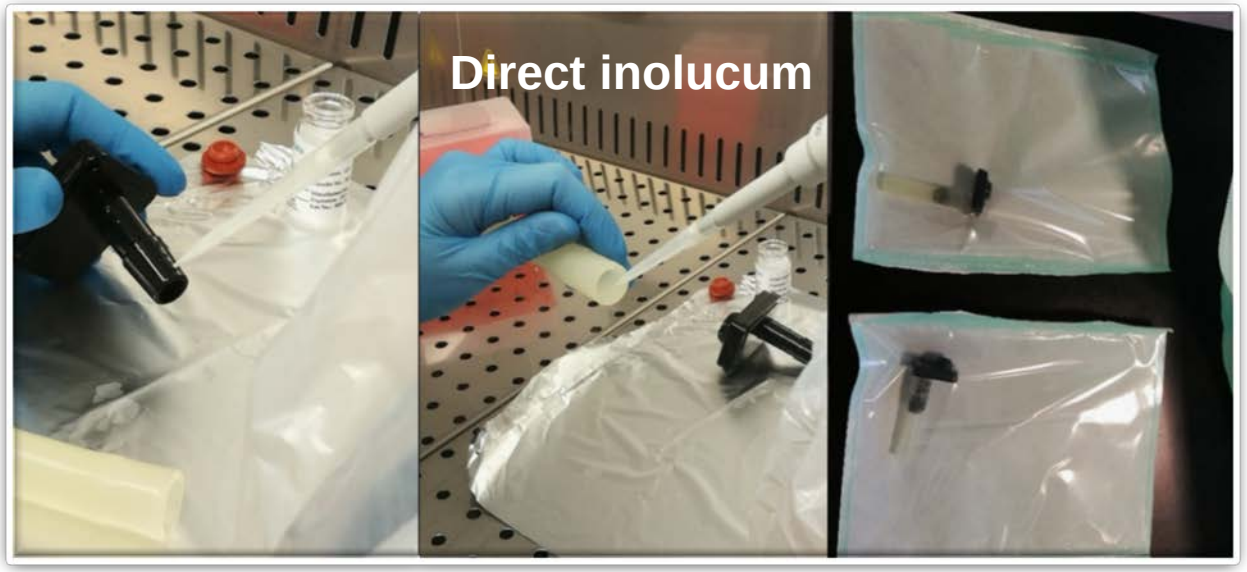
- Plastic bioreactor to be sterilized fully assembled
- 1 liter of water contained

CHOICE OF THE BIs



A case study

- Plastic bioreactor to be sterilized fully assembled
- 1 liter of water contained



CHOICE OF THE BIs

A case study

Anything else to be tested?

Performance qualification: biological approach

USP 41

PDA

CFPP- 01- 01 Part 3

EP 9.2

EU GMP

CFPP -01-01 part 3

This test is designed to be used in exceptional circumstances as an additional PQ test for steam sterilizers. The microbiological test should ideally follow a satisfactory thermometric test

There may be situations where thermometric tests are not possible, for example with narrow-lumened instruments, where it is not physically possible to place a thermocouple or temperature sensor into the lumen without altering the nature of the load. Reference should be made to BS EN 556-1 for sterility assurance requirements.

Use of biological indicators

2.100 Biological indicators are designed to show whether specified sterilization conditions have been attained, by the survival of test microorganisms. However, they should not be used for routine monitoring of steam sterilization processes. In exceptional circumstances where the use of biological monitors could be considered, advice should be sought from the Microbiologist (Decontamination).

EU GMP, Annex 1

"Before any sterilisation process is adopted its suitability for the product and its efficacy in achieving the desired sterilising conditions in all parts of each type of load to be processed should be demonstrated by physical measurements and by biological indicators where appropriate"

Annex 1 – EU GMP

European Pharmacopoeia 9.2 chapter 5.1.1

In cycle validation, the relevant positions in the load that are the most difficult to sterilise are determined and adequate biological effectiveness is verified by biological indicators in these positions or products, whichever is relevant.

USP 41

The goal of a validation activity is the confirmation of acceptable heat penetration using temperature measurements and biological indicator challenges.

Biological indicators may also be used to monitor established sterilization cycles and in period revalidation of sterilization processes.

PDA

"Performance qualification consists of two elements: physical qualification and biological qualification"

PDA, TR # 1, revised 2007

Performance qualification, biological approach

Consistency between physical and microbiological

result is central to sterilization validation.

Physical data taken from temperature and pressure measurements cannot alone provide confirmation that specified conditions required for lethality have been achieved in items where steam penetration or heat penetration may be difficult.

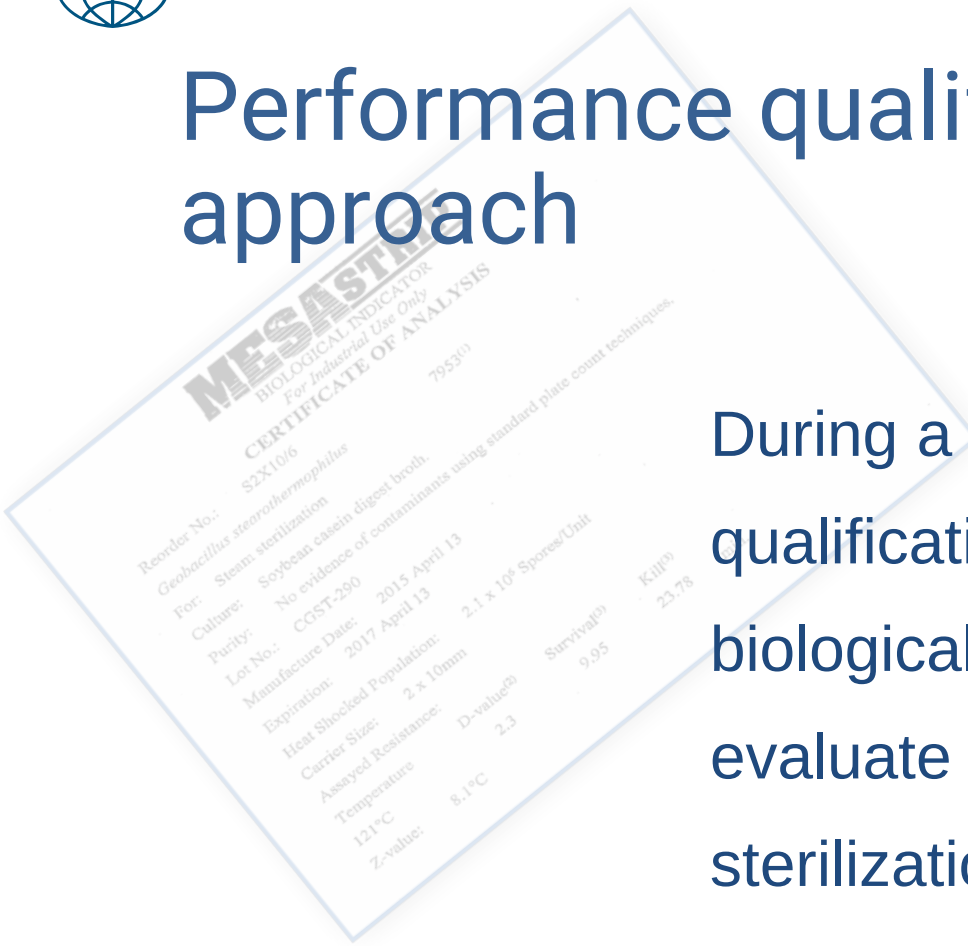
Performance qualification, biological approach

Consistency between physical and microbiological

result is central to sterilization validation.

Likewise, the destruction of a BI without consideration of the physical parameters needed to kill the BI does not provide sufficient evidence of the suitability of the cycle.

Performance qualification, biological approach



During a biological performance qualification, after having chosen the biological approach and the BI to use, evaluate the effectiveness of the sterilization cycle using the same batch of BI, if possible.

Performance qualification, biological approach

Monitor the process leaving biological indicators in the same position considered for thermal qualification: worst case locations, cold spots, should be monitored.

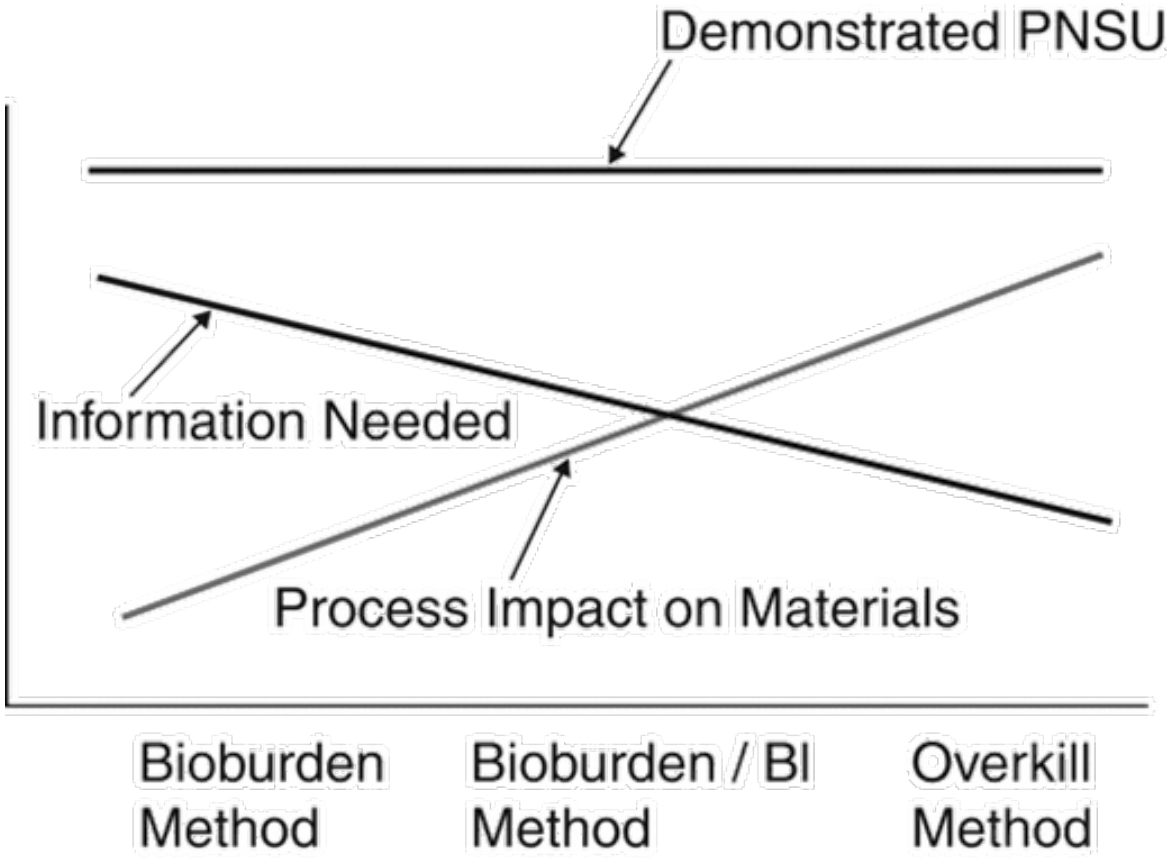
Validation methodologies

Validation methodologies: which is the best one? A microbiological point of view

Validation methodologies

The different approaches were developed because of the differences in the heat resistance of the materials to be sterilized.

Validation methodologies



OVERKILL STERILIZATION

Microbiological target: *the objective is a maximum PNSU of ≤10−6 for the bioburden*

Product: inert to the sterilizing agent

Bioburden knowledge: some bioburden knowledge, not so extensive as bioburden data required for bioburden process or BI/BB process

Use of BIs: process-resistant biological indicators with 10^6 spores and is demonstrated biologically based upon the spore log reduction of calibrated biological indicators.

Overkill is generally defined as a process that would deliver a minimum of F_0 of 12 minutes and is demonstrated biologically based upon the spore log reduction of calibrated biological indicators.

BI/BB APPROACH

Microbiological target: *the objective is a maximum PNSU of ≤10−6 for the bioburden*

Product: heat- labile one

Bioburden knowledge: it requires detailed knowledge of the bioburden and biological indicator populations and their relative resistance.

The relative resistance of the selected biological indicator to that of the bioburden must be established on or in the product.

Use of BIs: frequentely, biological indicators bearing approximately 10^6 spores with D_{121}-value > 1 minute are used in the development of such processes.

BB/BI is a method in which the incomplete destruction (or destruction of a modest population) of a resistant biological indicator can be used to demonstrate the capability of the process to reliably destroy any bioburden.

BIOBURDEN APPROACH

Microbiological target: *the objective is a maximum PNSU of ≤10−6 for the bioburden*

Product: heat- labile one

Bioburden knowledge: requires extensive knowledge of product bioburden, routine monitoring of the bioburden population and its resistance to the sterilization process is mandatory. The bioburden-based method requires the user to develop suitable critical control points within the process to control the bioburden titer.

Use of BIs: none

The bioburden-based method is used when material stability is limited or when there are no suitable biological indicator microorganisms available to use with the sterilizing process.

Syringes validation

OVERKILL APPROACH

If the liquid product is *not heat sensitive* and you are using the overkill method, then you could either directly inoculate the product or you could use sealed ampoule BI that is of a similar volume as the prefilled syringes, such as 1 mL MagnaAmp or 4 mL ProSpore, and place the BI in the chamber next to the syringes. In this way, the BI acts as a surrogate and experiences the same conditions as the prefilled syringes.

There are a couple of considerations to this method.

Will the syringe and glass ampoule heat at the same rate? ⟵ ***Primary packaging***

Will the product and the BI media heat at the same rate? ⟵ ***The product***

You may need to perform studies to show that spores in the liquid will be killed in the same amount or less time than the spores in the BI.

If the product has antimicrobial properties, then you will need to use a surrogate liquid that closely represents the actual product.

Syringes validation

BI/BB APPROACH

For heat – labile products

If you are using the BI/bioburden method then you must determine the D-value of the BI spores in the product and, with this information, select a BI with equal or greater resistance.

Again, if the product has antimicrobial properties, then you will need to use a surrogate liquid that closely represents the actual product.

Syringes validation

BIOBURDEN APPROACH

For heat – labile products.

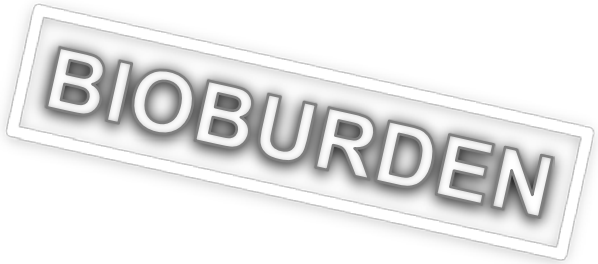
If you are using the bioburden method, then you will need to determine your most resistant bioburden and this would be used to directly inoculate the product.

And of course, if the product has antimicrobial properties, then you will need to use a surrogate liquid that closely represents the actual product

Overkill sterilization

The overkill process is frequently used when the article to be sterilized is completely inert to the sterilizing agent and sterilization cycle conditions without any concern for loss of product attributes or quality.

Overkill sterilization



…..When using this process, some bioburden knowledge should be available to ensure that the materials are not adulterated before sterilization….

(USP 41, General Chapter 1222)

….Where overkill sterilisation parameters are set for terminally sterilised products, bioburden might be monitored only at suitable scheduled intervals…..

(Annex 1, EU GMP)

Overkill sterilization

Overkill sterilization is a method in which the destruction of a high concentration of a resistant biological indicator can be used to demonstrate the capability of the process to reliably destroy any bioburden initially present on or in the load items.

Generally, process-resistant biological indicators containing approximately 10^6 spores with a determined D – value are used to establish the effectiveness of the sterilization process.

Overkill is generally defined as a process that would deliver a minimum **F_0 of 12 minutes** and is demonstrated biologically based upon the spore log reduction of calibrated BIs.

Overkill sterilization

"The objective of the overkill design approach is to assure a level of sterility assurance regardless of the number and heat resistance of the actual bioburden in the load." (PDA TR # 1 rev. 2007, Clause 4.1.1.1)

To convert this objective in practical criteria, it is assumed a microbial population with these values for population and resistance:

$N_0 = 10^6$

$D_{121} = 1'$

$z = 10^\circ C$

PDA TR#1

Using the above values, the design requirements for the delivered lethality, Fphy, Fbio, can be calculated as follow:

$F_0 = 1.0 \times \text{Log} (10^6 / 10^{-6}) = 12'$

Overkill sterilization: examples

- Calculated to provide a minimum 12 log reductions of microorganisms having a D-value of one minute at 121° C.
- Demonstration of 121° C for 15 minutes throughout all parts of a load.
- Through the complete inactivation of a microbial challenge of 10^6 spores of Geobacillus Stearothermophilus throughout the load.
- A process which demonstrates a minimum F_0 of 12 minutes throughout the load.

PDA TR#1

Overkill sterilization

The reference cycle for steam sterilisation is 15 min at 121°C in saturated steam determined in the coldest position of the chamber.

Bioburden/Biological indicator sterilization

"The bioburden-based method is used when material stability is limited or when there are no suitable biological indicator microorganisms available to use with the sterilizing process."

USP 41, General Chapter 1229

Bioburden/Biological indicator sterilization

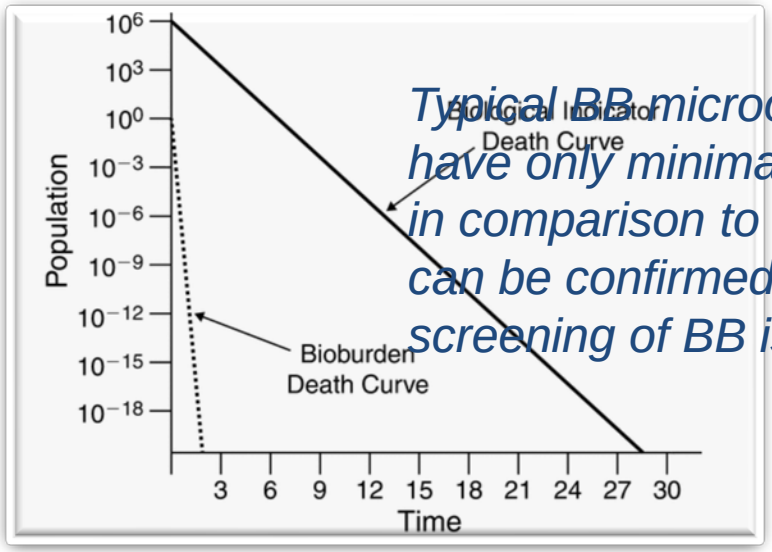
"Bioburden/biological indicator based sterilization is an approach in which the incomplete destruction (or destruction of a modest population) of a resistant biological indicator can be used to demonstrate the capability of the method to reliably destroy the bioburden present.

This is accomplished using detailed knowledge of the bioburden and biological indicator populations and their relative resistance.

USP 41, General Chapter 1229

Bioburden/Biological indicator sterilization

It relies on substantial differences between the population of the bioburden present and the biological indicator used during validation.



Typical BB microorganisms have only minimal resistance in comparison to BIs, and this can be confirmed by heat screening of BB isolates.

Bioburden/Biological indicator sterilization

The conventional BIs for terminal sterilization using BB/BI method are:

Clostridum sporogenes ATCC 7955

Bacillus Subtilis ATCC 5230

although other strain can be used.

The use of *G. stearothermophilus* is uncommon for the specific application because its strong resistance to moist heat makes it poorly suited for this application.

USP 41, General Chapter ⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS

Bioburden approach

This process is better suited for clean or ultra-clean products containing a consistently low level of colony forming units (cfu) per product unit. Also, this process may be necessary to permit terminal sterilization of a product that may potentially lose key qualities or attributes as a result of a more rigorous sterilization process.

USP 41, *General Chapter* ⟨1222⟩ TERMINALLY STERILIZED PHARMACEUTICAL PRODUCTS PARAMETRIC RELEASE

Bioburden approach

BB method is similar to the BB/BI method. The difference lies in the isolation and characterization of the most resistant bioburden microorganism.

USP 41, *General chapter* ⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS

Bioburden approach

The worst case isolate is used as the biological indicator in the evaluation of the process.

For use in this manner, it must be cultured to produce a suitable challenge population.

The bioburden of each process must be closely controlled with respect to population and must be monitored for resistance.

USP 41, General chapter ⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS

Bioburden approach

The bioburden-based method requires the user to develop suitable critical control points within the process to control the bioburden titer.

Products that readily permit bioburden survival require more controlled manufacturing environments and more precise in-process control.

USP 41, *General Chapter* ⟨1222⟩ TERMINALLY STERILIZED PHARMACEUTICAL PRODUCTS PARAMETRIC RELEASE

Product specific approach

For design purposes, the values selected for N_0 and D_T are based on values determined by bioburden analysis plus additional safety margins that are based on: 1) professional judgment 2) the extent of the bioburden data and 3) the degree of product bioburden testing that will be conducted on ongoing basis.

How to develop a cycle?

Ongoing tests for bioburden population and resistance, when?

Product specific approach: example1

a) bioburden testing of product

$N_0 < 10^1$ resistant microorganisms per unit of product

$D_{121°C} < 0.25$ minutes

b) values used for process design

$N_0 = 10^2$ microorganisms

$N_F = 10^{-6}$ (PNSU)

$D_{121°C} = 0.4$ minutes

c) calculated minimum lethality to achieve a PNSU of less than 10^{-6}

$F_{121°C} = (\text{Log } N_0 - \text{Log } N_F) \times D_T$

$(\text{Log } 10^2 - \text{Log } 10^{-6}) \times 0.4 \text{ minute} = 3.2 \text{ minutes}$

Since the design value for resistance is only slightly higher than the heat resistance of microorganisms found in the product, ongoing monitoring of BB population shoud be often conducted.

Product specific approach: example 2

a) bioburden testing of product

$N_0 < 10^1$ microorganisms per unit of product

$D_{121°C} < 0.25$ minutes

b) values used for process design

$N_0 = 10^2$ microorganisms

$N_F = 10^{-6}$ (PNSU)

$D_{121°C} = 1.0$ minute

c) calculated minimum lethality to achieve a PNSU of 10^{-6}

$F_{121°C} = (\text{Log } N_0 - \text{Log } N_F) \times D_T$

$(\text{Log } 10^2 - \text{Log } 10^{-6}) \times 1.0 \text{ minute} = 8.0 \text{ minutes}$

The need for BB monitoring is reduced but it has still to be monitored periodically

Parametric release

Parametric release is defined as the release of terminally sterilized batches or lots of sterile products based upon compliance with the defined **critical parameters of sterilization without** having to perform the requirements under ***Sterility Test***.

USP, 40 – General Chapter 1222

Parametric release

Requirements: Sterilization Microbiology Control

https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2008_11_25_gmp-an1_en.pdf

10.4 For parametric release systems, the bioburden assay should be performed on each batch and considered as an in-process test. Where appropriate, the level of endotoxins should be monitored.

Does not matter if it is BB or Overkill cycle!

Thank you

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