Biological Indicators and Validation

A. Cina, Technical Customer Support, Fedegari Autoclavi Spa

PDA Parenteral Drug Association

CONNECTING PEOPLE SCIENCE AND REGULATION®

Introduction to Steam Sterilization & Validation

All sterilization processes should be validated. Particular attention should be given when the adopted sterilization method is not described in the current edition of the European Pharmacopoeia, or when it is used for a product which is not a simple aqueous solution.
Where possible, heat sterilization is the method of choice.

[Annex 1 EU GMP, Clause 8.35/8.36]

"To validate a sterilization process, **physical conditions** are chosen that are expected to sterilise the items in the load to achieve a **sterility assurance level (SAL) equal to or less than 10^{-6}.**

[Eu Ph. 11.0 - Chapter 5.1.2]

The objective of a validation study is to demonstrate that the sterilization effectiveness anticipated from the physical process parameters is equivalent to the biological sterilization effectiveness.

[Eu Ph. 11.0 – Chapter 5.1.2]

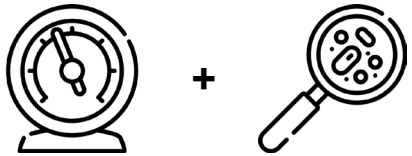
Physical VS microbiological results

Validation of sterilization processes links **physical measurements** with **biological indicator performance** to establish method lethality.

[USP 43, Chapter 1229]

*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 and repeatability of the cycle.



Spores don’t lie and probes don’t always know (or tell) the truth

The **probes** in your sterilization equipment are state-of-the-art, accurately calibrated, highly sensitive instruments and without them, controlling one's cycle would not be possible. But each one measures a limited environment and is only capable of sensing and measuring one parameter in a cycle.

Only the **Biological Indicator spores** can **accurately monitor the delivery of lethality** at various locations throughout the load.

[MesaLabs Spore News Vol 2 Issue 4 “ spores don’t lie and probes don’t always know (or tell) the truth]

Biological Indicators for the sterilization process

In addition to the physical sterilization parameter the effectiveness of a sterilization process is dependent of a large numbers of variables, which may include, but is not necessarily restricted to:

1. The number and the resistance of the contaminant micro-organisms;
2. Penetration of the sterilant;
3. Time;
4. Temperature;

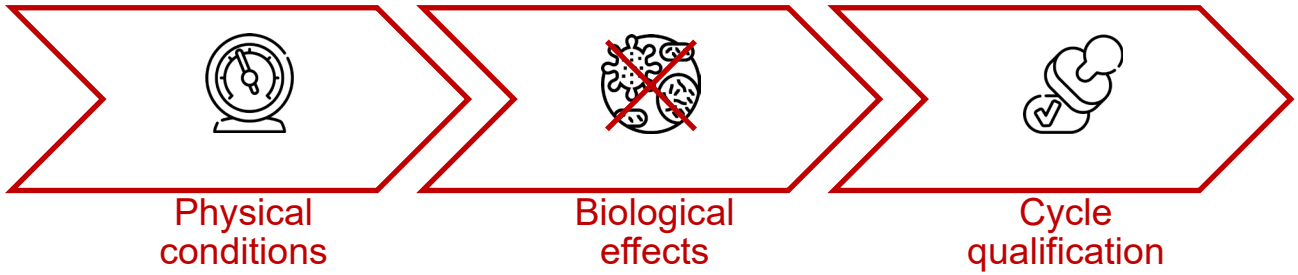
Biological Indicators for the sterilization process

In addition to the physical sterilization parameter the effectiveness of a sterilization process is dependent of a large numbers of variables, which may include, but is not necessarily restricted to:

5. Concentration;
6. pH;
7. Moisture content;
8. Chemical composition of the product or item.

Biological qualification

The objective of the biological component of cycle qualification is to **obtain biological data confirming that the developed cycle achieves the actual biological lethality requirements** established during cycle design.



Biological qualification

Biological qualification using microbiological challenges follows a straightforward sequence:

- An appropriate microbial challenge system is devised based on the **desired lethality** (F-value) determined during the design of the process;
- The load is exposed to minimal or subminimal **sterilization conditions**;
- After completion of the cycle, the **microbiological challenges are retrieved**;
- …

Biological qualification

Biological qualification using microbiological challenges follows a straightforward sequence:

- …
- Each microbiological challenge is **individually incubated** in appropriate media and conditions for growth of survivors;
- The results are evaluated to ensure that the **spore log reductions** achieved for the microbiological challenges **meet predetermined acceptance criteria**;
- Growth of the microbiological challenge organism is required **in positive controls**.

Biological indicators at glance

What is a Biological Indicator?

Test systems that provide a defined challenge to verify required effectiveness of a specified sterilisation process.

[Eu Ph. 11.0 5.1.2.]

Which type of microorganisms?

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

[USP 43, Chapter 1229.5]

Why to use Biological Indicators?

BIs are used to demonstrare the effectiveness of processes that render a product sterile.

[USP 43, Chapter 1229.5]

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 selection

The **selection of the types of biological indicator** used will depend on:

- The **nature of the sterilizing agent** (*Moist heat – G. stearothermophilus, Dry Heat – B. atrophaeus, Ethylene Oxide – B. atrophaeus, H2O2 – G. stearothermophilus*);
- The expected **effectiveness of the treatment** (*e.g. the* F_{Phys} *calculated from the process parameters*);
- The **characteristics** of the pharmaceutical **product** or item to be sterilized. (*e.g. final container, packaging material, utilities such us tubes or pumps*).

[Eu Ph. 11.0, Chapter 5.1.2]

Biological Indicators selection

PAY ATTENTION!

The selection of a BI requires knowledge of the **resistance of the BI** system to the specific sterilization process.

It must be established that the **BI system provides a challenge** to the sterilization process greater than the resistance of the native bioburden.

[USP 43, Chapter 1229.5]

Use and Placement of Biological Indicators

For cycles designed using the **overkill design approach**, the challenge system is typically spores of *G. stearothermophilus.*

Lower levels of thermal input may be delivered for the **product-specific design approach**. Consequently, the challenge organism used in qualification is often less resistant than spores of *G. stearothermophilus*.

Typical microorganisms used for qualification of cycles using the product-specific design approach include: *Clostridium sporogenes, Bacillus smithii* (formerly *Bacillus coagulans*) and *Bacillus subtilis 5230*.

[PDA TR No.1 par. 3.2.1]

Biological indicators: Microorganisms & Regulatory References

	ISO 11138-3	Eu. Ph. XI ed.	USP 43
Strain 	G. stearothermophilus	G. stearothermophilus (ATCC 7953, 12980, NCTC 10007, CIP 52.81, NCIMB 8157)	G. stearothermophilus (ATCC 12980, ATCC 7953)
			C. sporogenes (ATCC 7955)
			B. atropheus (ATCC 9372)
			B. subtilis (ATCC 5230)
Population 	≥1,0*10^5		
D value at 121°C 	≥1,5 min	1,5 min to 4,5 min	
z value 	≥6°C		

Biological Indicators: Types

Spore suspension

Self contained

Inoculated carriers

Custom–made

Inoculated carriers

Consist of a **defined population** of bacterial spores inoculated into or onto a **suitable carrier** and in most cases in a protective envelope.

The **type of carrier** (and the envelope if used) may **influence the resistance** of the bacterial spores and must be compliant with the chosen sterilization process.

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

After the exposure the carrier is aseptically handled according to the manufacture's instruction, transferred to a suitable culture medium and incubated for a sufficient period of time at appropriate temperature.

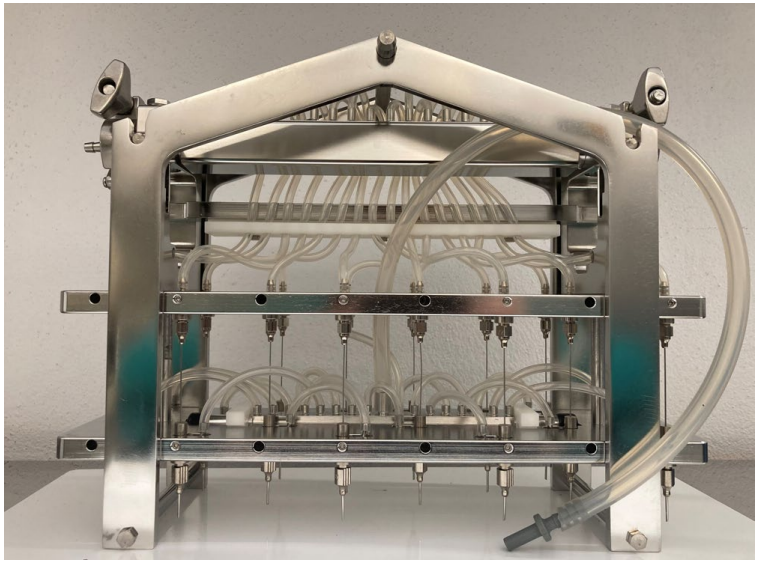
[Eu Ph. 11.0, Chapter 5.1.2]

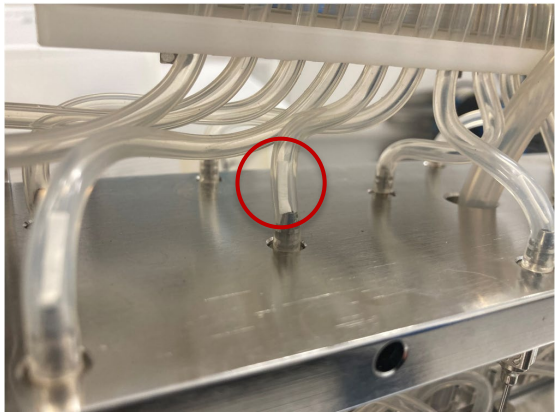
Practical example

Load: Dosing system

BIs: Inoculated carriers

Cycle: Steam sterilization by direct contact



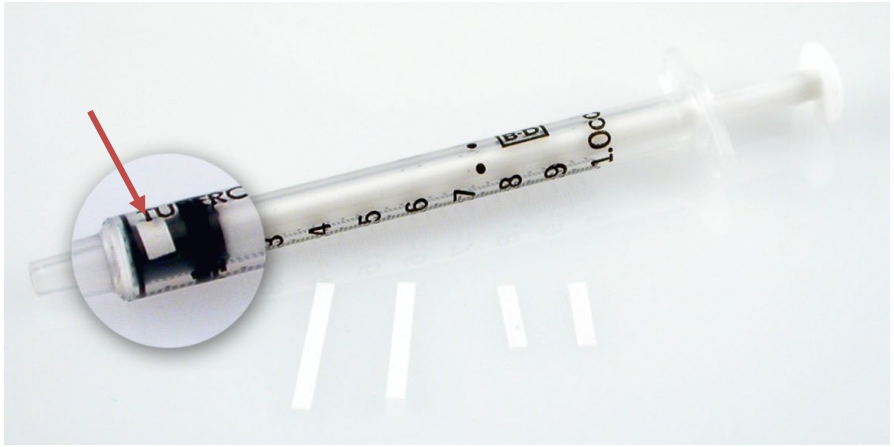


Practical example

Load: Syringes

BIs: Inoculated carriers

Cycle: Air over steam sterilization



Practical example

Load: Stoppers in double bags

BIs: Inoculated carriers

Cycle: Steam sterilization by direct contact

Self-container BIs

System consisting of an **inoculated carrier and a container** (e.g. ampoule) with a nutrient medium suitable for the test micro-organism used.

The sterilizing agent comes in contact with the inoculated carrier while the growth promoting properties of the nutrient medium are not adversely affected by the sterilization process. After the exposure the carrier is brought into contact with the nutrient medium **by simple manipulation**.

[Eu Ph. 11.0, Chapter 5.1.2]

TIPS: Using an SCBI provides an alternative option for customers who do not have the capability to perform aseptic culturing as the system is 'self-contained' and this eliminates the chance of post-process contamination.

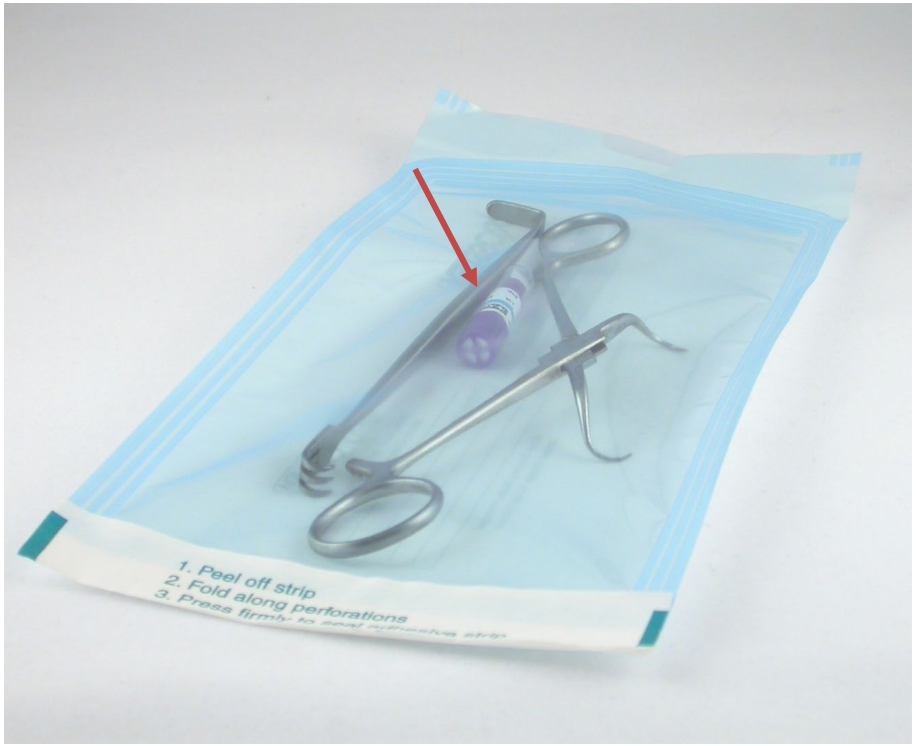
[MesaLabs spore news 'biological indicator selection' vol 12 issue 5]

Practical example

Load: Metal items in Tyvek bag

BIs: Self contained

Cycle: Steam sterilization by direct contact



Self-container BIs

Container (e.g. ampoule) of a population of the test micro-organism **in an appropriate nutrient medium.**

After the exposure the container is incubated without any further manipulation.

This type of biological indicator is sensitive only to an exposure time and temperature and may be used primarily to monitor **sterilization of aqueous fluids**.

[Eu Ph. 11.0, Chapter 5.1.2]

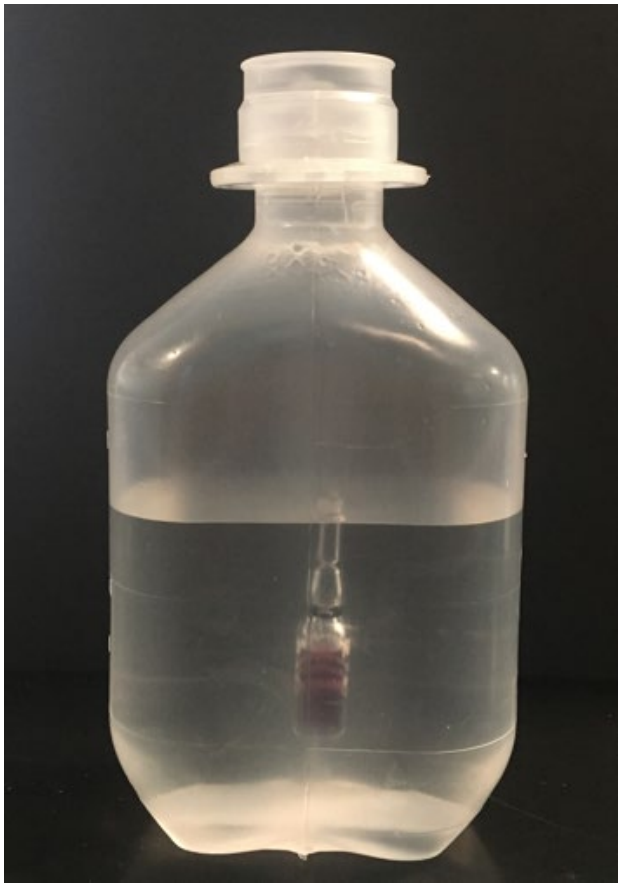
Practical example

Load: Liquid loads

BIs: Self contained ampoules

Cycle: Steam sterilization by direct contact

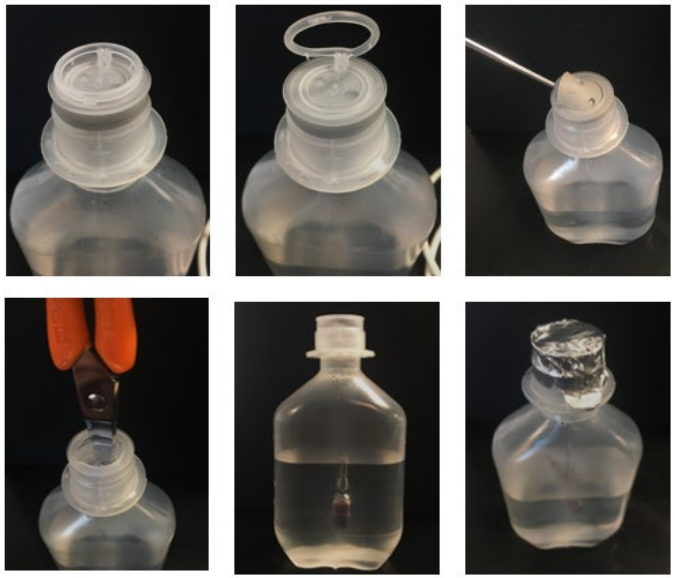
Practical example



Load: 250 ml plastic bottles (PP) filled with water

BIs: Self contained ampoules

Cycle: Super-heated water sterilization



Spore suspension

Consist of a **defined population of bacterial spores**, prepared from clearly characterized ad suitable maintained strain of a spore forming bacterial species in a **stable suspension**.

[Eu Ph. 11.0, Chapter 5.1.2]

Custom made BIs

Test items (e.g. rubber stoppers), **or products, inoculated with a suitable test micro-organism**, usually from a characterized spore suspension.

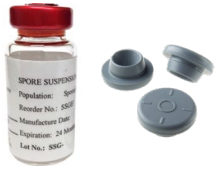
The spore suspensions can be prepared from isolated environmental monitoring or other microbiological testing using a well-defined procedure designed to give satisfactory sporulation. The D- value and, when appropriate the z-value of the spore suspension must be determined.

The D-value and the z-value (if appropriate) of the spores of the inoculated items/products must be determined as this may be different from the spores in suspension.

After the exposure to the sterilization cycle, the custom-made biological indicator is enumerated or tested for the presence/absence of surviving test-microorganism using a validated, appropriate microbiological technique.

[Eu Ph. 11.0, Chapter 5.1.2]

Practical example



Load: Stoppers
BIs: Spore suspension
Cycle: Steam sterilization by direct contact

Spores do not have an intrinsic D-value

A major misunderstanding in the industry is that spores have an intrinsic D-value. This is not true if for no other reason than it is impossible to test the resistance of individual spores suspended in space. **The spores are placed onto a surface or suspended in liquid and this system becomes the biological indicator (BI)**.

By definition, *the D-value for a BI is the time (or dose) required at a specified set of exposure conditions to reduce the viable spore population by one log or 90%.*

Don't forget that the D-value is a measure of resistance performance that refers to the entire BI package and is not a value for the spore itself.

[MesaLabs Spore News, Vol. 5 N. 1 - January 2008]

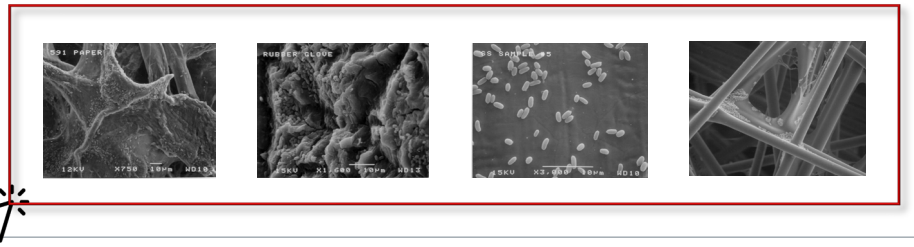
Spores do not have an intrinsic D-value

Solid Surfaces

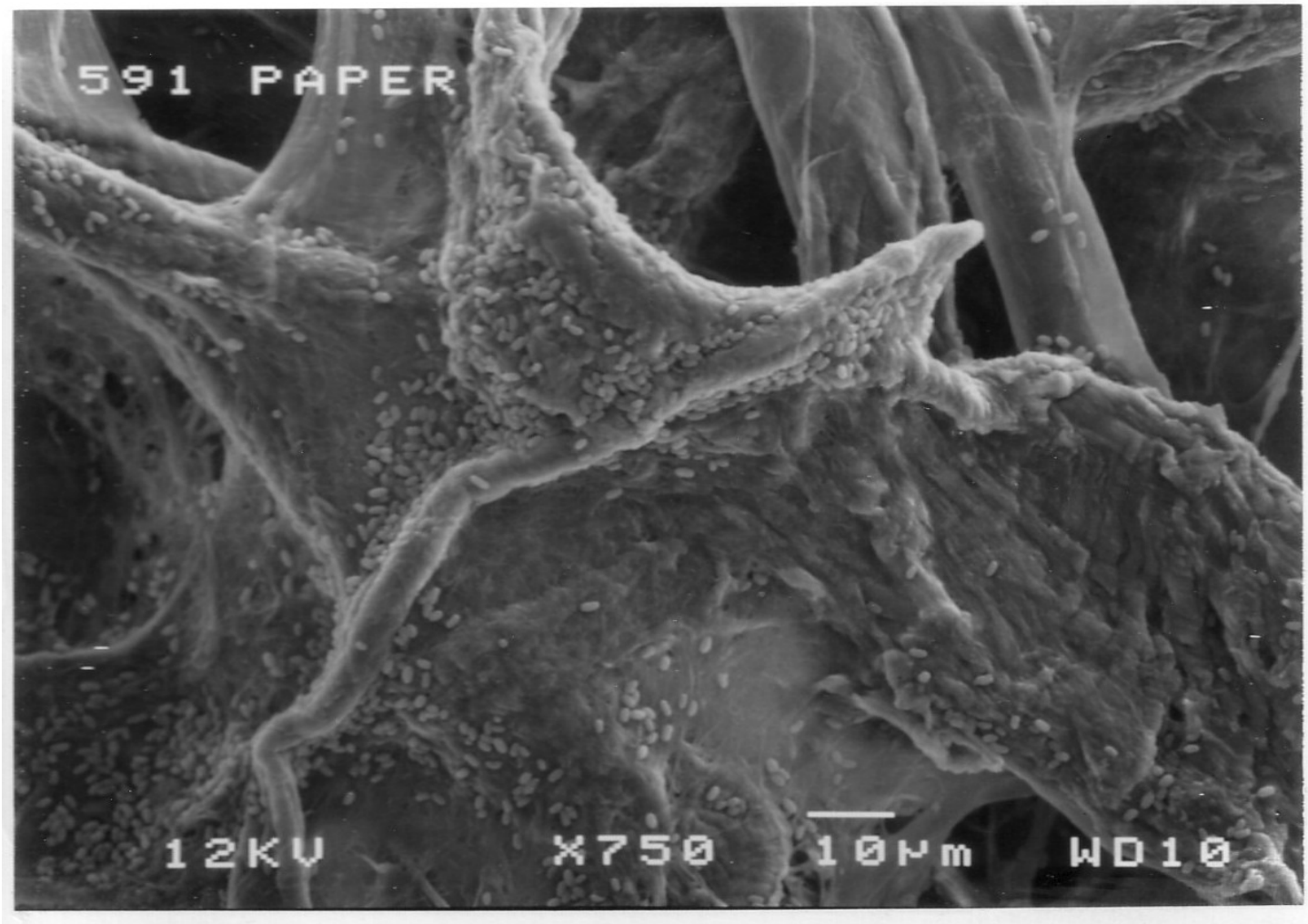
Not all surfaces are equal. **Spores will interact differently with a surface** depending on many factors:

- Surface topography
- Surface hydrophilicity
- Different elastomers type
- Etc…

[MesaLabs Spore News, Vol. 5 N. 1- January 2008]



PAPER



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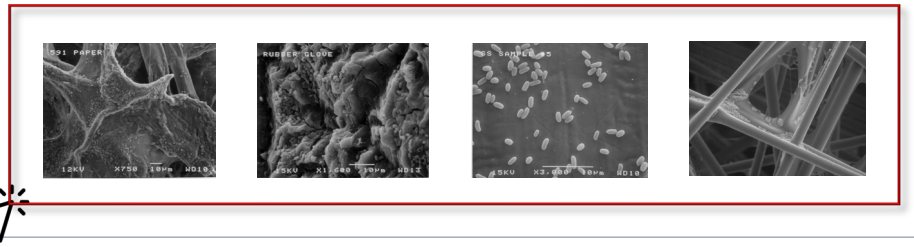
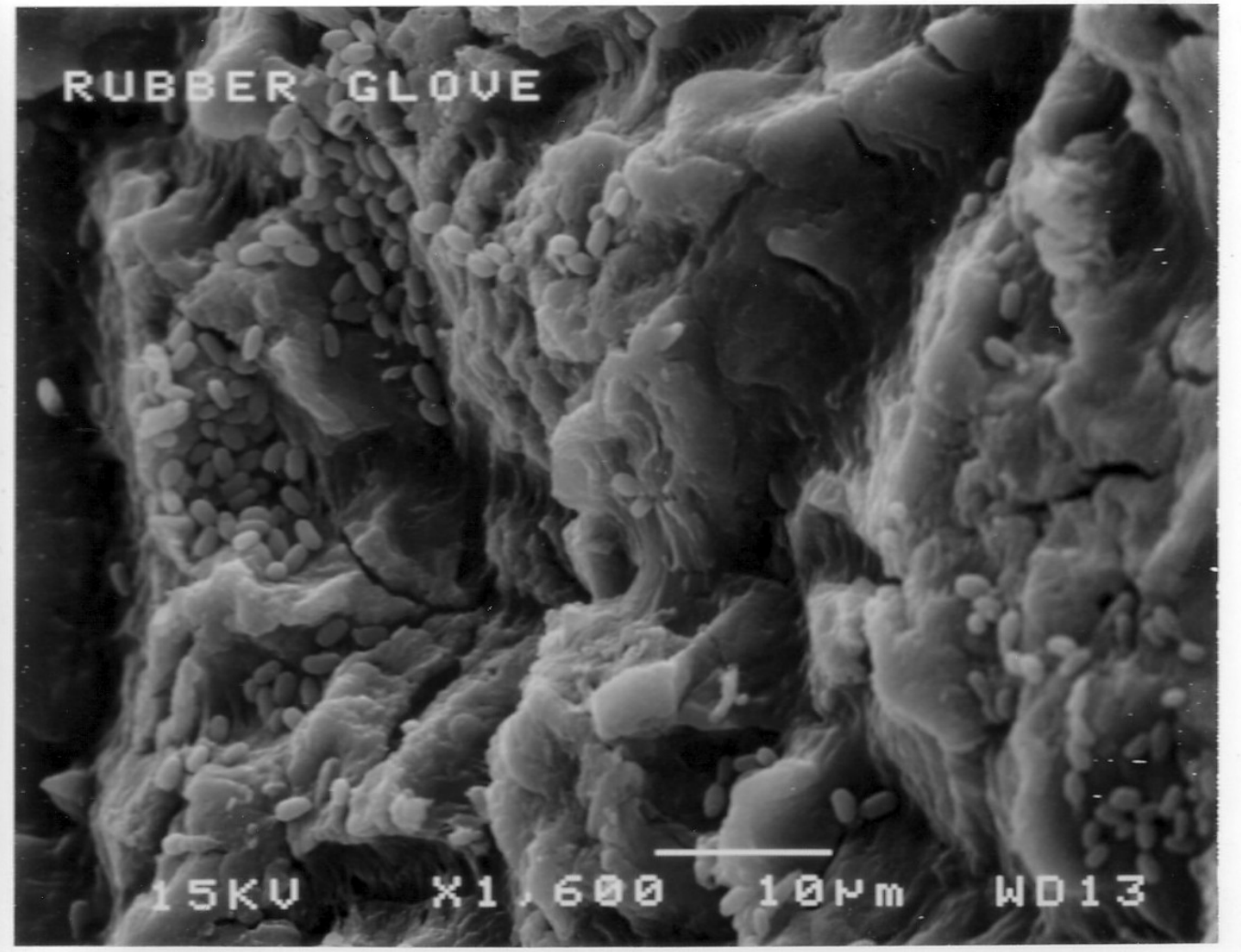


RUBBER GLOVE



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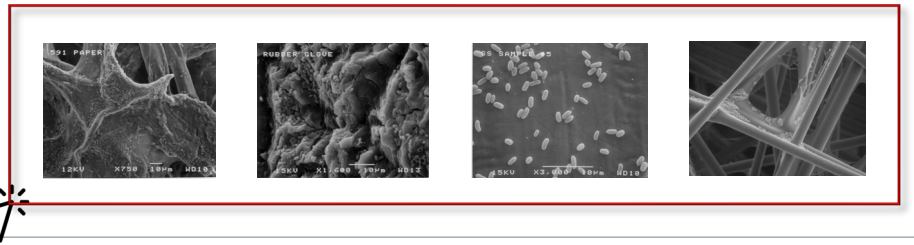
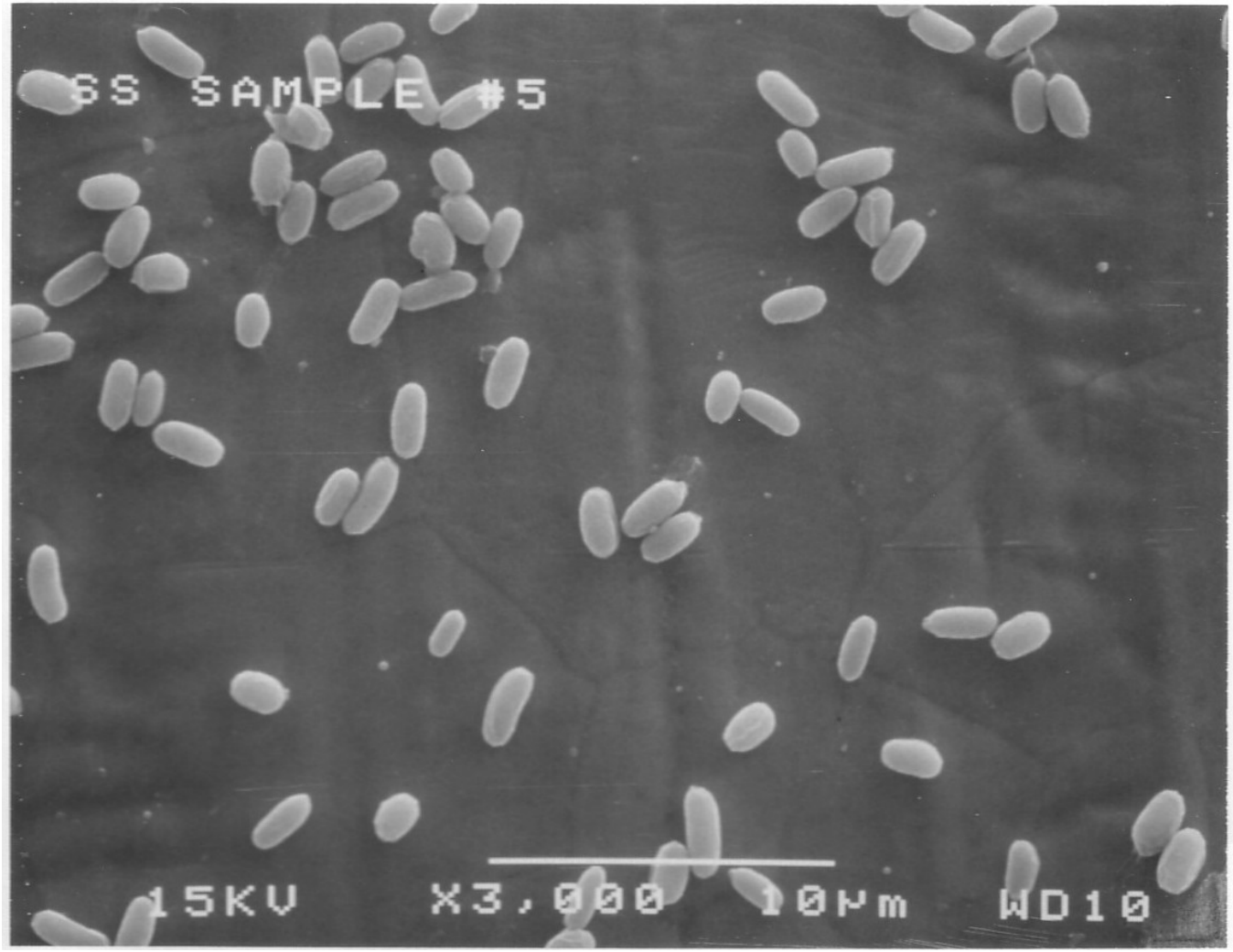


STAINLESS STEEL



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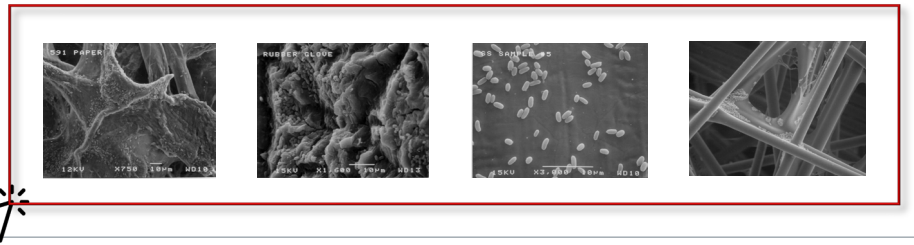
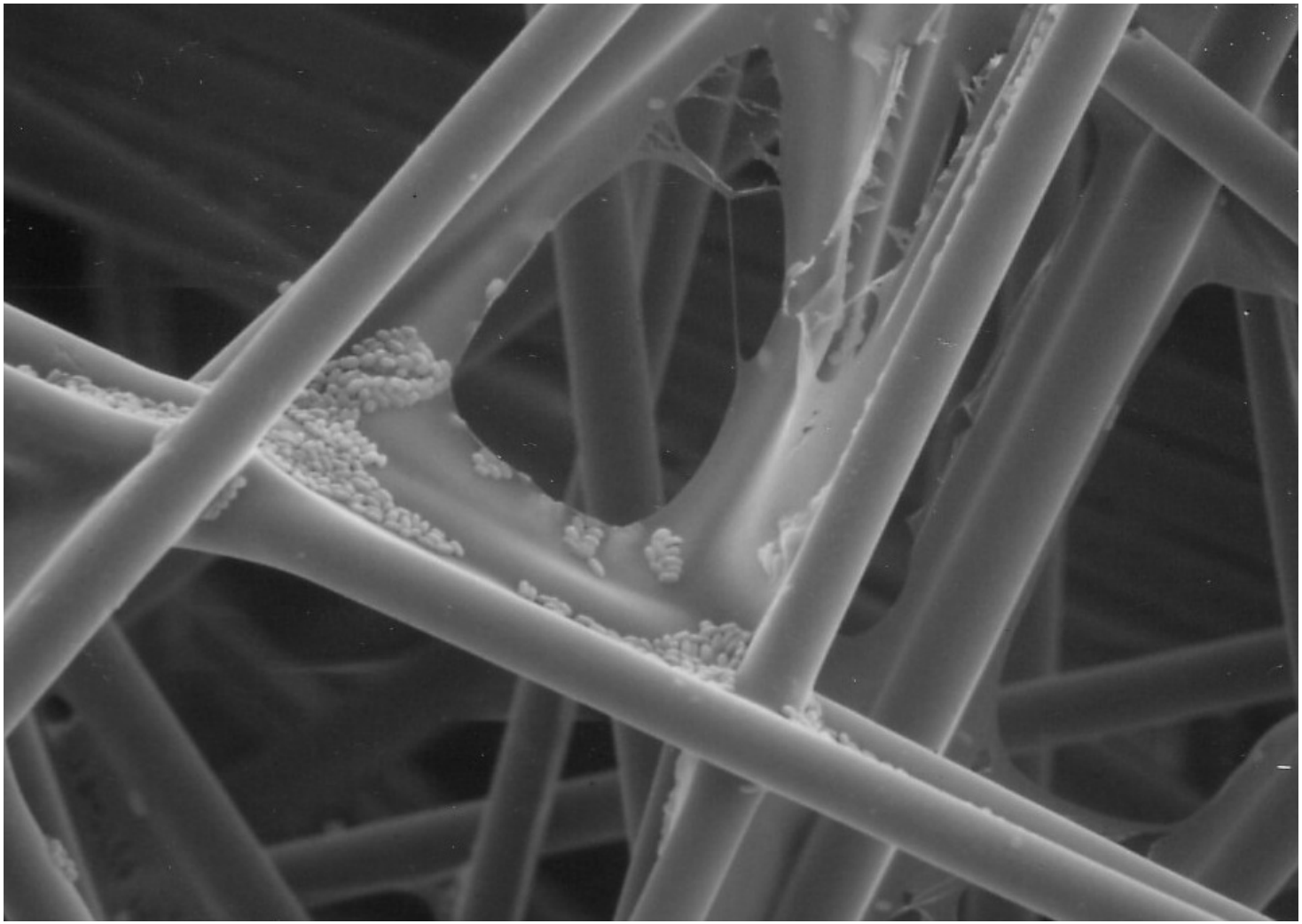


FIBERGLASS



Biological indicators: Responsibility

Manufacturer

Determining the **performance characteristics** of each BI lot;

Provide information concerning the **microbial population and resistance** (D and z values, respectively, where appropriate);

The **resistance** of the BI should be determined by the manufacturer under defined conditions;

[USP 43 Chapter 1229.5]

Biological indicators: Responsibility

Manufacturer

Storage and **expiry** information;

Should provide **directions for use**, including the medium and conditions used for the recovery of microorganisms after exposure to the sterilization process;

Disposal instructions also should be provided by the manufacturer of the BI;

The manufacturer should provide, with each lot of BIs a **certificate of analysis** that attests to the validity of BI performance claims.

[USP 43 Chapter 1229.5]

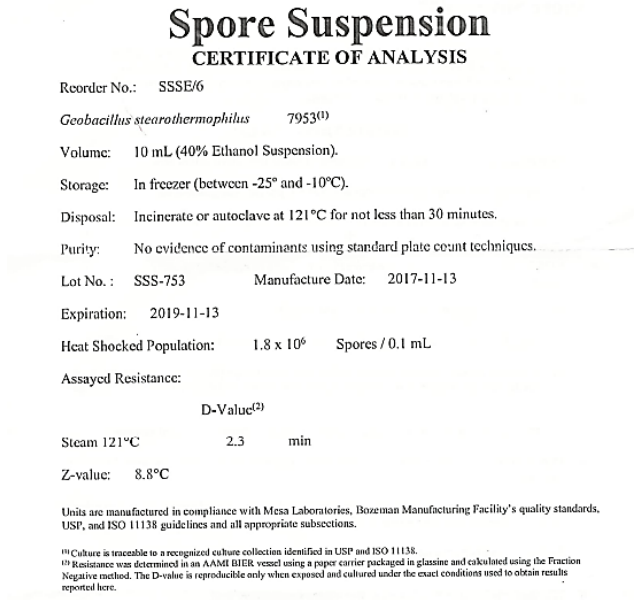
Characterization of biological indicators

Packaging and storage

Store under the conditions recommended on the label or under validated conditions, and protect from light, toxic substances,excessive heat, and moisture;

Expiration date

Use within the BI's labeled or determined expiration date;



Spore Suspension

CERTIFICATE OF ANALYSIS

Reorder No.: SSSE/6

Geobacillus stearothermophilus 7953[1]

Volume: 10 mL (40% Ethanol Suspension).

Storage: In freezer (between -25° and -10°C).

Disposal: Incinerate or autoclave at 121°C for not less than 30 minutes.

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

Lot No. : SSS-753 Manufacture Date: 2017-11-13

Expiration: 2019-11-13

Heat Shocked Population: 1.8×10^6 Spores / 0.1 mL

Assayed Resistance:

	D-Value[2]	
Steam 121°C	2.3	min

Z-value: 8.8°C

Units are manufactured in compliance with Mesa Laboratories, Bozeman Manufacturing Facility's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections.

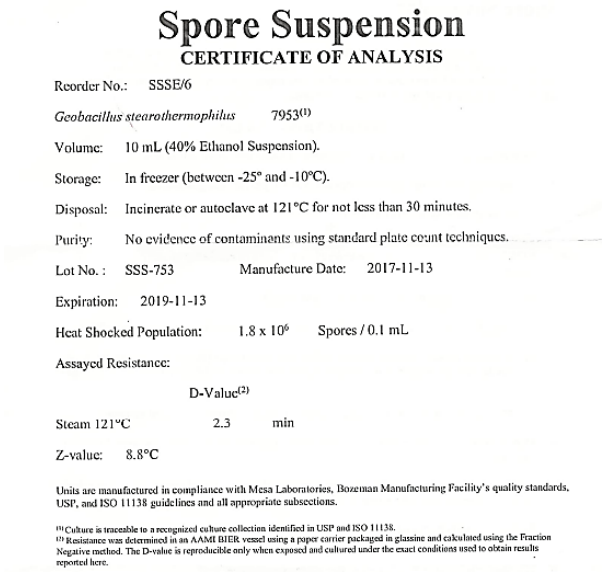
[1] Culture is traceable to a recognized culture collection identified in USP and ISO 11138.
[2] Resistance was determined in an AAMI BIER vessel using a paper carrier packaged in glassine and calculated using the Fraction Negative method. The D-value is reproducible only when exposed and cultured under the exact conditions used to obtain results reported here.

[USP 43 Chapter 1229.5]

Characterization of biological indicators

Identification

Where identification of the BI species is deemed necessary, as in the course of an investigation into unusual results, use either a phenotypic or genotypic identification method;



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[USP 43 Chapter 1229.5]

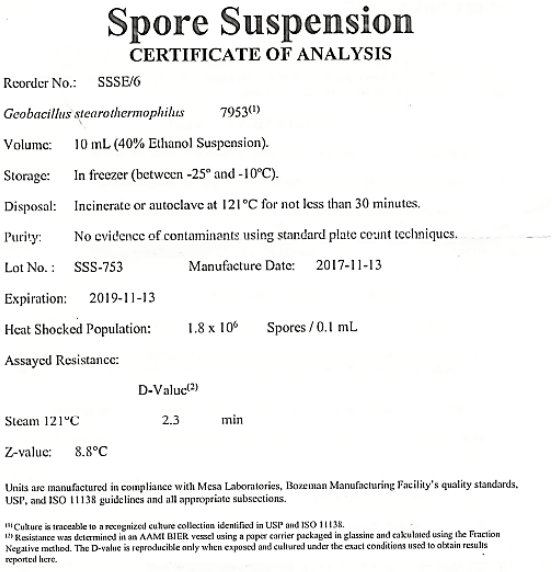
Characterization of biological indicators

Purity

By examination of the colonies derived from the spores on a suitable plate culture medium, determine that there is no evidence of contamination with other microorganisms;

Disposal

Prior to discarding used spores, sterilize using a method recommended by the BI manufacturer or other equivalent means.



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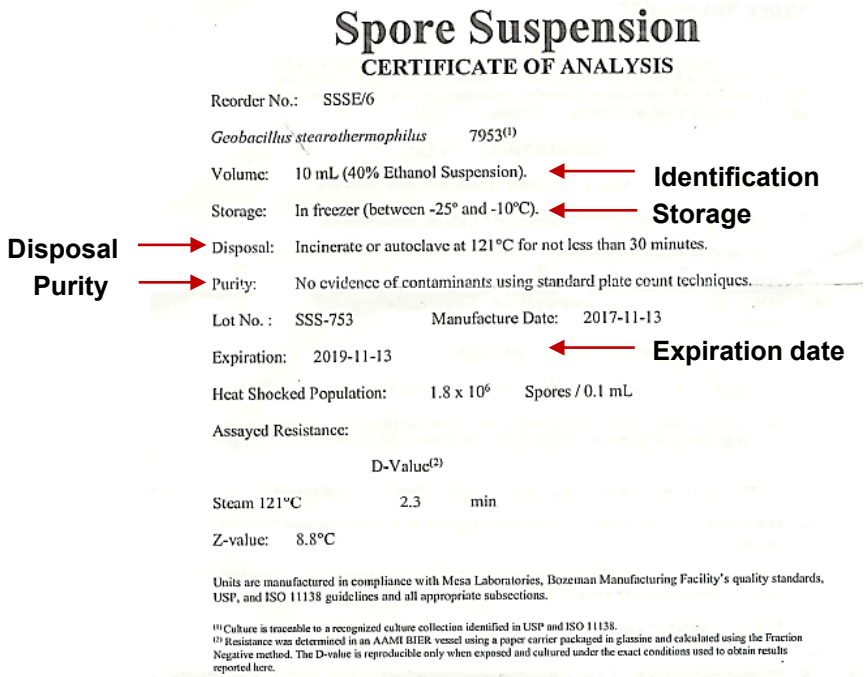
[USP 43 Chapter 1229.5]

Characterization of biological indicators



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Biological indicators: Responsibility

User

When BIs are purchased, their **suitability for use** in a specific sterilization process must be established.

The BI user should obtain a **certificate of analysis** for each lot of BIs and verify the manufacturer's label claims for spore population

When BIs are **used in accordance** with the BI manufacturer's directions, the **resistance of the BI need not be reconfirmed**.

[USP 43 Chapter 1229.5]

Biological indicators: Responsibility

User prepared biological indicators

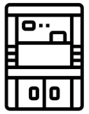
A user of BIs may elect to propagate spore crops of a single species for use as a suspension. Alternatively, these spore suspensions may be purchased from a BI manufacturer. **When liquid suspensions are applied to a substrate, it is the user's responsibility to determine the population and resistance of the microorganism used**. The resistance determined for liquid suspensions relates only to other lots of the same suspension and is not representative of how that microorganism will perform on a substrate or in a different suspending medium. In these circumstances, the BI resistance and population should be re-established.

[USP 43 Chapter 1229.5]

Tech Tips

The carrier and primary packaging **should not be damaged** or degraded by the specific sterilization process;

The **time** between completion of sterilization process and incubation should be within the manufacturer's stated time or should be justified;



Transfer of microorganisms exposed to the sterilization process to the appropriate recovery medium should be done using aseptic technique.

Tech Tips

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.

[Eu Ph. 11.0, Chapter 5.1.1]

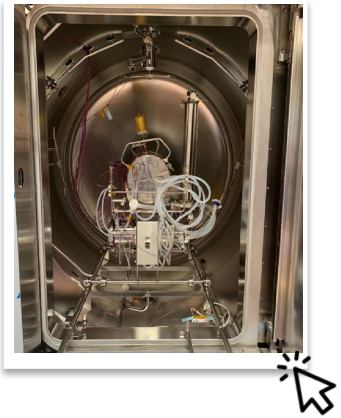
Monitor the process leaving biological indicators in the **same position considered for thermal qualification**: **worst case locations** and **cold spots** should be monitored.
The identification of the worst-case position of each standard load is part of the validation exercise of the sterilization process.

Performance Qualification

PQ is the most demanding part of validation exercise, because it refers to **actual loads** and **operating conditions**. Basically, it includes:

Physical qualification→ by **measuring physical parameters;**

Biological qualification → by measuring the **actual kill of microorganism in a biological indicator.**



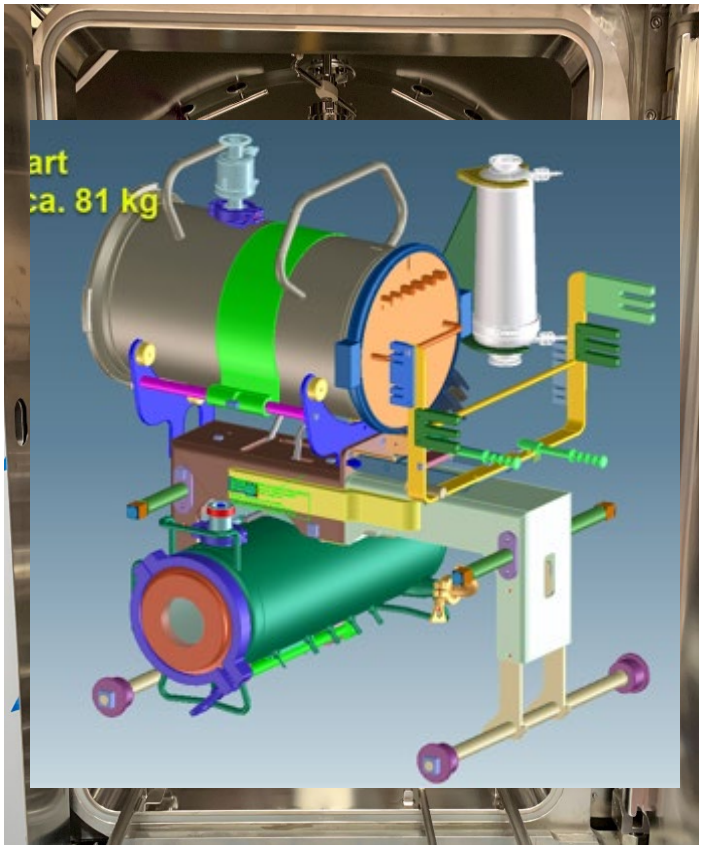
Both **physical and biological qualification** are concerned with definition of number of TE, TC, BIs and their placement in test with standard loads.

PQ on an actual load

Dosing canister for vials filling process

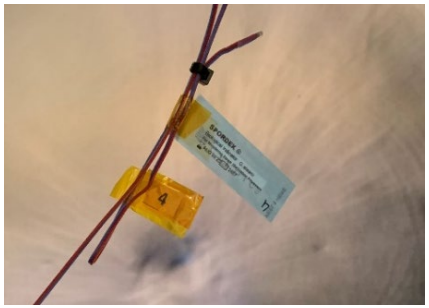
Use of Biological Indicators

Use of Thermocouples

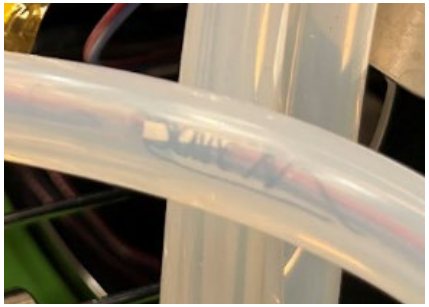


Biological Indicators used

Inoculated carrier with envelope
Hanged on the surfaces of the trolley and the chamber and inserted in the pipes with larger diameter



Inoculated carrier without envelope
Inserted in the pipes with small diameter



Aim of the PQ

Physical qualification

Temperature monitoring in different points of the load and the chamber.

Biological qualification

Measure of the killing effect on the BIs – investigation in the same locations of the TC probes.

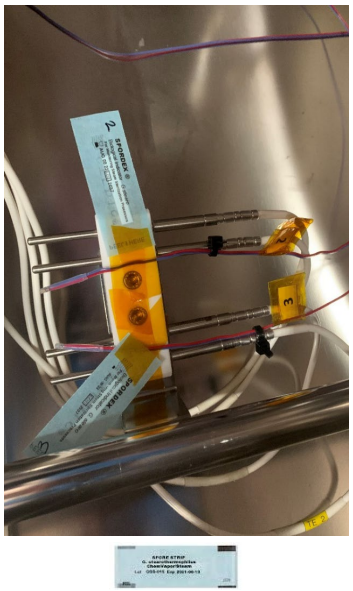
Dryness and Integrity of the load

To match the chosen process with the efficiency in terms of thermal and biological overcomes.

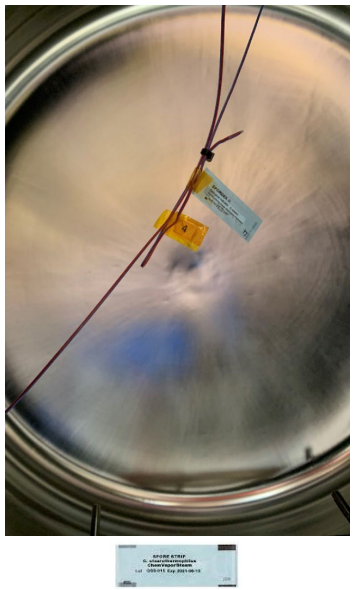
Position of validation TC and BI

TC and BI 1
Drain of the autoclave

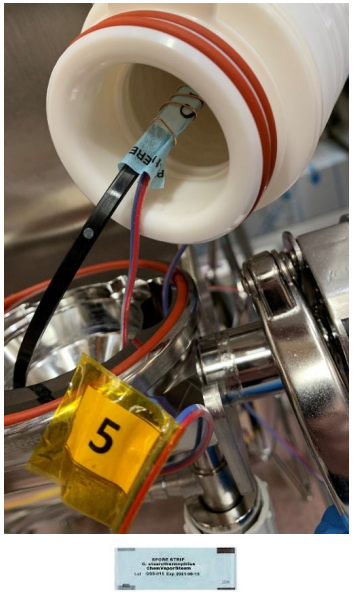
TC and BI 2 and 3
TE4 and TE2 plant probe



TC and BI 4
In the centre of the autoclave chamber

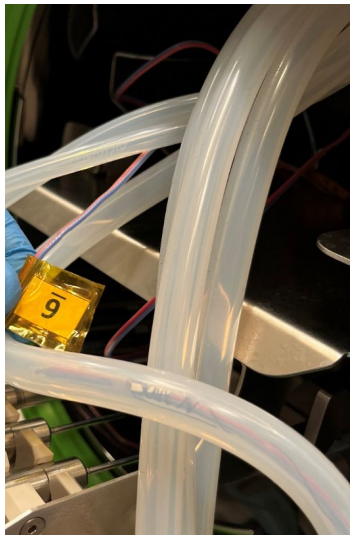


TC and BI 5
Pall filter –
Internal part of the cartridge

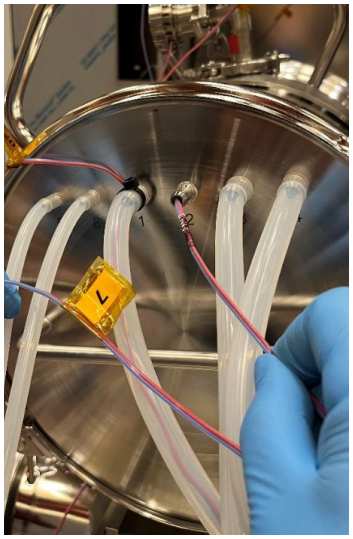


Position of validation TC and BI

TC and BI 6
Inside plastic pipe

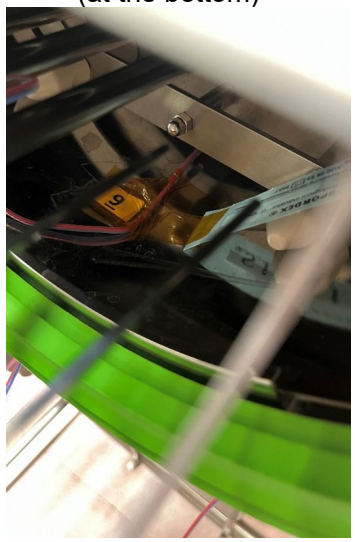


TC and BI 7
Inside needle No.2



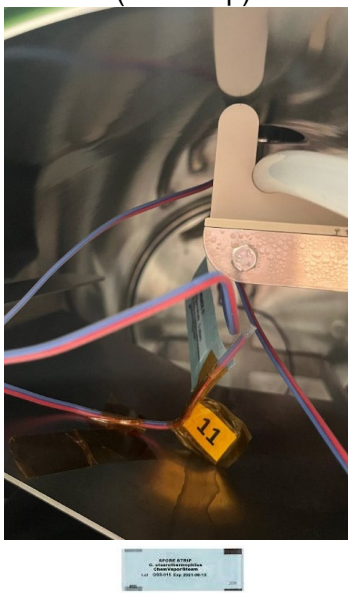
TC and BI 8
Inside disposable filter -
External part of the cartridge

TC and BI 9
Inside upper vessel
(at the bottom)

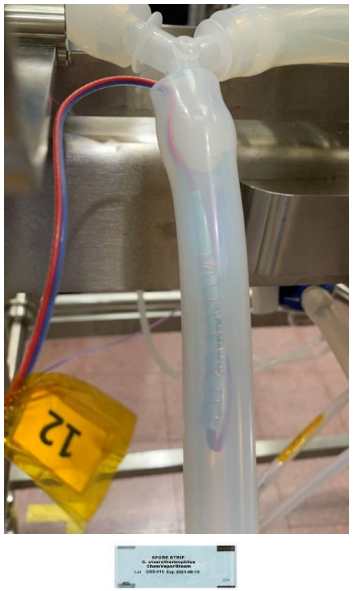


Position of validation TC and BI

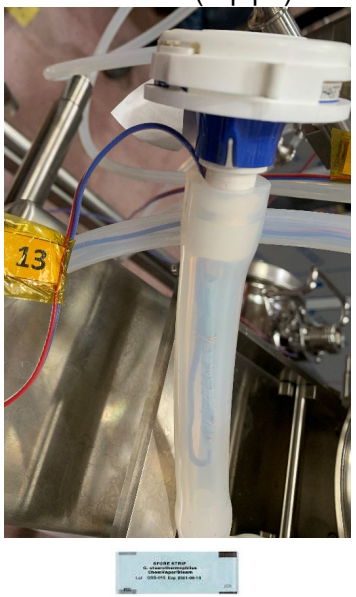
TC and BI 11
Inside upper vessel
(at the top)



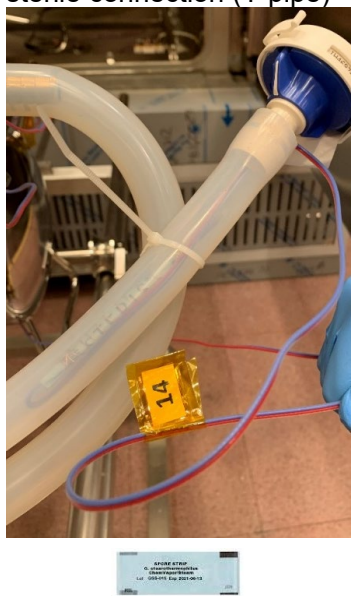
TC and BI 12
Inside Y connection



TC and BI 13
Inside plastic pipe with sterile connection (Y pipe)

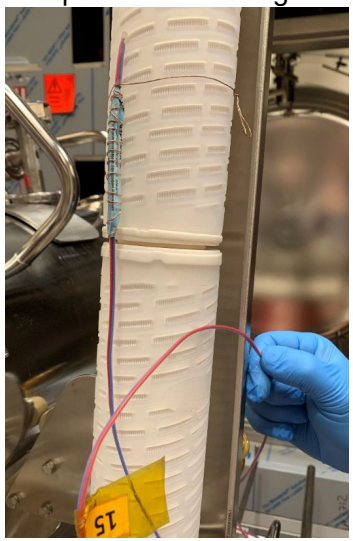


TC and BI 14
Inside plastic pipe with sterile connection (Y pipe)

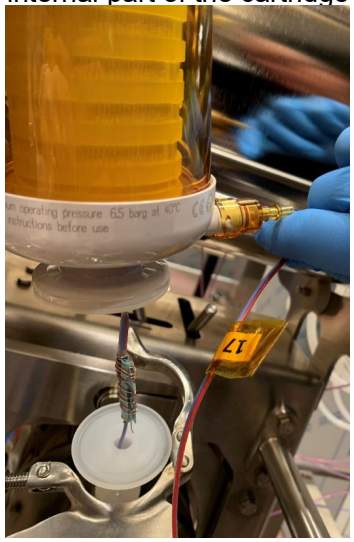


Position of validation TC and BI

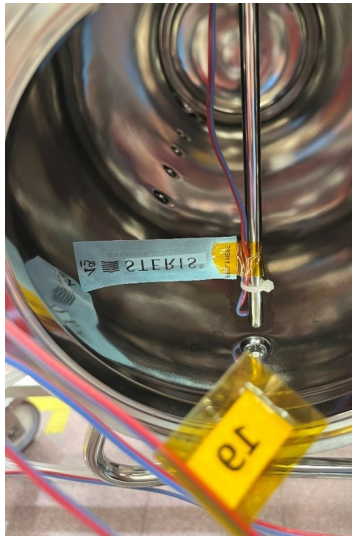
TC and BI 15
Pall filter – External part of the cartridge



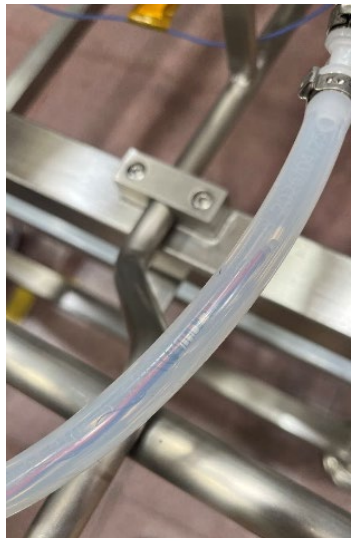
TC and BI 17
Inside disposable filter – internal part of the cartridge



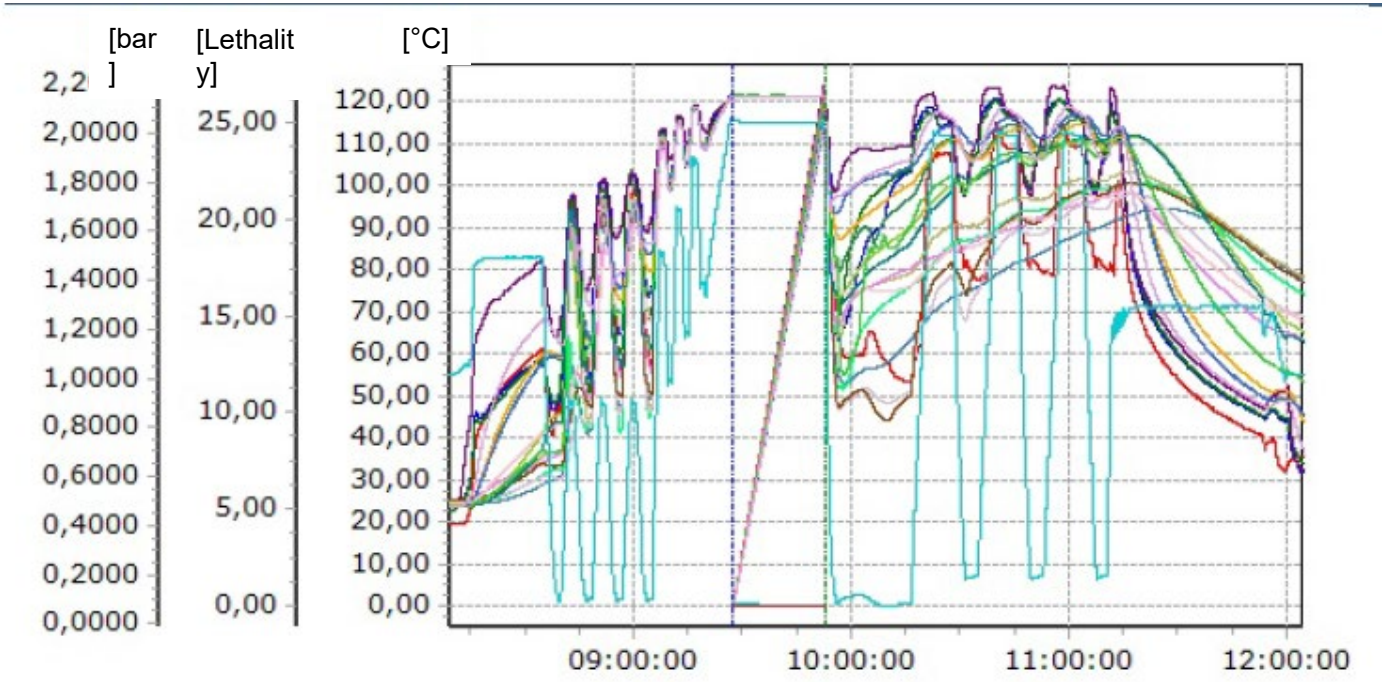
TC and BI 19
Close to level sensor probe



TC and BI 20
Inside plastic pipe



Temperature mapping



Good thermometric results: 6 seconds of equilibration time → every TC was within the sterilization range (120°C – 122°C).

Outcomes after BIs incubation

No Growth in any of the investigated positions

Outcomes after BIs incubation

No Growth in any of the investigated positions

Aim of the PQ VS Final results

Physical qualification

Sterilization temperature reached in every investigated point.

Biological qualification

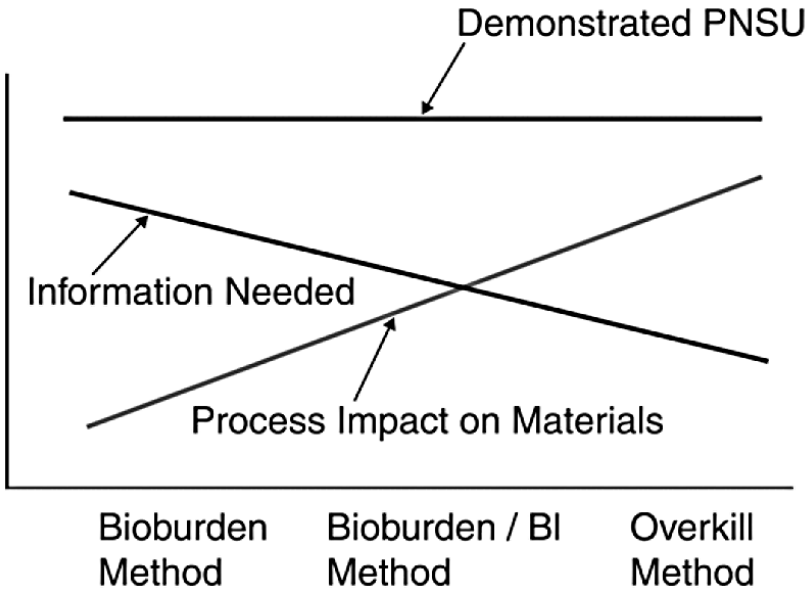
No growth in any investigated position.

Dryness and Integrity of the load

Dry load, filters not damaged.

Validation Methodologies at glance

- Overkill method
- Bioburden/BI method
- Bioburden based method



[USP 43 Chapter 1229]

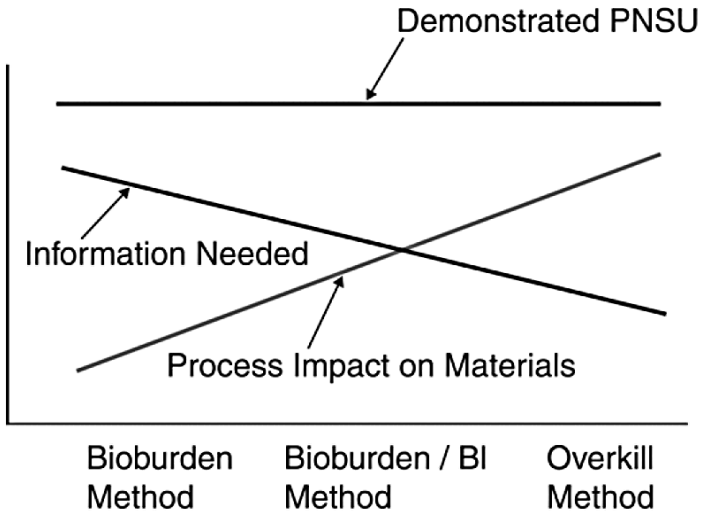
The different approaches were developed because of the **differences in the heat resistance of the materials** to be sterilized.

Overkill approach

Overkill sterilization is a method in which the **destruction** of a **high concentration** of a **resistant microorganism is correlated with the destruction of reasonably anticipated bioburden** present during routine processing.

When the load items can withstand substantial **heat without adverse consequence**, overkill is the **method of choice** for steam sterilization because of its ease of execution, reduced considerations for bioburden control, and overall simplicity.

[USP 43, Chapter 1229.1]



[USP 43 Chapter 1229]

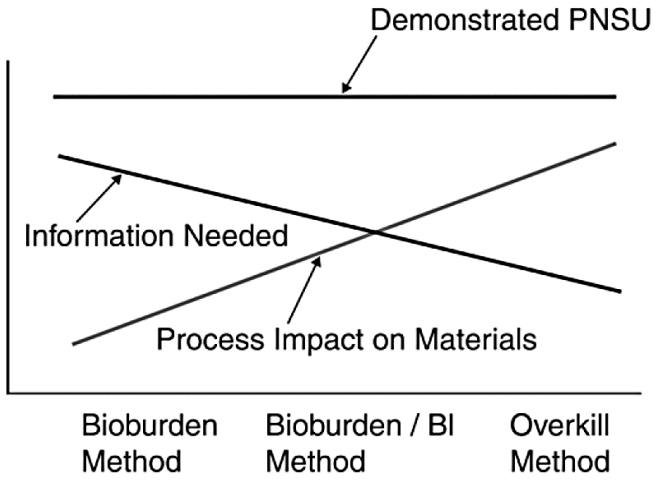
Overkill approach

Microbiological target: PNSU of ≤10^{-6};

Product: items that are unaffected by the process exposure (inert items);

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

Use of BIs: G. stearothermophilus (ATCC 12980 or ATCC 7953).



[USP 43 Chapter 1229]

Overkill design approach

"…a cycle design with the overkill design approach can be defined as a sterilization cycle that is demonstrated to deliver an **F_{PHY} and F_{BIO} of at least 12 minutes** to the items being sterilize"

[PDA TR No 1, Paragraph 4.1.1.1]

DATA

$N_0 = 10^6$

$D_{121°C}$ = 1.0 minute

z= 10°C

GOAL

$N_F = 10^{-6}$

CALCULATION

$F_0 = D_{121°C} * (\text{Log } N_0 - \text{Log } N_F)$

F_0 = 1.0 minute * (Log 10^6 – Log 10^{-6}) = 12 minutes

[Overkill design approach, PDA TR No 1]

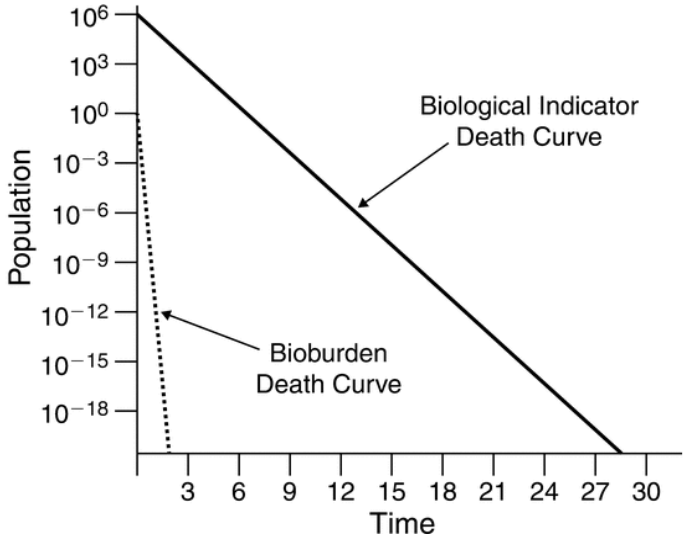
"The objective of the overkill design approach is to assure a level of sterility assurance regardless of the number and heat resistance of the actual bioburden in the load."

[PDA TR No 1 rev. 2007, Clause 4.1.1.1]

Product specific approach: BB/BI

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.

[UPS 43, Chapter 1229]



[Relative resistance and population of typical biological indicator and bioburden microorganisms. USP 43, Chapter 1229- Figure 4]

Product specific approach: BB/BI

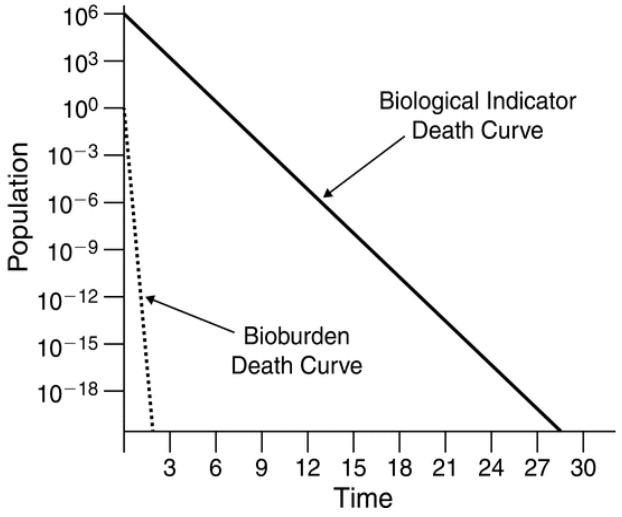
Microbiological target: PNSU of ≤10^{-6} ;

Product: heat-sensitive 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: The conventional BIs are Clostridium sporogenes ATCC 7955 and Bacillus subtilis ATCC 5230, although other strains can be used.

[USP 43, Chapter 1229.2]



[Relative resistance and population of typical biological indicator and bioburden microorganisms. USP 43, Chapter 1229- Figure 4]

Product specific approach: Bioburden

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

[USP 43, Chapter 1229.2]

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

Product specific approach: Bioburden

Microbiological target: PNSU of $\leq 10^{-6}$ for the bioburden;

Product: heat-sensitive 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.

Product design approach: Example (I)

BIOBURDEN TESTING OF PRODUCT

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

$D_{121°C} < 0.25$ minutes

VALUES USED FOR PROCESS DESIGN

$N_0 = 10^2$ MOs

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

$D_{121°C} = 0.4$ minutes

CALCULATED MINIMUM LETHALITY TO ACHIEVE A PNSU OF LESS THAN 10^{-6}

$\mathbf{F_{121°C} = (Log\ N_0 - Log\ N_F) * D_T} = (Log10^2 - Log10^{-6}) * 0.4\ min = \mathbf{3.2\ min}$

[Product- specific design approach , PDA TR No 1]

Since the design value for resistance (0.4 minutes) is only slightly higher than the heat resistance of the Mos present in the product, it is necessary to conduct continuous monitoring of the bacterial load population to ensure that no drift occurs in the product. size of the population or resistance over time.

Product design approach: Example (II)

BIOBURDEN TESTING OF PRODUCT

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

$D_{121°C} < 0.25$ minutes

VALUES USED FOR PROCESS DESIGN

$N_0 = 10^2$ MOs

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

$D_{121°C} = 1$ minutes

CALCULATED MINIMUM LETHALITY TO ACHIEVE A PNSU OF LESS THAN 10^{-6}

$\mathbf{F_{121°C} = (Log\ N_0 - Log\ N_F) * D_T} = (Log10^2 - Log10^{-6}) * 1\ min =$ **8 min**

[Product- specific design approach , PDA TR No 1]

Since the design value selected far heat resistance is very conservative (1.0 minute), the need for ongoing product bioburden heat resistance testing is significantly reduced, but should still be monitored periodically.

Steam Sterilization & Validation Methodologies

Terminal sterilization processes require greater consideration of the **effects** of the treatment on **material properties**.

The preferred method for steam sterilization is the **overkill method**.

When the processed materials are susceptible to **damage** by moist heat at the overkill conditions, the **BB/BI** method is better suited because it results in reduced heat input while affording the same degree of process efficacy but with different controls.

European Medicines Agency –

Guideline on the sterilization of the medicinal product, active substance, excipient and primary container.

Cycle	Type of process	Information in dossier*	Bioburden level before steam sterilisation or terminal heat treatment	Bioburden Characterised	Process hold temperature
Ph. Eur. 5.1.1 Reference Cycle	Sterilisation	1, 6	100 CFU/100ml (non-routine)	No	≥ 121 °C for ≥15 minutes
Overkill cycle F_o >12 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (non-routine)	No	≥ 121 °C
F_o > 8 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (routine)	No	> 115 °C
F_o > 8 min	Sterilisation	1, 2, 3, 5, 7, 8	100 CFU/100ml (routine)	Yes**	> 115 °C
F_o > 8 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (routine)	Yes	> 110 °C
F_o > 8 min	Sterilisation	1, 2, 3, 5, 7, 8	100 CFU/100ml (routine)	Yes**	> 110 °C
F_o <8 min	Post-aseptic processing terminal heat treatment	1, 2, 3, 4, 7, 8	0 CFU/100ml, aseptic filtration and processing prior to terminal heat treatment (routine)	Yes***	> 110 °C****
F_o <8 min	Post-aseptic processing terminal heat treatment	1 2, 3, 5, 7, 8	0 CFU/100ml, aseptic filtration and processing prior to terminal heat treatment (routine)	Yes***	> 110 °C****

* For clarification of the code numbers, see below
** In-process control demonstrating acceptable heat resistance of bioburden
*** The bioburden prior to the sterilisation step (i.e. filtration) should be characterised for heat resistance
**** Temperatures below 110 °C may be used if justified. The requirement for additional documentation for such cycles is evaluated on a case by case basis
Clarification of the information to be presented in the quality dossier
1: Sterilisation time, temperature profile
2: Sterilisation method (for instance saturated steam cycle, air/steam-overpressure cycle, vacuum phase) description including SAL
3: Validation of F_{0Phys} and F_{0Bio}
4: Biological indicator with a $D_{121} \geq 1.5$ minutes used in the validation
5: Biological indicator with a $D_{121} < 1.5$ minutes used in the validation
6: No validation data requested in the dossier, only a confirmation that validation has been performed.
7: Validation data to be provided in the dossier is presented below
8: Additional validation data to be provided in the dossier is presented below

Table 1: Cycles for steam sterilisation and post-aseptic processing terminal heat treatment and corresponding data required in the quality dossier

Biological indicators for moist heat sterilisation

in Ph. Eur. 5.1.2, 3-2-1

1. **MOs**: *G. stearothermophilus* is the most widely accepted biological indicator micro-organism for moist heat sterilisation processes;

2. **Heat resistance**: Reported $D_{121°C}$-values for its spores are in the range of 1.5 min to about 4.5 min;

- *EMA guideline refers to these concepts for the selection of suitable BIs;*

- *EN ISO Standard 11138-3:2017, "Biological indicators for moist heat sterilization processes", prescribes as test organism "G. stearothermophilus or other strains of microorganisms of demonstrated equivalent performance". To comply with this standard the minimum $D_{121\,°C}$-value shall be 1.5 min and the minimum viable count 1 x 10^5.*

Biological indicators for moist heat sterilisation

in Ph. Eur. 5.1.2, 3-2-1

3. **Population**: It is recognised that a 10^5 or 10^6 population of G. stearothermophilus may not be suitable for sterilisation processes delivering an F0 between 8 and 15, therefore a lower spore number (i.e. 10^3 or 10^4) or a different test micro-organism may be used;

4. **Choice**: The charactertistics of the sterilisartion process [T, t, SAL, F0] are the basis for the choice of the biological indicator [Type, M.O., Population].

- *EMA guideline refers to these concepts for the selection of suitable BIs;*

- *EN ISO Standard 11138-3:2017, "Biological indicators for moist heat sterilization processes", prescribes as test organism "G. stearothermophilus or other strains of microorganisms of demonstrated equivalent performance". To comply with this standard the minimum $D_{121\,°C}$-value shall be 1.5 min and the minimum viable count 1 x 10^5.*

Conclusions

Validation of sterilization processes requires **knowledge of sterilization technology** and use of the **appropriate instrumentation and equipment** to control and verify critical sterilization process parameters.

Validation of sterilization processes links **physical measurements** with **biological indicator** performance to establish method lethality.

Product-oriented validation “Terminal sterilization processes require greater consideration of the effects of the treatment on material properties”.

Validation methods: **overkill, BB/BI and BB**.

Validation lifecycle should be documented.

Thank you!