Biological indicators and biological validation

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Index

Bis Standards

***ANSI/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
- *11138-7* - Guidance for the selection, use and interpretation of results

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

ANSI/AAMI/ ISO 17665 Sterilization of health care products -- Moist heat Requirements for the development, validation and routine control of a sterilization process for medical devices

***EMA, March* 2019** Guidelines on the sterilization of medicinal product, active substance, excipient and primary container

United States Pharmacopeia NF - 2021

European Pharmacopeia 10th edition

What is a Biological Indicator?

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

USP NF-2021 General 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.

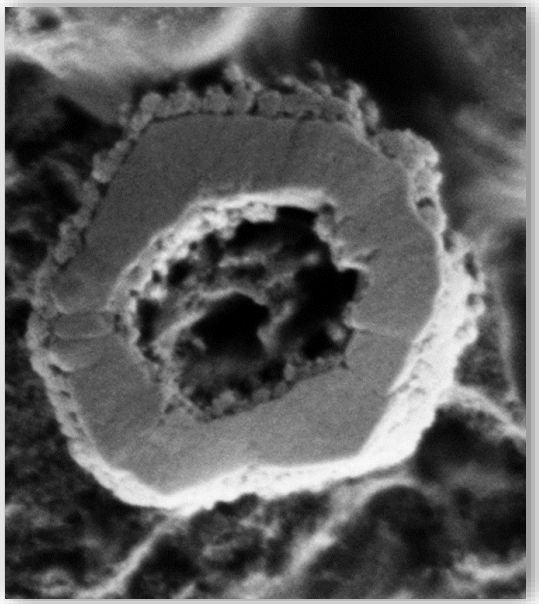
Biological Indicators: purpose

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 recognized as suitable for BIs are spore-forming bacteria, because the spores of these microorganisms are significantly more resistant than the vegetative cells that comprise the majority of bioburden in or on materials.



Microbial forms

Vegetative cells

- Actively growing and reproducing cells

Spores

- Dormant forms
- No measurable physiological activity
- Extremely resistant to environmental stress

What are Biological Indicators?

Typical characteristics for commercial supplied BIs

Strain	Population (spores per carrier)	D - value at 121°C (minutes)
EP 10.0; 5.1.2		
Geobacillus Stearothermophilus **Other strains can be used**		**Reported D - values are in the range of 1,5 min - 4,5 min**
USP NF - 2021; 1229.5		
Geobacillus Stearothermophilus		**-**
(for Steam Sterilization by Direct Contact)		
Clostridium Sporogenes		
Bacillus Subtilis		
Bacillus Atropheus		
(for Moist Heat sterilization of Aqueous Liquids)		
ISO 11138 - 3		
Geobacillus Stearothermophilus	**≥ 1,0 × 10^5**	**≥ 1,5 min; z ≥ 6°C**
Bacillus Subtilis		

The resistance of the microorganism is evaluated to ensure its suitability for the process

Type of BIs

There are at least three types of Bis

Type of BIs

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

Type of BIs

Carriers and primary packaging

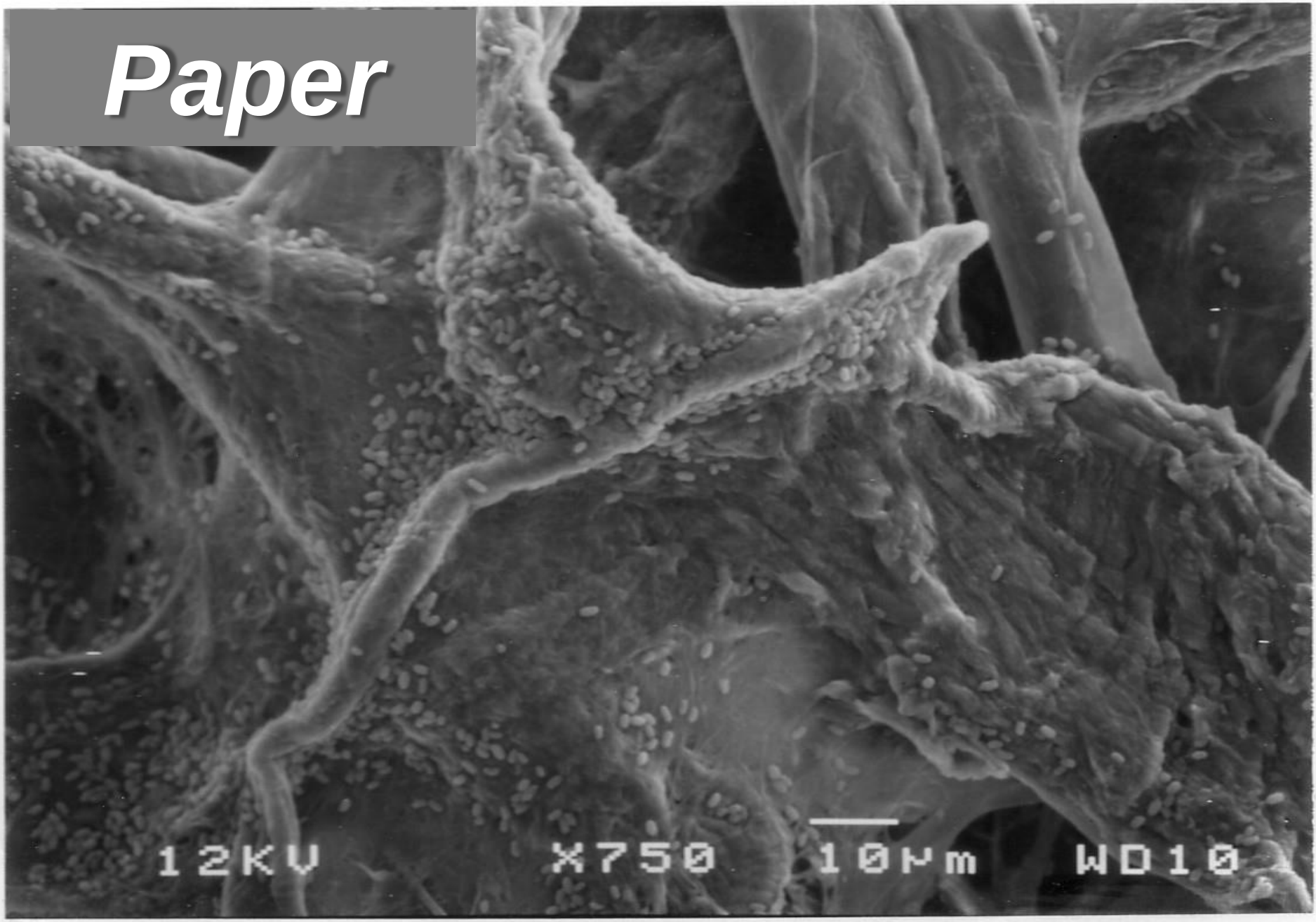
- no chemical/microbial contamination
- not degraded by the sterilization process
- they should minimize the loss of the original inoculum during transport, handling and shelf life storage

Type of BIs

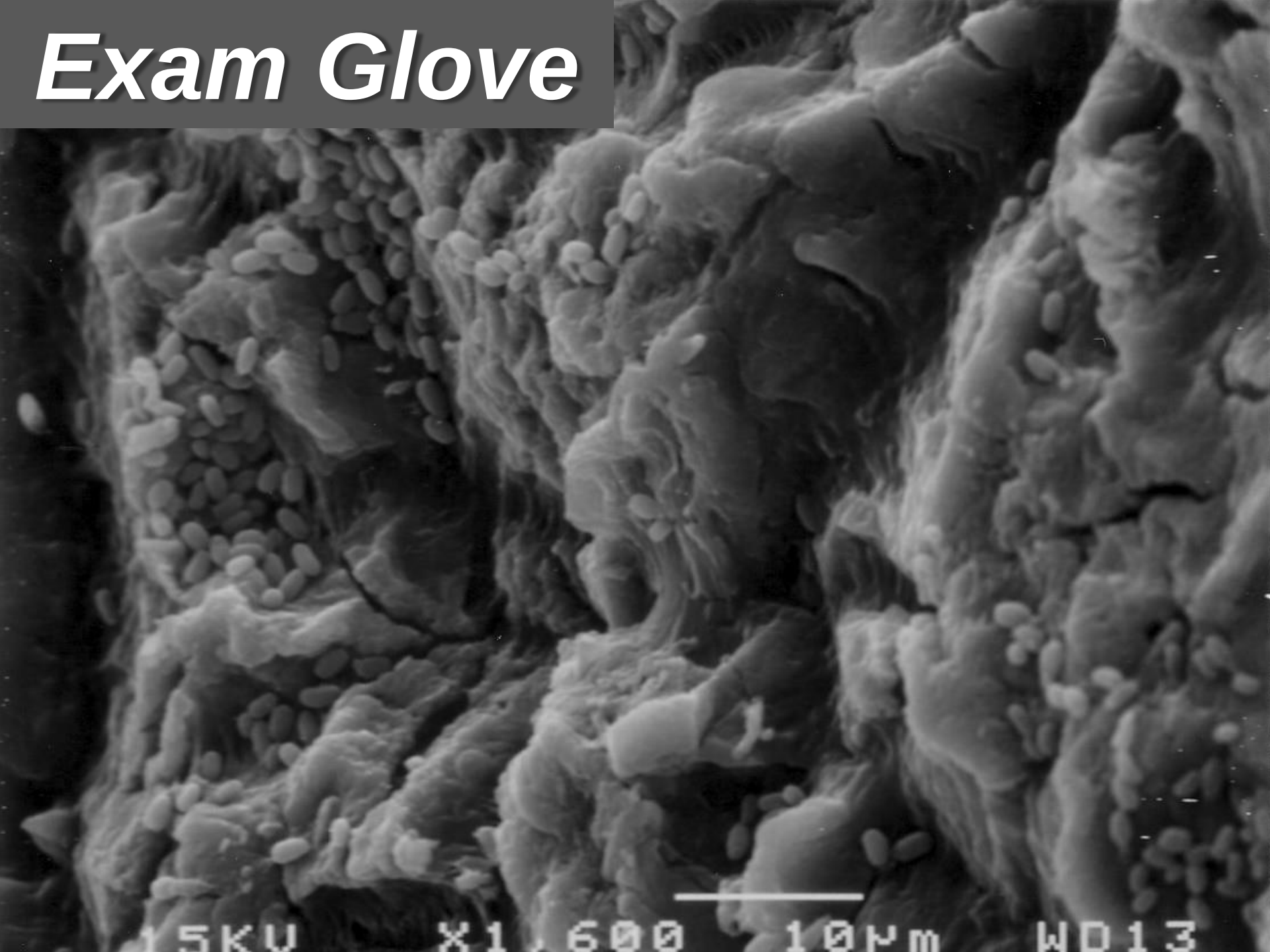
Carriers and primary packaging

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

Paper

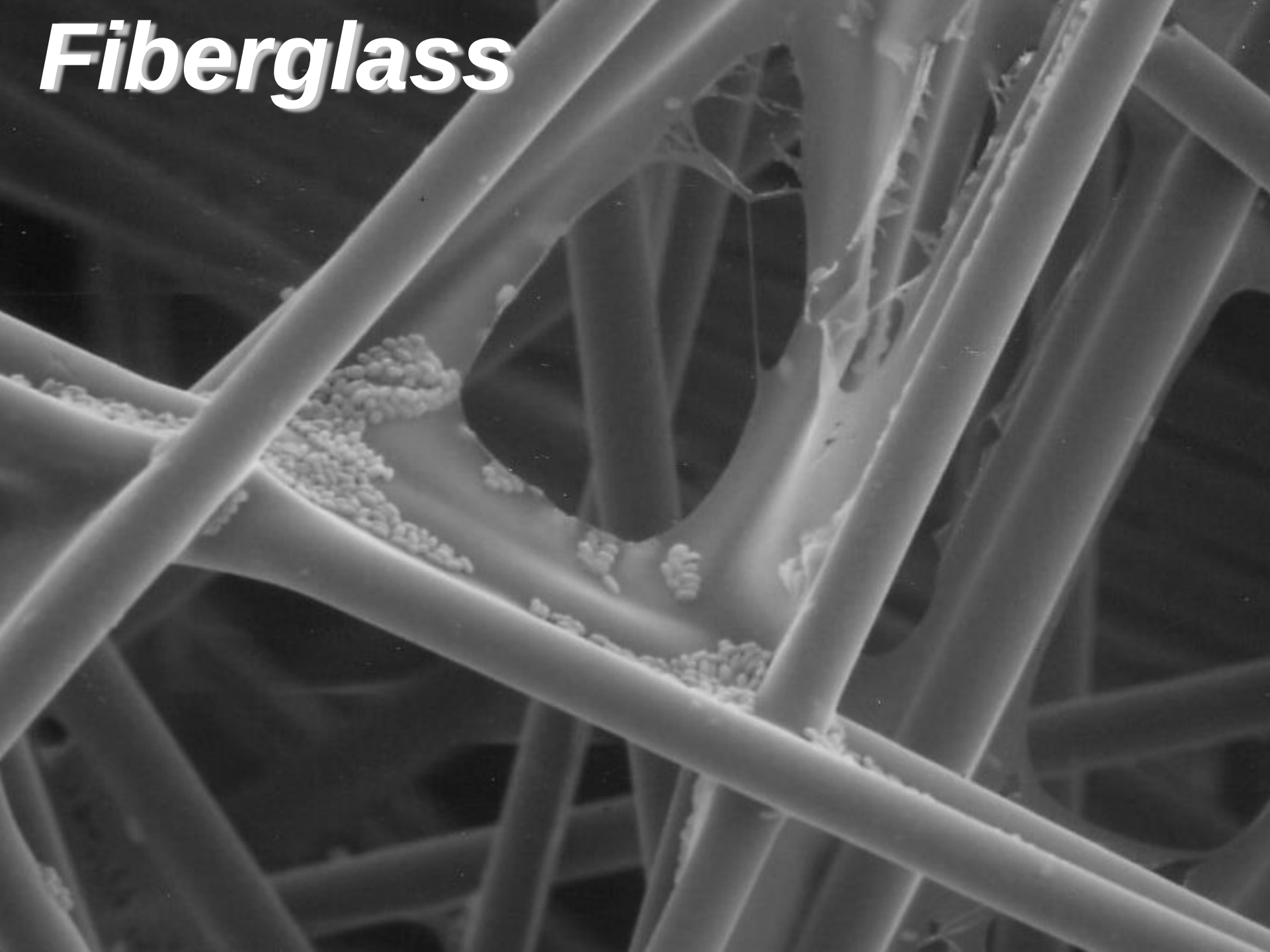


Exam Glove



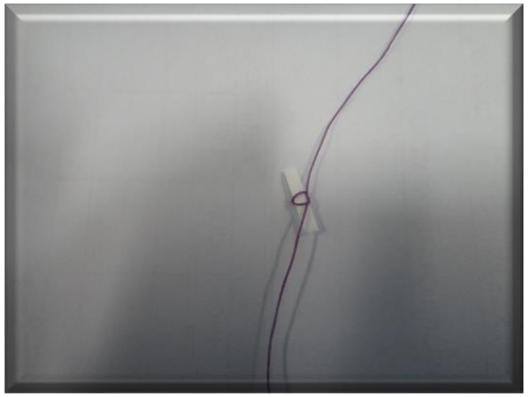
Stainless Steel

Fiberglass

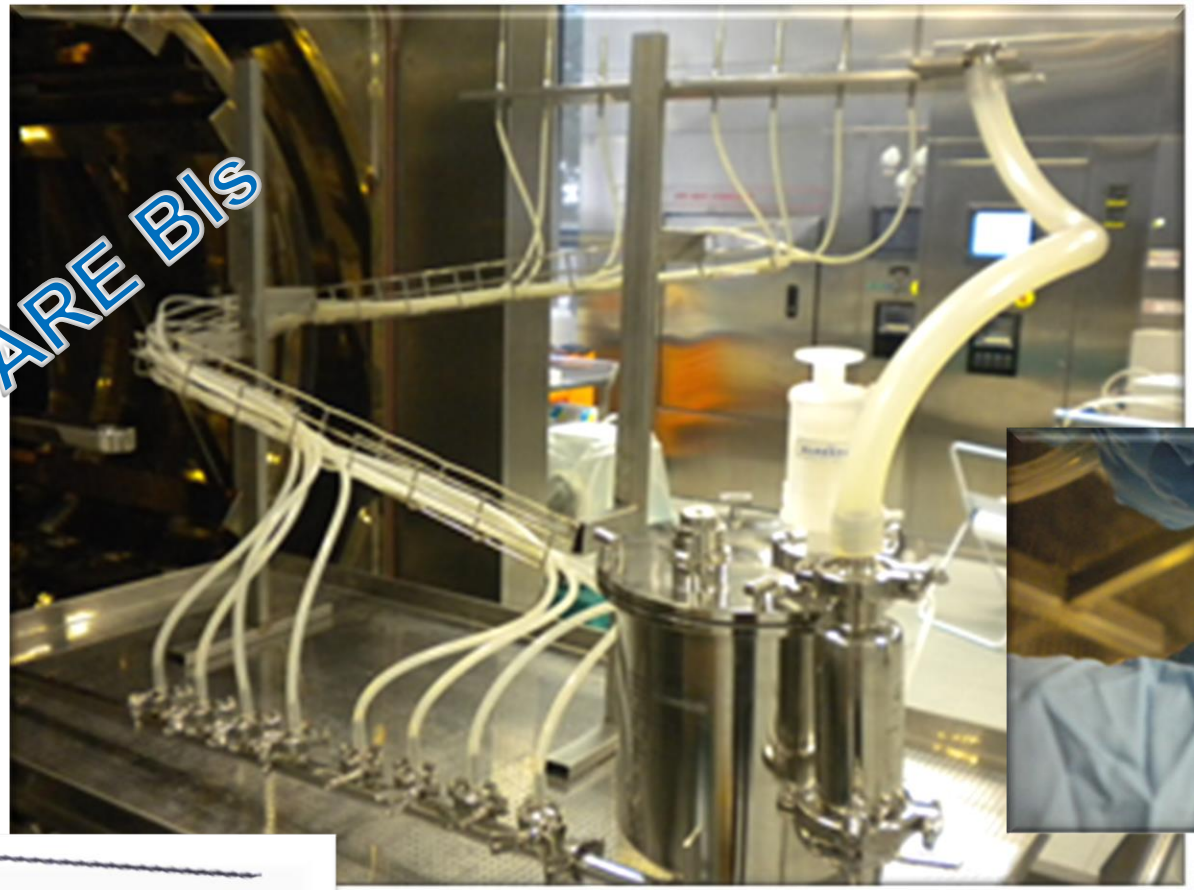


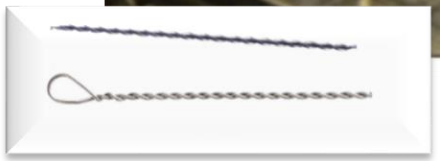
Type of BIs

Bare Bis: no packaging



Type of BIs





Type of BIs

Spore suspensions

They consist of a defined population of bacterial spores, prepared from a clearly characterised and suitably mantained strain of spore-forming bacterial species in a stable suspension. EP 10.0, 5.1.2

They are inoculated on or into representative units of the product to be sterilized.

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

The resistance characteristics of a test organism in suspension can be considerably changed upon deposition on or in carriers. Several factors can influence the resistance characteristics, such as **the surface on to which the suspension is inoculated (e.g. solid materials, viscous products or fluids), the way the spores are dispersed, etc**.

Spore suspensions and D value calculation

ISO 11138-7:2019 *Sterilization of health care products - Biological indicators - Guidance for the selection, use and interpretation of results*

5.3.2 The resistance characteristics of a test organism in suspension can be considerably changed upon deposition on or in carriers. Several factors can influence the resistance characteristics, such as the surface on to which the suspension is inoculated (e.g., solid materials, viscous products or fluids), the way the spores are dispersed and otherwise treated, the method of drying, etc.

United States Pharmacopoeia 2021

GENERAL CHAPTER <1229.2> MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS BIOLOGICAL INDICATORS

The biological challenge is either directly inoculated into a liquid-filled container or is introduced via self-contained units provided there is adequate correlation between their resistance and the resistance that would occur in the process fluid. The liquid can be either the product or a surrogate fluid. The resistance of the indicator in the product (and surrogate fluid, where used) must be known.

Spore suspensions and D value calculation

European Pharmacopoeia 10:2021

5.1.2 BIOLOGICAL INDICATORS AND RELATED MICROBIAL PREPARATIONS USED IN THE MANUFACTURE OF STERILE PRODUCTS

2. BIOLOGICAL INDICATORS FOR STERILISATION PROCESSESS

Spores inoculated into a product or onto surfaces are known to react differently to sterilizing conditions as compared to biological indicator units. In these cases, commercially available biological indicator units may not be suitable to test sterilization effectiveness and an inoculated test product/item prepared from a well-characterized spore suspension may be a better model to evaluate the effectiveness of the sterilization cycle.

$D_{substrate} > D_{BI}$ or $D_{BI} > D_{substrate}$

Type of BIs

Type of BIs

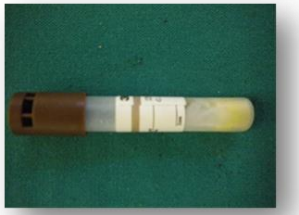
Self-contained indicators

Type of BIs

An ampoule containing growth medium and a carrier inoculated with test organisms contained within an outer vial so that the sterilizing agent obtains access to the inoculated carrier through a sterile barrier or a tortuous path.

After exposure to the sterilization process, the growth medium is brought into contact with the inoculated carrier by breaking the ampoule of growth medium, thereby eliminating the need to aseptically transfer the inoculated carrier to a separate vial of growth medium.

The biological indicator manufacturers' recommendations should be followed for incubation of self-contained biological indicators.



Type of BIs

How to read the results?
Usually, by a visual inspection: they change their colour.

To have a permanent results, some instruments are in the market...

Type 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

Type 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, if any, in the self contained unit)

BI User's responsability

- 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.
- The user should consider the particular **sterilization process as the basis for the choice of biological indicator**
- A certificate of analysis should be obtained for each lot of indicators.
- Users who employ biological indicators **outside the manufacturer's labelled** recommendations should thoroughly characterize the resistance of the biological indicators to the particular sterilization process. ISO 11138-7: 2019 – 4.6

BI User's responsability

When the user has established a high level of confidence in the **supplier** the testing performed by the **user can be minimal**.

At a minimum, the user should have a mechanism to ensure that a shipment of biological indicators contains **all agreed-upon documentation**, such as appropriate label information, packet inserts, storage and handling instructions, etc.

There should be a **mechanism to ensure that the BI supplier continues to maintain the expected quality standards**, such as a BI supplier or BI manufacturer's declaration of conformity to standards. If the user has not established the supplier relationship required to be ensured of consistent biological indicator performance, additional testing could be necessary until an appropriate assurance can be established that the biological indicators meet the BI manufacturer's label claim and/or user requirements.

ISO 11138-7: 2019 – 6.1.4

BI User's responsability

Testing by the user, if deemed necessary, can consist of **population assays and defined resistance tests such as D value or survival-kill time** on samples from each new batch of biological indicators received (see also 8.6 and Clause 11). Testing should be conducted **under exact conditions specified by the manufacturer**. Provided that the biological indicator manufacturer produces the based upon detailed standard specifications, i.e. the ISO 11138 series, and the user uses the biological indicator as intended by the biological indicator manufacturer, testing of the resistance characteristics by the user is considered unnecessary.

ISO 11138-7: 2019 – 6.1.5

BI User's responsability

Prior to use of a **new batch/lot of BIs, the population and identity of the indicator organism of the batch/lot should be verified**. For other critical parameters, e.g. D-value, Z- value, the batch certificate provided by the qualified supplier can normally be used.

Annex 1, draft, 8.42

BI User's responsability

Quality control for biological indicators consists of testing for **purity, identity and estimation of the number of viable cells**.

European pharmacopoeia 10.0; 5.1.2

BI User’s responsability

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** (see Biological Indicators—Resistance Performance Tests ⟨55⟩). When a BI is used in accordance with the BI manufacturer's directions, the resistance of the BI need not be reconfirmed.

USP NF 2021 ⟨1229.5⟩ BIOLOGICAL INDICATORS FOR STERILIZATION

BI User's responsability

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

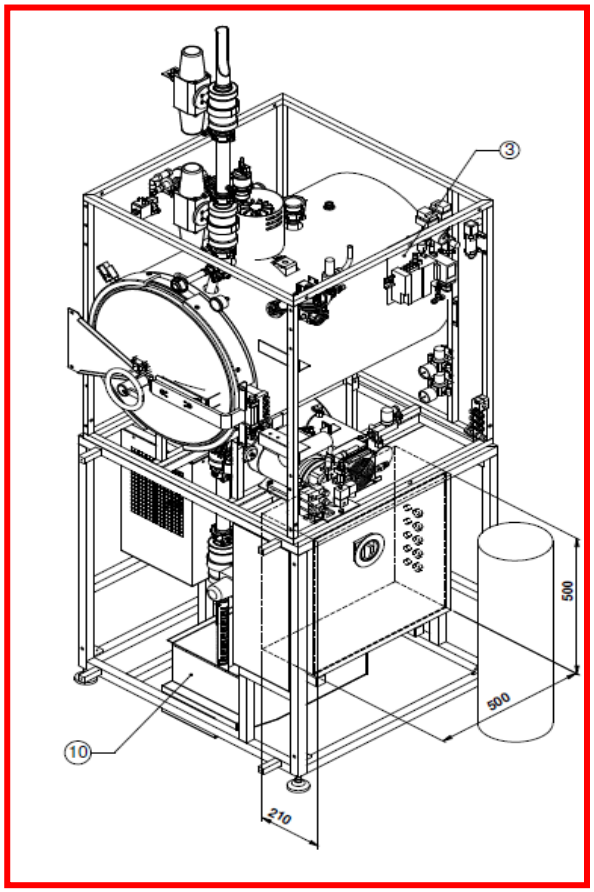
Carrier Size: 2 x 10mm

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 vessel:

Biological

Indicator

Evaluator

Resistometer

D-value determination

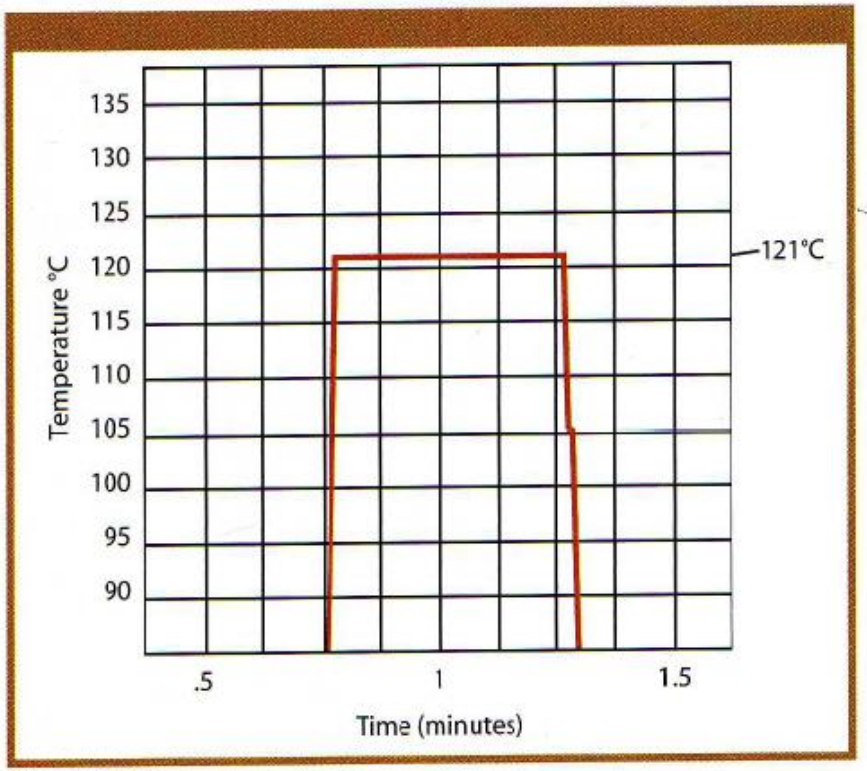


Figure 1: The BIER vessel's square-wave system. Samples placed in the BIER vessel are taken from ambient conditions, brought to the sterilizing condition, and returned to the ambient conditions.

According to **ISO 11138-1**

What are you sterilizing?

Is it a solid load or a liquid one?

What are you sterilizing? A culture media

Culture medium

What are you sterilizing? A culture media

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 NF – 2021 General chapter ⟨1117⟩ MICROBIOLOGICAL BEST LABORATORY PRACTICES

Choice of the BI for a culture medium

Customer's choice:

Choice of the BI for a culture medium

AFTER INCUBATION

PANTONE®
13-0755
Primrose Yellow

SUPPOSED

PANTONE
UNIVERSE
266 C

OBTAINED

PANTONE®
16-1144
Oak Buff

At the end of the sterilization cycle… Why Bis 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

Choice of the BI for a culture medium

What could have been another choice?

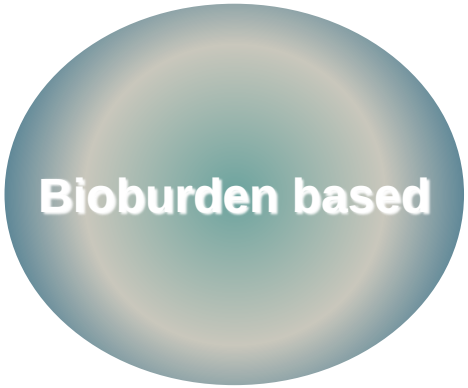
- Inoculating a spore suspension in the culture medium and incubating it after the sterilization cycle

- Because laboratory media are considered self-indicating with respect to sterility, the use of internal biological indicators during validation is not required (*USP NF-2021 - General Chapter 1229.2*)

Choice of the BI for blood bags

A case study and an open discussion:
Blood bags

Validation methodologies

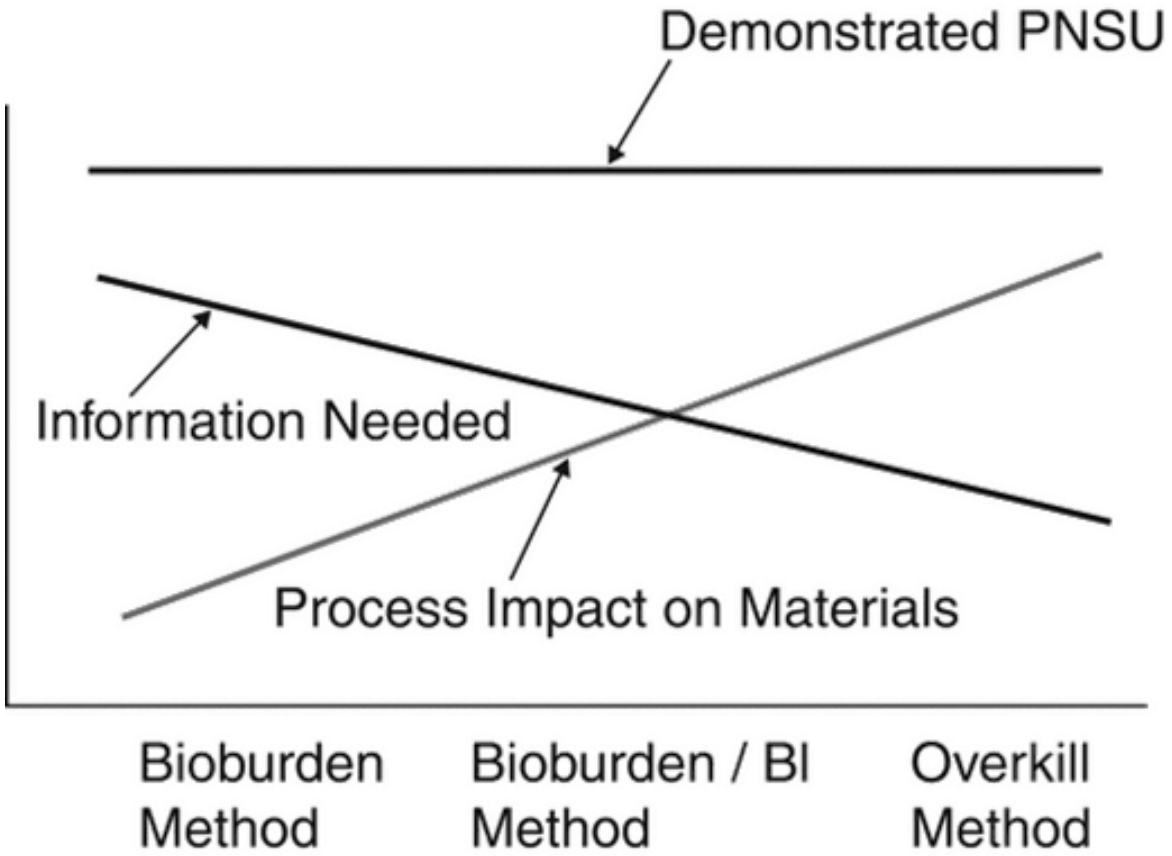


Validation methodologies: which is the best one? A microbiological point of view

Validation methodologies

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

Validation methodologies



USP NF - 2021, General Chapter ⟨1229⟩ STERILIZATION OF COMPENDIAL ARTICLES

Overkill sterilization

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

Overkill sterilization

The overkill method can be confidently used without detailed consideration of the presterilization bioburden.

USP NF - 2021, ⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS

Where the overkill method is used, bioburden controls can be less rigorous.

USP NF - 2021, ⟨1229⟩ STERILIZATION OF COMPENDIAL ARTICLES

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

EUDRALEX, VOLUME 4, ANNEX 1: draft

Overkill sterilization

Overkill sterilization can be defined as a method in which the destruction of a high concentration of a resistant microorganism supports the destruction of reasonably anticipated bioburden present in routine processing.

USP NF - 2021, ⟨1229⟩ STERILIZATION OF COMPENDIAL ARTICLES

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:

$N_0 = 10^6$

$D_{121} = 1'$

$z = 10^{\circ}C$

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

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

Bioburden/Biological indicator sterilization

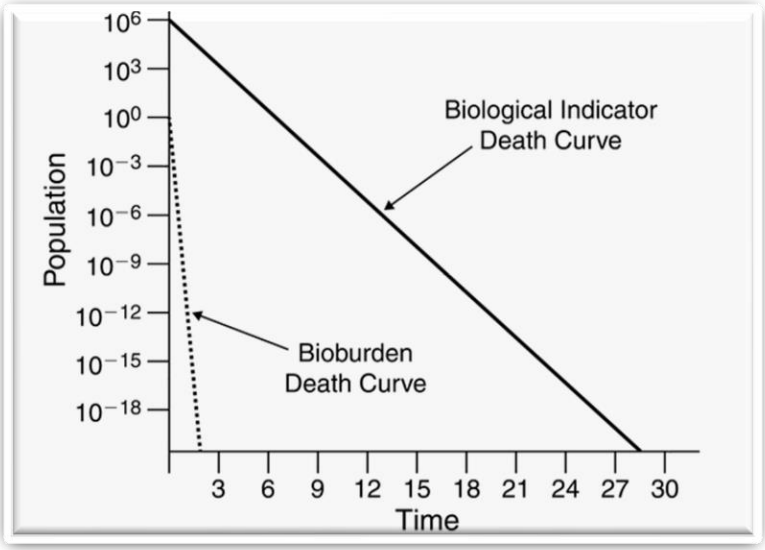
"Bioburden/biological indicator based sterilization is an approach in which the incomplete destruction (or destruction of a modest population) of a resistant biological indicator can be used to demonstrate the capability of the method to reliably destroy the bioburden present.

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

USP NF-2021, ⟨1229⟩ STERILIZATION OF COMPENDIAL ARTICLES

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 NF - 2021*, ⟨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.

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 NF - 2021, ⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS

Bioburden 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 NF-2021, ⟨1229⟩ STERILIZATION OF COMPENDIAL ARTICLES

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 NF-2021, ⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS

European Medical Agency: validation approach and BIs use

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

6 March 2019
EMA/CHMP/CVMP/QWP/850374/2015
Committee for Medicinal Products for Human use (CHMP)
Committee for Medicinal Products for Veterinary use (CVMP)

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

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

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

Sterilization cycles other than Ph. Eur 5.1.1 “Reference Cycle” (T ≥ 121°C, t ≥ 15 min)

❖ ***Requirements for sterilization process “quality dossier” in EMA “Guidance on Sterilisation”, Par. 4.1.1 & Table 1***

- Bioburden not higher than 100 CFU /100 ml
- Load mapping of the sterilizer chamber
- Load mapping distribution of items in it (“Standard loads”)
- Cycle description: Temperature, time, method
- Demonstration of actual compliance of physical parameters
- Determination and biological justification of SAL
- Validation of F_{0phy} and F_{0bio} for repeatable compliance with minimum values and repeatable attainment of a SAL ≤ 10^{-6}
- Acceptable temperature differences in the load
- Acceptable F_0 variability in the load
- Relationship between physical and biological validation

Sterilization cycles other than Ph. Eur 5.1.1 “Reference Cycle” (T ≥ 121°C, t ≥ 15 min)

Requirements for Sterilization Process “quality dossier” in EMA “Guidance on Sterilisation”, par. 4.1.1 & Table 1

Validation by inactivation of biological indicators:

- D_{121} ≥ 1.5 minutes if:
  F_0 > 12 (“overkill cycle”) at a temperature ≥ 121° C
  F_0 > 8:
  T > 115 °C, bioburden not characterized
  T > 110 °C, bioburden characterized for heat resistance
- D_{121} < 1.5 minutes if:
  - F_0 > 8:
    - T > 110 °C, bioburden characterized for heat resistance with “in-process” control and additional validation data in the quality dossier for justification of starting T for F_0 calculation and suitability of BIs at the actual temperature.

EMA “Guideline on Sterilisation”, Par. 4.1.1 & Table 1

Acceptable bioburden limits (“without further justification”)

“Before steam sterilisation” (defined by $F_0 \geq 8$ & $T \geq 110$ °C):

100 CFU / 100ml *(to be non-routine or routine monitored, and characterized or not for heat resistance depending on actual sterilization process parameters)*

“After aseptic filtration and processing prior to terminal heat treatment” (defined by $F_0 < 8$; $T < 110$ °C may be used if justified):

0 CFU / 100ml *(to be routine monitored and characterized for heat resistance)*

The bioburden limit should be in line with any pre-sterilization bioburden reduction process capability (e.g. filtration). For aqueous solutions, the limits stated in table 1 are acceptable for active substances and drug product formulations without further justification. Other testing regimes and limits to control bioburden at the defined level should be justified.

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 NF - 2021 – ⟨1222⟩ TERMINALLY STERILIZED PHARMACEUTICAL PRODUCTS—PARAMETRIC RELEASE

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

mbr@fedegari.com