BIOLOGICAL VALIDATION

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BI 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 43

European Pharmacopeia 10th edition

FEDEGARI GROUP

Performance qualification: biological approach

USP 43

PDA, TR 1 rev. 2007

HTM 01-01 Part C

Eu. Ph. X ed.

EU GMP, Annex 1

EMA guidance on sterilization

ISO 17665 - 1

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Health Technical Memorandum 01-01: Management and decontamination of surgical instruments (medical devices) used in acute care Part C: Steam sterilization

Microbiological test for PQ

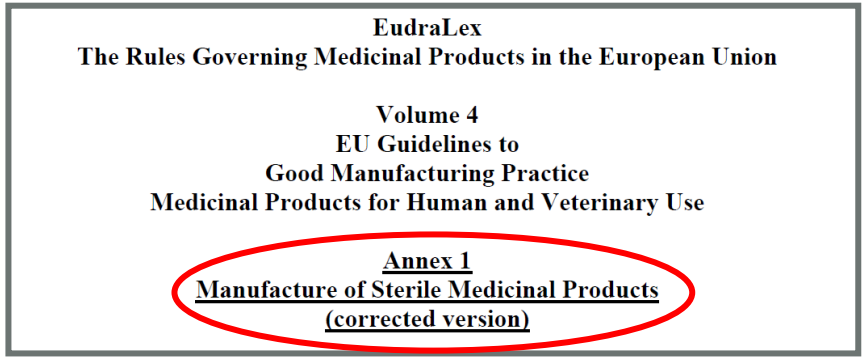
2.51 This test is designed to be used in exceptional circumstances as an additional PQ test for steam sterilizers. The microbiological test should ideally follow a satisfactory thermometric test, using the identical loading condition and operating cycle. There may be situations where thermometric tests 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).

Eudralex: Volume 4, EU Guidelines to Good Manufacturing Practice, 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"


EudraLex
The Rules Governing Medicinal Products in the European Union

Volume 4
EU Guidelines to
Good Manufacturing Practice
Medicinal Products for Human and Veterinary Use

Annex 1
Manufacture of Sterile Medicinal Products
(corrected version)

New draft

Annex 1 : Manufacture of Sterile Products

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European Pharmacopoeia X ed. 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.

United States Pharmacopoeia 43

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

BIs may also be used to monitor established sterilization cycles and are used for periodic reassessment of sterilization process effectiveness.”

⟨1229.5⟩ BIOLOGICAL INDICATORS FOR STERILIZATION

Parenteral Drug Association

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

PDA, TR # 1, revised 2007

ISO 17665-1: 2006

Sterilization of health care product – Moist heat

9.4 Performance qualification

If, in addition to the measurement of physical parameters, the sterilization process is to be based on bioburden, or verified by microbiological methods, biological indicators shall be positioned in and/or on the product in locations identified in 9.4.4, and then exposed to one of the following:

- *a treatment that is reduced relative to that in the sterilization process*; the outcome of this treatment is extrapolated to demonstrate that, on application of the sterilization process, the specified requirements for minimum microbicidal effectiveness are met;

- *the full extent of the treatment at the lower tolerances of the sterilization process parameters,* the outcome of this treatment is used to confirm a prediction that, on the application of the sterilization process, the specified requirements for minimum microbicidal effectiveness are met;

- *or an "overkill" process*.

ISO 17665-1: 2006

Sterilization of health care product – Moist heat

9.4 Routine monitoring and control

Delivery of the sterilization process shall be verified from the results of chemical indicators or biological indicator system, if used, and by conforming that within specified tolerances.

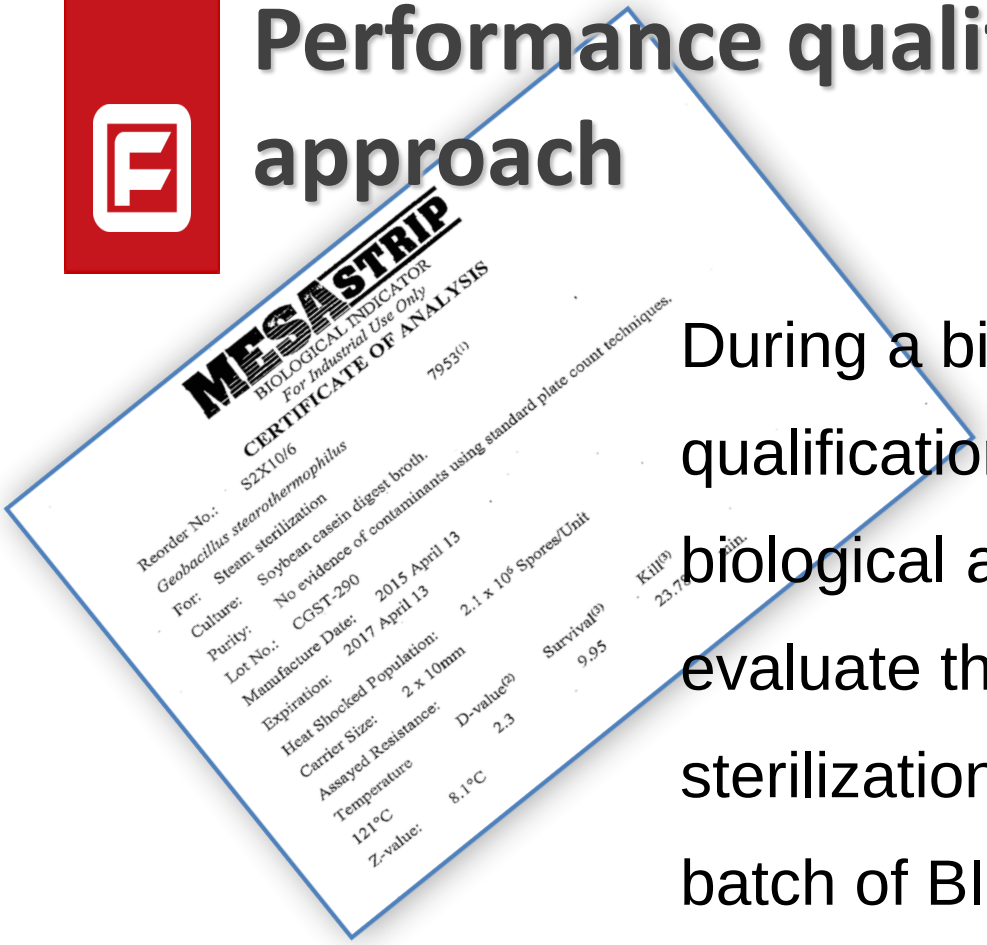
Performance qualification, biological approach

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


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: D-value(2) 2.3 Survival(3) 9.95 Kill(3) 23.7 min.
Temperature 121°C
Z-value: 8.1°C

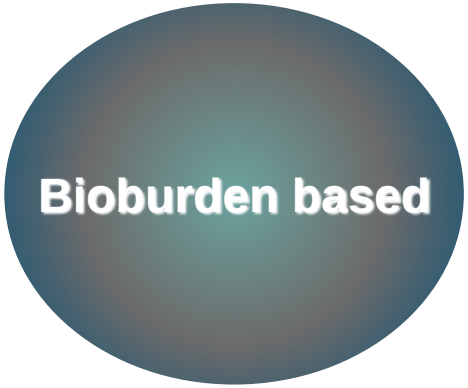
During a biological performance qualification, after chosen the biological approach and the BI to use, evaluate the effectiveness of the sterilization cycle using the same batch of BI, if possible.

Performance qualification, biological approach

Monitor the process leaving biological indicators in the same position considered for thermal qualification: worst case locations 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.

Validation methodologies

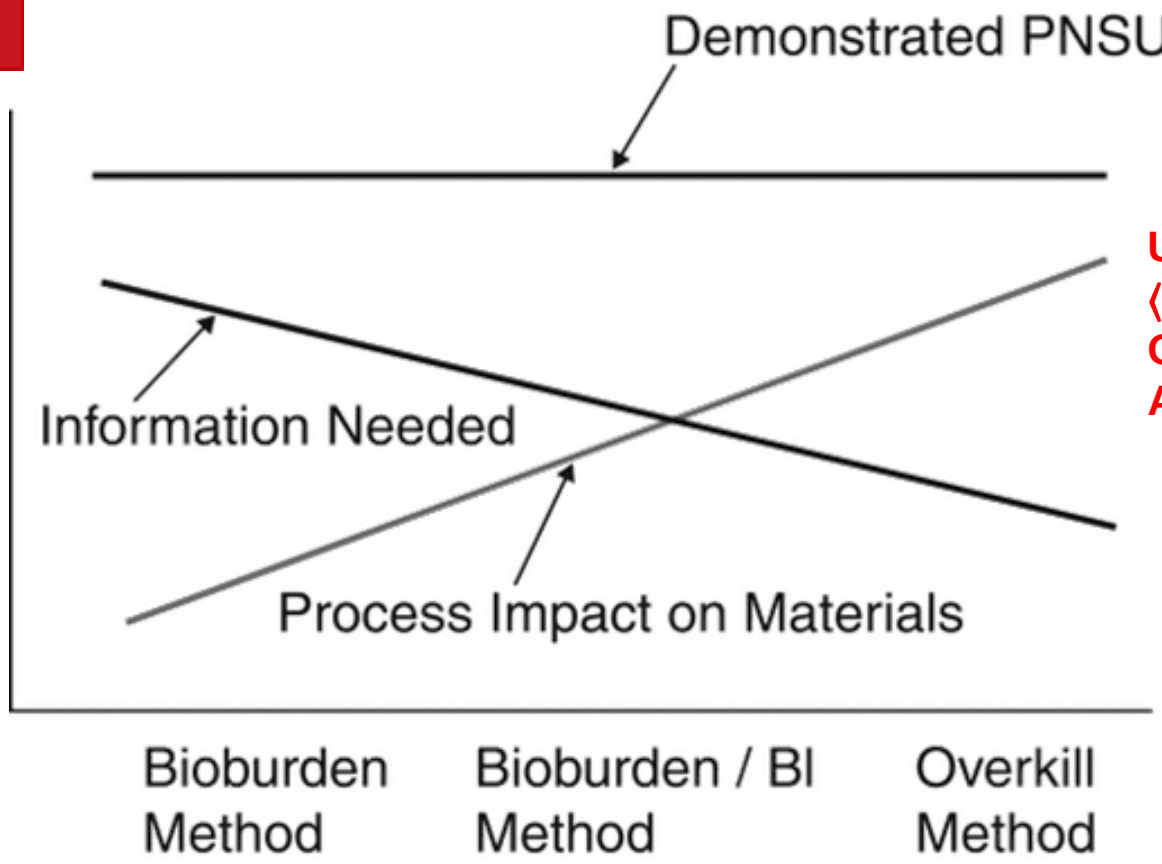


Validation methodologies: which is the best one? A microbiological point of view

Validation methodologies

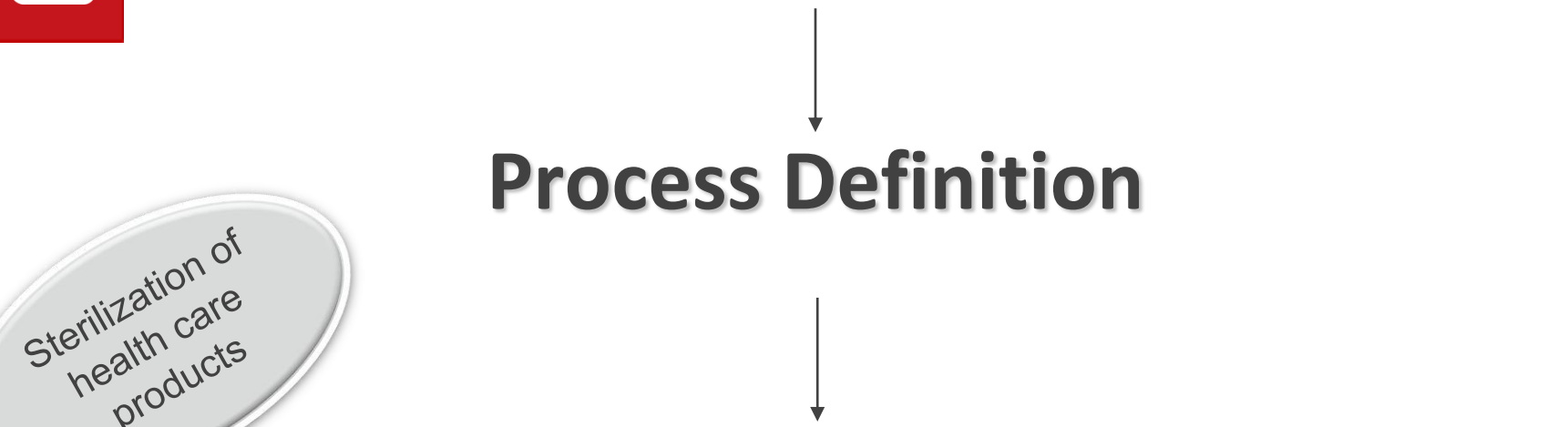
The different approaches were developed because of the differences in the heat resistance of the materials to be sterilized.

Validation methodologies



USP 43, General Chapter ⟨1229⟩ STERILIZATION OF COMPENDIAL ARTICLES

ISO 17665 -1 : validation methodologies and biological approach



Validation methodologies: ISO 17665 -1

8 PROCESS DEFINITION
8.11 The SAL attained on and/or within the product during the sterilization process shall:

a) be established by knowledge of the bioburden (see Annexes B or C)

Annex B speaks about a sterilization method which requires extensive knowledge of the naturally occuring bioburden (on going monitoring and control over the bioburden) and of the thermal resistance of the components.

Annex C requires to locate Bis in the worst case positions of the load, running cycles under conditions selected to deliver less lethality than is delivered during routine sterilization in order to have reference microorganisms still not inactivated.

The number of microorganism surviving should be determined by direct enumeration or estimated by most probable number technique.

Validation methodologies: ISO 17665 -1

Annex B

Process definition based on inactivation of the microbial population in its natural state (bioburden-based method).

– Extensive knowledge of the naturally occurring bioburden is required

– Bioburden of representative production determined ⟶ ISO 11737-1

– Product exposed to the sterilizing agent in predetermined increments of the anticipated sterilization process; sterility test on product is required (see ISO 11737-2)

– Relationship between the proportion of product exhibiting no growth in tests of sterility and the extent of exposure to the sterilizing agent

– Three runs

– Periodical monitoring of representative products (requalification and bioburden control)

Validation methodologies: ISO 17665 -1

Annex C

*Process definition based on the inactivation of a reference microorganism and a knowledge of bioburden on product items to be sterilized (combined **bioburden/biological indicator based method**)*

– Location within the product at which sterility is most difficult to achieve should be established

– Challenge to the sterilization process with a known number of microorganisms with known resistance to the sterilizing agent should be created

– Challenge packaged the same as routinely produced product and included within the sterilization load in the location where it is most difficult to achieve sterilizing conditions

– Exposure under conditions selected to deliver less lethality than is delivered during routine sterilization, so not all reference microorganisms will be inactivated.

–Three runs

–The number of microorganisms surviving either determined by direct enumeration or estimated by the most probable number technique.

–The rate of inactivation of the reference microorganisms calculated

– From a knowledge of the bioburden and the rate of inactivation of the reference microorganisms, process definition

Validation methodologies: ISO 17665 -1

b) be determined by an "overkill" method (see Annex D)

This annex describes the process of overkill based on the inactivation of reference microorganisms.
This process is widely employed and is often used to sterilize re-usable items.
It describes two conservative methods which use BIs located in the worst case position of the load:

• partial cycle approach: from a reduced level of treatment delivered to a defined microorganism

• full cycle approach: mathematically based on an empirical microorganism

Key points:
• Establish location within the product at which sterility is most difficult to achieve
• Create a challenge to the sterilization process (B.I. / inoculation); the biological indicator provides a greater challenge than the bioburden
• Challenge packaged as routine products

Validation methodologies: ISO 17665 -1

b) be determined by an "overkill" method (see Annex D)

Partial cycle approach

The sterilization load should be exposed to the sterilizing agent under conditions designed to deliver a reduced level of treatment.

The extent of treatment needed to inactivate 10^6 microorganisms on a biological indicator that complies with ISO 11138 - 3 should be confirmed.

At half cycle, the user can typically demonstrate, at least, 6 log reductions (ISO 11138-7: 2019).

The level of treatment identified should be carried out in triplicate to demonstrate reproducibility

If the inactivation of 10^6 viable microorganisms is confirmed, determine the extent of treatment for the sterilization process by extrapolation to a predicted probability of survival of 10^{-6} or better, taking into account the nature of the inactivation kinetics of the sterilizing agent and the number and resistance of the microorganisms on the biological indicator.
The extent of treatment can be defined conservatively as twice that used by the reduced level of treatment.

Validation methodologies: ISO 17665 -1 and USP 43, (1229.11)

HALF CYCLE

The half-cycle validation method requires the destruction of a suitable concentration of a resistant microorganism under defined, minimum conditions for a complete kill. Then, in routine operation, the minimum lethal time period is arbitrarily doubled, which supports a doubling of the spore log reduction of the BI, and is more than sufficient to inactivate the bioburden.

Validation methodologies: ISO 17665 -1

b) be determined by an "overkill" method (see Annex D)

Full cycle approach

The sterilization load should be exposed to the sterilizing agent under conditions designed to deliver a level of treatment that will inactivate a biological indicator complying with ISO 11138-3.

The thermal dose must not be lower than 12' and with this value we can calculate the minimum population of a BI with a known D value.

Fbio is determined from the equation:

$$Fbio = D_{121}(\log No - \log N)$$

Validation methodologies: ISO 17665 -1

b) be determined by an "overkill" method (see Annex D)

Full cycle approach

$Fbio = D_{121}(\log N_o - \log N_F)$

D_{121} is the D value of the biological indicator at an exposure temperature of 121°C;
N_o is the pre-exposure viable population of the biological indicator
N_F is the post-exposure viable population of the biological indicator

If $N_F = 10^0$
If D_{121}= 2 minutes

$\log N_o = Fbio / D_{121} + \log 1 = 12/ 2 = 6$
$N_o = 10^6$

PDA TR#01:2007, par. 5.2.1.1

N_F = the population of the biological challenge after exposure. For calculation purposes, if the biological challenge is killed, then it can be assumed that there is less than one surviving microorganism, which is depicted as $N_F = 10^0$ in this equation.

Validation methodologies: ISO 17665 -1

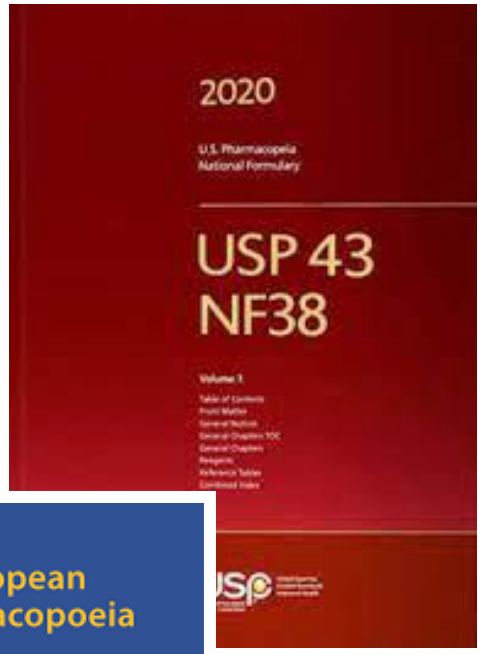
b) be determined by an "overkill" method (see Annex D)

Full cycle approach

The nominal population on the biological indicator should exceed by at least 0,5 × log to the base 10 of the population, calculated from Fbio = 12 and the certified D_{121} value for the biological indicator. This takes into account variations in microbiological manipulations and changes in D value for the test microorganism, which can be caused by contact with the product or a contaminating material.

We are adding a safety margin because of different manipulations of the Bis and/or changes of the D value (e.g. because the inoculum on the product).

Validation methodologies: ISO 17665 -1



PROCESS DEFINITION
8.11 The SAL attained on and/or within the product during the sterilization process shall…

c) be defined by demonstrating that during the holding time all parts of the product are exposed to process parameters selected from an official national or regional pharmacopoeia

d) be deemed to be equal to or to exceed the requirements specified in c), provided that the product is assigned to a product family for which a sterilization process is specified and that the equilibration time does not exceed the maximum for products assigned to the same product family.

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Overkill sterilization: other references

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

USP 43, ⟨1222⟩ TERMINALLY STERILIZED PHARMACEUTICAL PRODUCTS—PARAMETRIC RELEASE

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

Annex 1, draft, 10 Quality Control, 10.3

USP 43: 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.»

USP 43, ⟨1229.1⟩ STEAM STERILIZATION BY DIRECT CONTACT

«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.»

USP 43, ⟨1222⟩ TERMINALLY STERILIZED PHARMACEUTICAL PRODUCTS—PARAMETRIC RELEASE

USP 43: Bioburden/Biological indicator sterilization

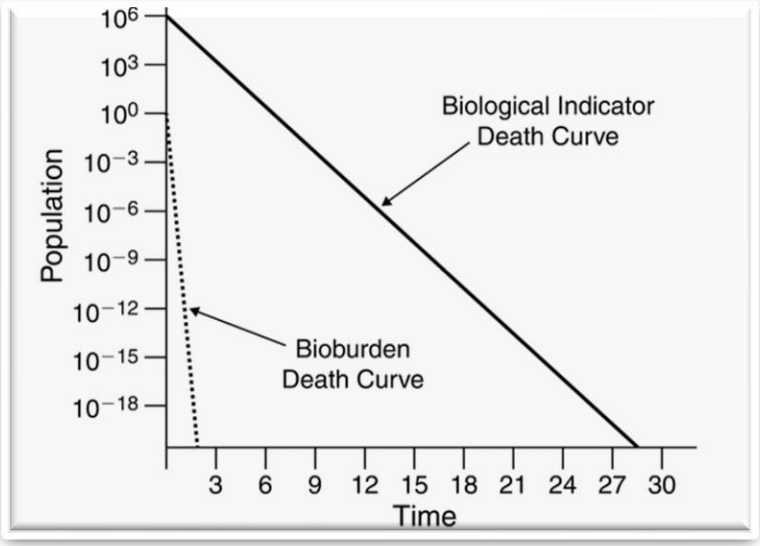
"Bioburden/biological indicator based sterilization is an approach in which the incomplete destruction (or destruction of a modest population) of a resistant biological indicator can be used to demonstrate the capability of the method to reliably destroy the bioburden present.

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

USP 43, ⟨1229⟩ STERILIZATION OF COMPENDIAL ARTICLES

USP 43: 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.

USP 43: 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 43, **⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS**

USP 43: 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 43: 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 43, ⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS

USP 43: 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 43, ⟨1229⟩ STERILIZATION OF COMPENDIAL ARTICLES

USP 43: 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 43, ⟨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; validation data to be provided in the quality dossier.***

- 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:

$D_{121} \geq 1.5$ 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.

Thank you

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