Introduction to current sterilization methods

Hydrogen peroxide decontamination

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Agenda

Decontamination

Hydrogen peroxide chemical properties & Sporicidal Mechanism

Technologies & Process

Fedegari Isolation technology

Development & Validation of a decontamination process

Normative references & practical case studies

Decontamination

…"decontaminated" refers to an **item** or **surface** that has been subjected to a **process that eliminates viable bioburden**.

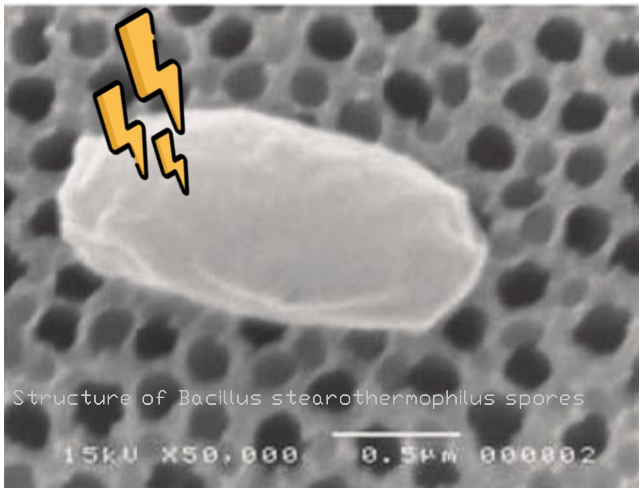
USP43 ⟨1208⟩ Sterility Testing – Validation of Isolator System

….. The process is applied to *internal surfaces of the isolator* and **external surfaces of materials** *inside the isolator*, when conventional sterilization methods are not required.

The application of a sporicidal process to isolators is not considered to be a sterilization process in the same way as, for example, a sealed container subjected to a validated dry heat, moist heat or irradiation process.

PIC/S - Recommendation used for aseptic processing and sterility testing, Glossary

Mechanism of lethality



Penetration of the outer protective layers of the bacterial endospores

↓

Oxidation of microbial components

or

Alkylation of macromolecules within the microorganisms

Mechanism of lethality

- **Oxidation of microbial components**

i.e. *Hydrogen peroxide and ozone*

The microbiocidal action is attributable to free radical reaction mechanisms. The presence of free radicals results in the oxidation of proteins, DNA and other components within the spore leading to its inactivation.

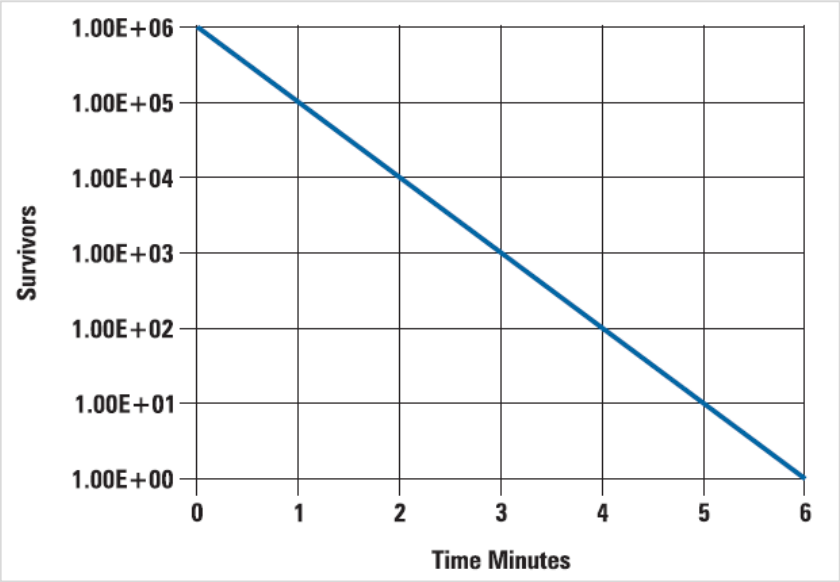
- **Alkylation of macromolecules within the microorganism**

i.e. *Formaldehyde*

These compounds will alkylate a number of functional groups such as amino, hydroxyl, and sulfhydryl groups of proteins and nucleosides of DNA and RNA, adding alkyl groups.

PDA TR. No. 51

Kinetics of Sporicidal Agent



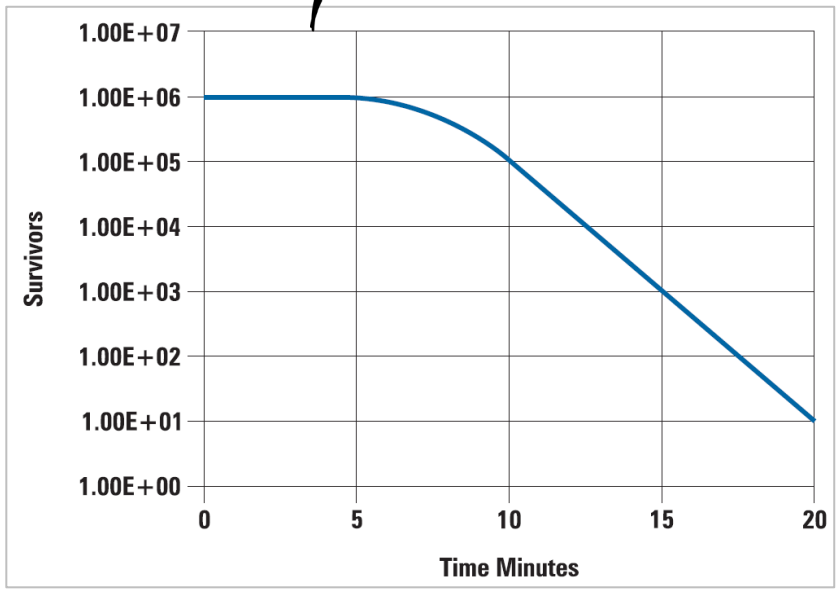
Idealized Plot of Death Kinetics_PDA TR N. 51

Ideal lethality kinetic under steady-state conditions

- Fist order kinetic
- Exponential relationship
- Asymptotic trend

Kinetics of Sporicidal Agent

The rate of lethality progressively increases due to the increase of the concentration



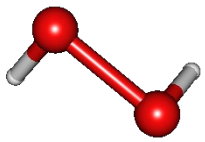
Typical Survival Curve - PDA TR N. 51

It is not possible to generate a constant lethality rate during the biodecontamiantion of an isolator.

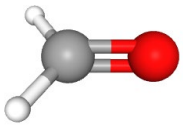
If the process relies on condensation, the concentration and mass of the liquid layer will vary during the cycle.

Common sporicidal decontamination agents

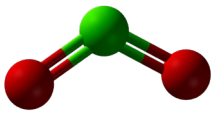
- Hydrogen peroxide vapor
- Peracetic acid vapor
- Chlorine dioxide gas
- Formaldehyde vapor
- Hydrogen peroxide and peracetic acid vapor in combination



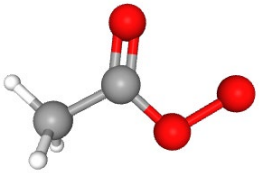
Hydrogen Peroxide
H_2O_2



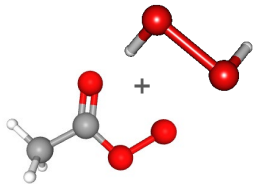
Formaldehyde
CH_2O



Chlorine dioxide
ClO_2

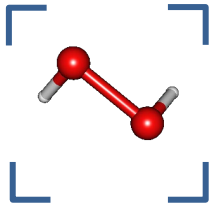


Peracetic acid
$C_2H_4O_3$



Hydrogen Peroxide +
Peracetic acid

Hydrogen Peroxide – H_2O_2



Sterilizing, disinfectant and decontaminating agent

Strong oxidant active against a broad range of micro-organisms (ex. bacteria, viruses, molds)

Vapor-phase H_2O_2
Dry mist H_2O_2 (compressed air+ H_2O_2)= microscopic drops

H_2O_2 Decontamination

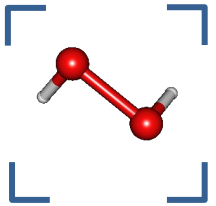
Heat-sensitive materials that should be transferred between classified areas in order to minimize the risk of contamination

Isolators, Sterility Test Isolators, Filling Line Isolators

Good and complete distribution
Sufficient contact time at a specified concentration

H_2O_2 Decontamination

Typical process conditions



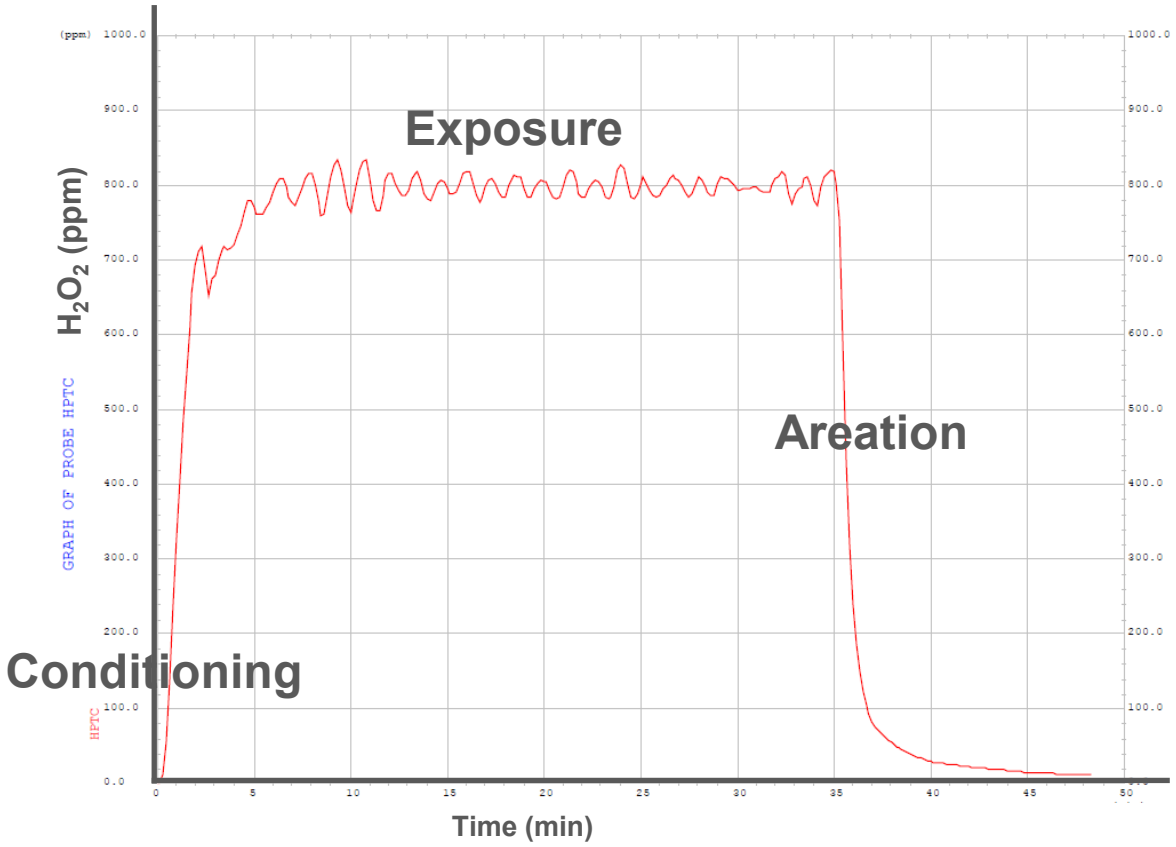
700 –1300 ppm H_2O_2 vapor during decontamination

≤ 5 – 90 % Humidity

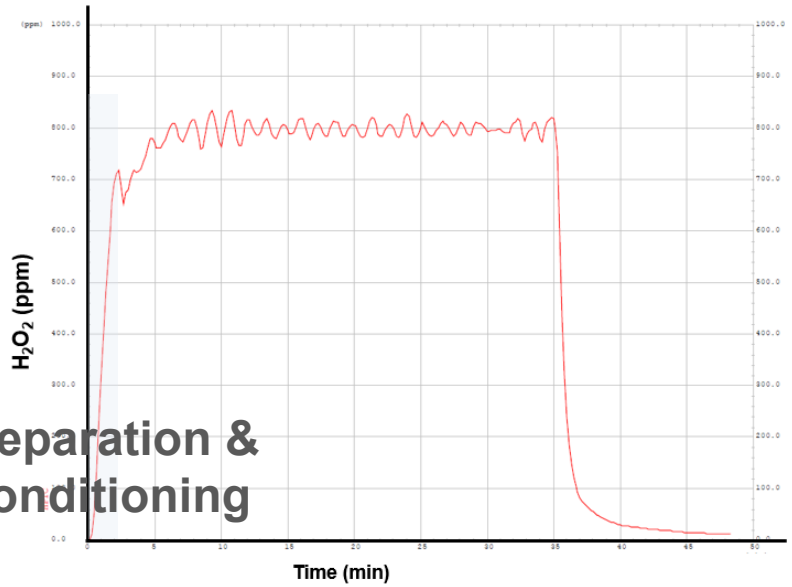
≤ 25°C Temperature during the process

Total cycle time: based on the product type

Decontamination Cycle



Preparation & Conditioning Steps

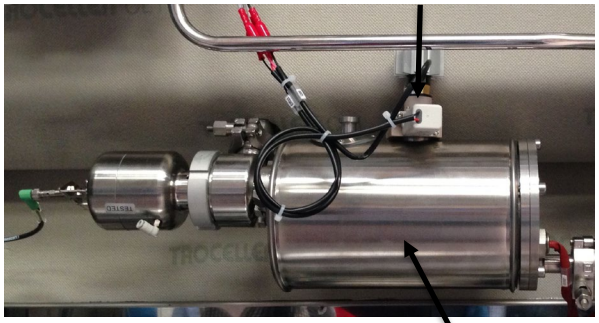


PREPARATION: Achievement of the pre-defined temperature and relative humidity value (set point)

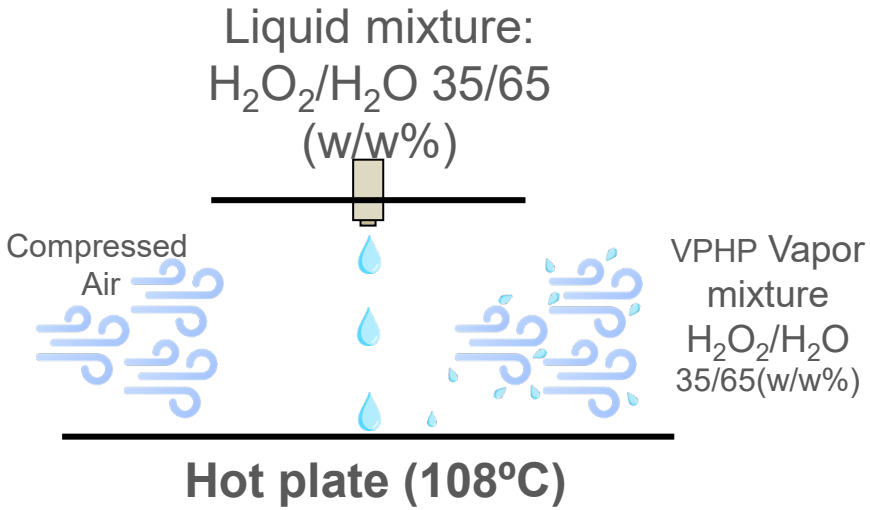
CONDITIONING: VHPH injection at high speed and achievement of the pre-defined Hydrogen peroxide vapor concentration

VPHP Production: Flash vaporization

Reciprocating pump



Vaporizer



All the components are vaporized simultaneously

H_2O_2 Detection

Dräger

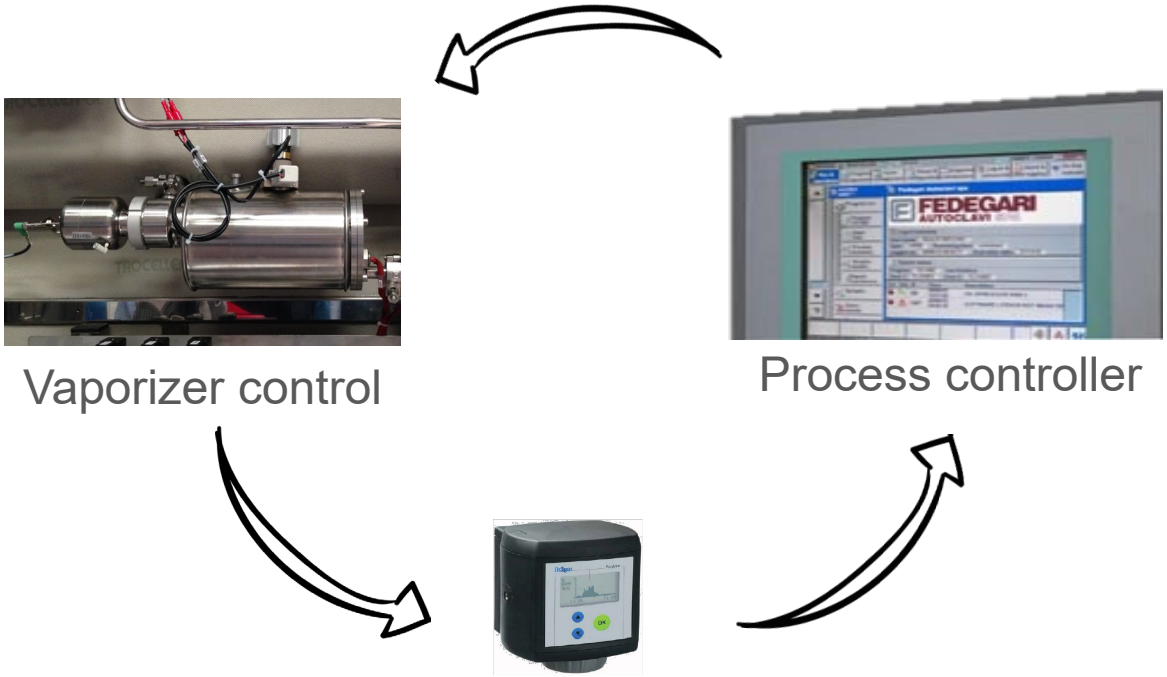
- **Electrochemical** sensor
- Measurement of H_2O_2 concentration (ppm)
- Low concentration sensor 0.1–300 ppm → Intended for **safety level measurement**
- High concentration sensor 100 – 7000 ppm
- Accuracy min 5% max > 20% of reading

VAISALA

- Capacitive thin-film polymer sensor
- Humidity sensors useful to detect hydrogen peroxide
- Measurement of H_2O_2 concentration (range 0–2000 ppm, with lower detection limit of 20 ppm)
  → **Not intended for safety level measurement**
- Measurement of *Relative Humidity* (RH), *Relative Saturation* (RS) and *Temperature*
- Accuracy (at +10…+25°C): ±10 ppm or 5 % of reading

Fedegari H_2O_2 Concentration Control

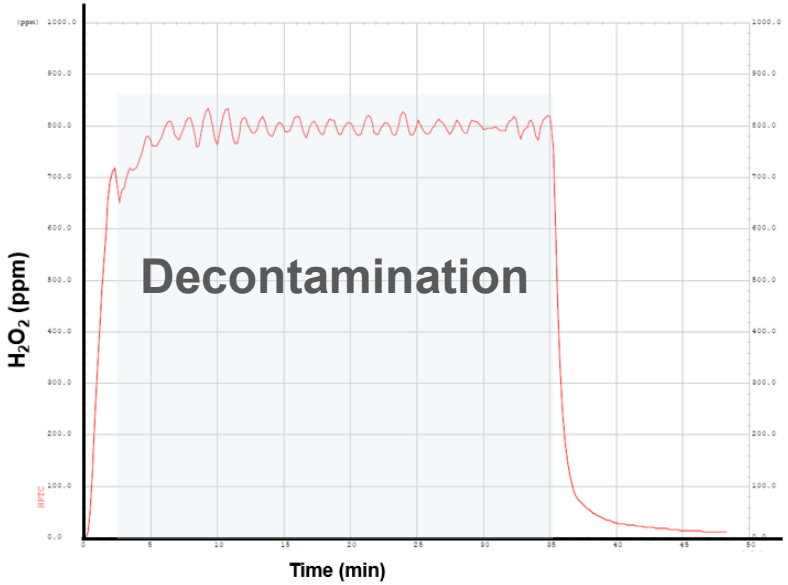
Continuous measuring of H_2O_2 ppm



Fedegari H_2O_2 Concentration Control

- Process strictly kept **under control**
- Dependent on the **instantaneous concentration of the biocide** (H_2O_2 vapour phase) that is measured in the chamber.
- A change in the H_2O_2 concentration (versus set-point) determines a change in the H_2O_2 injection rate by the generator (**feedback control loop**)
- Feedback control for superior process **repeatability** even if load patterns change.
- Higher **robustness** and **reproducibility** of the **cycle** (also for different loads)

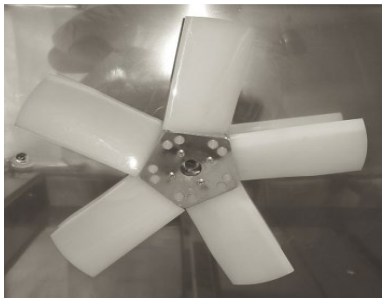
Decontamination step



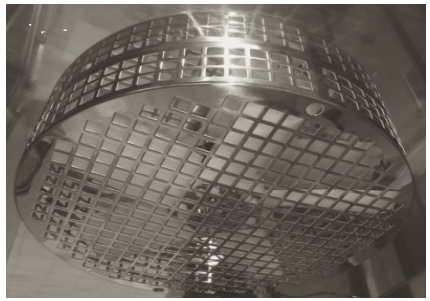
VPHP injection at a reduced rate
VPHP concentration is maintained constant for a pre-defined time

Biocide distribution: a critical issue for cycle reproducibility

A **fan** is used to guarantee a **homogeneous mixing** and **distribution** of **temperature**, **humidity** and **H_2O_2**



Magnetic coupling propeller



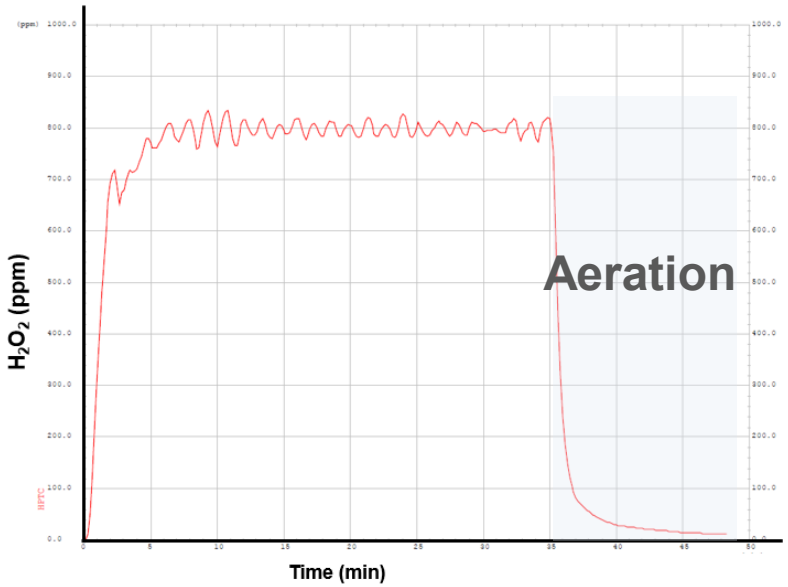
Vertical inlet Side delivery

No cold spots

No H_2O_2 stratification

Uniform material decontamination

Aeration step



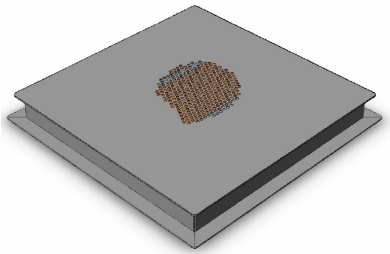
Air injection to replace (by dilution) H_2O_2

H_2O_2< 1ppm (TLV/TWA, threshold limit value/time-weighted average)

The time depends on both air exchange rate and H_2O_2 desorption from the decontaminated material (↑ temperature: ↑ vdes)

Focus on Biocide Elimination

- A catalytic converter is required to break H_2O_2 generated during the decontamination cycle into H_2O moisture and O_2.
- The "*catalytic converter*" is generally installed into the exhaust duct towards the atmosphere.



"Catalytic converter"
Activate alumina filter

- Composed of alumina microspheres
- Absorbs H_2O_2 vapors
- Electrical resistors accelerate H_2O_2 decomposition ($H_2O + O_2$) and to regenerate the "*catalyzer*"

*The **activate alumina** is characterized by a high surface layer, a high absorption power combined to a remarkable abrasion resistance.*

H_2O_2 residual concentration implications

Some materials are **adversely affected** by decontaminating agents, which can result in inhibition of microbial growth. Of concern are the **penetration** of decontaminating agents **into product containers**; accessory supplies such as filter sets and tubing; or any material that could come in contact with product, media, or dilution fluids used in the sterility test. It is the responsibility of the operator to verify that containers, media, and supplies are unaffected by the decontamination process.

USP 43 ⟨1208⟩ Sterility Testing—Validation of Isolator Systems

H_2O_2 residual concentration implications

Screw-capped tubes, *bottles*, or *vials sealed* with rubber stoppers and crimp overseals have proven **very resistant to the penetration** of commonly used decontaminating agents.

Wrapping materials in *metal foil* or *placing them in a sealed container* will **prevent contact with the decontaminating agent**; however, **these procedures may also result in some surfaces not being decontaminated**.

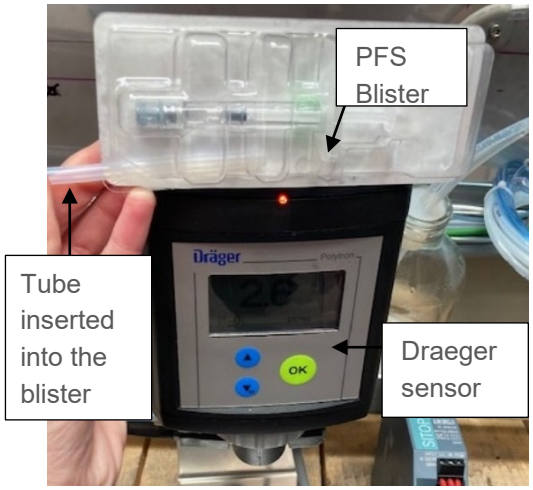
USP 43 ⟨1208⟩ Sterility Testing—Validation of Isolator Systems

H_2O_2 residual concentration implications

The effect of exposure to the H_2O_2 process on the physical or chemical properties of the product and packaging material shall be assessed and the outcome of the test shall be recorded.

- **Deterioration of elasticity** or strength **flexibility** → visible damages
- **Absorption** → Degassing time
- **Penetration** → Product damage
- **Microbiological effectiveness** → Surface finish

Measurement of H_2O_2 residual concentration



Into the blisters
Draeger sensor

Inside the product
Test Peroxydes strips

It is the responsibility of the operator to verify that containers, media, and supplies are unaffected by the decontamination process

USP 43 ⟨1208⟩ Sterility Testing—Validation of Isolator Systems

Validation

A **document program** that provides an **high degree of assurance** that a specified process, method or system will **consistently produce a result** meeting pre-determinated acceptance criteria.

PDA TR No. 54

USP 43, <1229.11> Vapor phase sterilization

Normative References

- USP 43 ⟨1072⟩ Disinfectant and Antiseptics
- USP 43 ⟨1208⟩ Sterility Testing – Validation of Isolator System
- USP 43 ⟨1229.11⟩ Vapor phase sterilization
- USP 43 ⟨1072⟩ Disinfectant and Antiseptics
- European Pharmacopoeia10.0 – 5.1.1 Methods of preparation of sterile products- Gas sterilisation (vapour phase sterilization)
- EudraLex Vol. 4 - Annex 1
- PIC/S - Recommendation used for aseptic processing and sterility testing
- PDA TR. No. 34 Design and Validation of Isolator Systems for the Manufacturing and Testing of Health Care Products
- PDA TR. No. 51 Biological Indicators for Gas and Vapor-Phase Decontamination Processes: Specification, Manufacture, Control and Use

Validation

The equipment qualification for vapor sterilization mimics that of other sterilization processes in order to **confirm that the equipment has been properly installed and operates as intended**.

USP 43 ⟨1229.11⟩ Vapor phase sterilization

Qualification at a glance

Process of establishing by objective evidence that all key aspects of the process equipment and ancillary system installation comply with the approved specification

ISO 22441 - Definition 3.19

Process of obtaining and documenting evidence that installed equipment operates within predetermined limits when used in accordance with its operational procedures

ISO 22441 - Definition 3.26

Process of establishing by objective evidence that the process, under anticipated conditions, consistently produces a product which meets all predetermined requirements

ISO 22441 - Definition 3.29

Process characterization

- The **process parameters**, together with their tolerances, **shall be specified**
- Processing at such process parameters shall **routinely yield** a **safe** and **functional product**

Description of the operating cycle

Process and **cycle parameters** (e.g. H_2O_2 concentration)

Load configuration

Location of **Chemical/Biological Indicators**

Validation

… Humidity and temperature measurements, along with chemical indicators, can provide a limited indication of sterilant distribution. BIs are not required in the evaluation of the empty chamber.

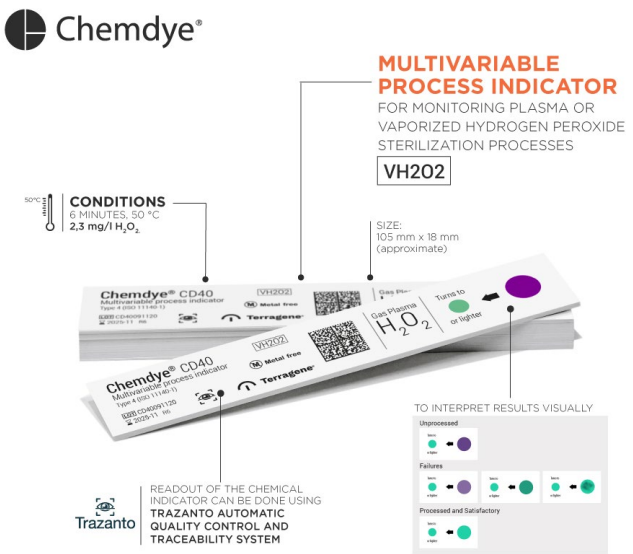
USP 43 ⟨1229.11⟩ Vapor phase sterilization

Chemical Indicator

Class 4: Multi-variable indicators

A multivariable indicator shall be designed to **react to two or more of the critical variables** (see 5.2) and is intended to indicate exposure to a sterilization cycle at stated values of chosen variables (see 5.7 and 5.8)

UNI EN ISO 11140-1, Clause 4.5



5.2 For the different sterilization processes, the following **variables** are defined as being critical:

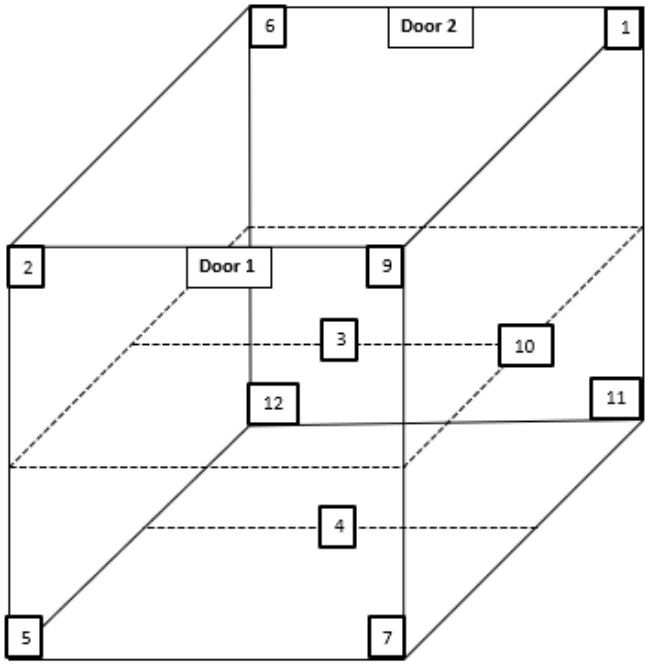
……

VAPORIZED HYDROGEN PEROXIDE: **Time**, temperature**, hydrogen peroxide concentration**, and, if applicable plasma

5.7 If the indicator is designed for use in specific sterilization cycles, this information shall be stated or coded on the indicator, e.g. STEAM 121°C 15 min

5.8 Each package of indicators or technical information leaflet supplied with the package shall provide the following information: a) the **change that is intended to occur**… b) the **critical variable(s)** to which the indicator will respond… c) the **class, process and intended use**… d) the **storage conditions**, before and after use; e) the **expiration date** or the manufacturing date plus the shelf life, under the specified storage conditions … f) a unique code (e.g. **lot number**) to provide traceability; g) **instruction for use** … h) any **interfering substances**… i) any **safety precautions** required during and/or after use; j) the **manufacturer's or supplier's name and address**; k) the nature of any change that can occur when completely/incompletely change indicators are stored according to the manufacturer's instructions.

Empty chamber mapping



Exposed

Not Exposed

Enzymatic Indicator

Enzymatic Indicators contain an enzyme that is inactivated during the bio-decontamination process, translating this into real-time quantifiable results and data.

60 Seconds

Quantifiable data

No manual interpretation

Validation

… Effects of load size and patterns should be assessed.

USP 43 ⟨1229.11⟩ Vapor phase sterilization

Validation

Biological indicators are required to **demonstrate the effectiveness of the process** they play a crucial role during the cycle development and validation.

USP 43 ⟨1229.5⟩ and PIC/S Recommendation clause 9.4.13

The use of **multiple BIs** at each test location is recommended to more adequately support the process lethality.

USP 43 ⟨1229.11⟩ Vapor phase sterilization

Biological Validation

… The process [sporicidal process] is normally validated using biological indicators containing bacterial spores. **The number of spore log reductions is not specified in this definition, but a target of six log reductions is often applied.** The process is applied to internal surfaces of the isolator and external surfaces of materials inside the isolator, when conventional sterilization methods are not required.

PIC/S Recommendation on Isolators Used for Aseptic Processing and Sterility Testing

Definition/Glossary clause 5.3 Sporicidal Process

Biological Validation

The decontamination methods used to treat isolators, test articles, and sterility testing supplies are capable of reproducibly yielding greater than a **three-log reduction against highly resistant biological indicators** (see Biological Indicators for Sterilization ⟨1229.5⟩), as verified by the fraction negative or total kill analysis methods. […]

USP 43 ⟨1208⟩, Sterility Testing - Isolator System

Biological Indicators at a glance

What is a Biological Indicator?

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

USP 43 ⟨1229.5⟩ Biological Indicators For Sterilization

Why use Biological Indicators?

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

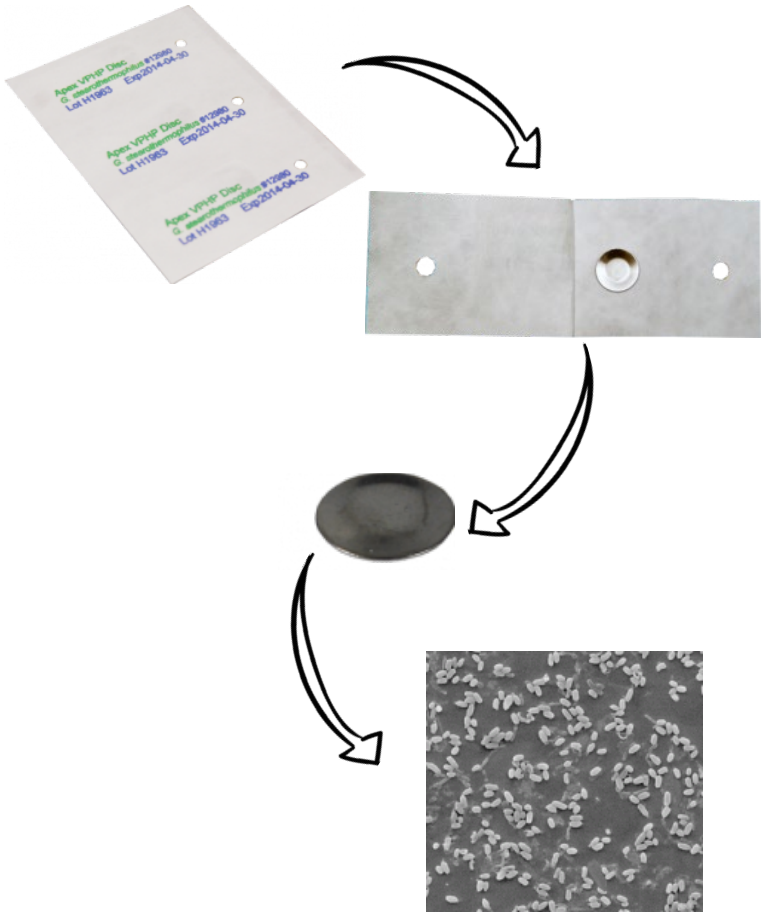
USP 43 ⟨1229.5⟩ Biological Indicators For Sterilization

How Biological Indicators are made?

Spores, Carrier for the spores, Primary pack

Anatomy of a Biological Indicator

- A **primary pack,** to protect and contain the spores while allowing the microbiocidal inactivation process to proceed unhindered.
- A **carrier** for spores. Carrier may be made from a variety of materials
- A number of **viable spores** of a species and strain demonstrated as being resistant to the chosen microbiological inactivation process.



PDA Technical Report No.51

Spore type

BIs should be suitably qualified microorganisms

BIs using either *G. stearothermophilus* or *B. atrophaeus* have been utilized in the evaluation of these processes.

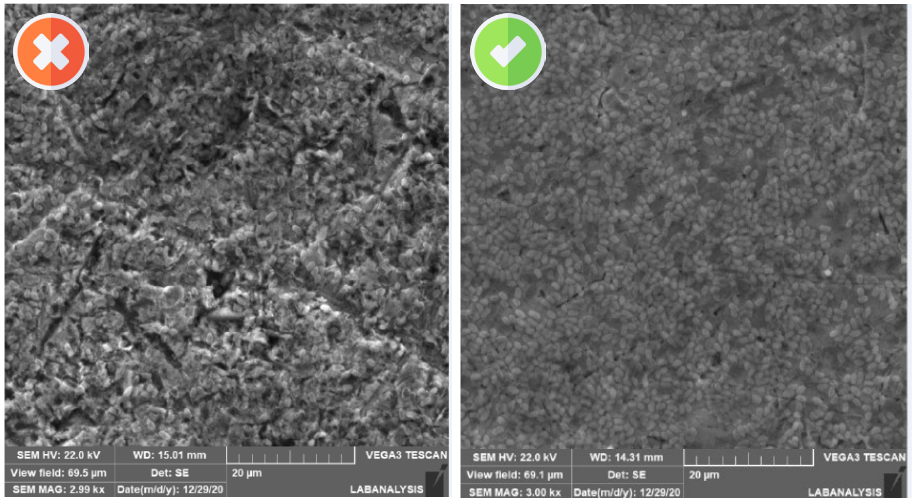
USP 43 ⟨1229.5⟩ Biological Indicators For Sterilization

Process	Selected Organism	ATCC Derivation
Peracetic acid Hydrogen peroxide	*Geobacillus stearothermophilus*	7953 or 12980 (Ph. Eur.)
Ethylene oxide Formaldehyde Peracetic acid	*Bacillus atrophaeus* *(formerly Bacillus subtilis var. niger)*	9372 (Ph. Eur.)

For applications where the surface to be decontaminated is not in direct contact with the product, a BI with a population of $<10^6$ may be considered with a supporting rationale ***(40)***.

PDA, Technical Report No.51 – Table 7.1-1 Recommended Microorganisms

General requirements for the BIs

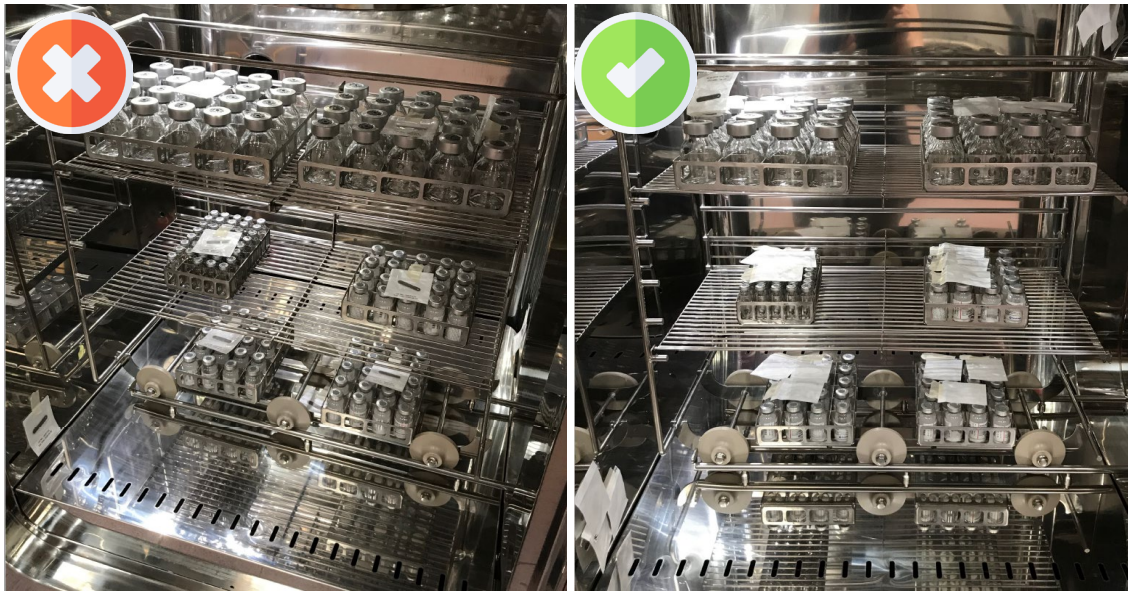


- BIs should consist of a ***monolayer* of spores** to guarantee direct contact of the hydrogen peroxide to the spores PDA, Technical Report No.51, clause 4.1.3
- Physical variations between *"even monolayer distribution of spores"* and *"varying degrees of clumping and spore aggregation […] contribute to unpredictable BI performance"* PDA, Technical Report No.51, clause 4.2.1

BIs & H_2O_2 Process Validation

*A **sufficient number of BIs** are used to prove statistical reproducibility and adequate distribution of the decontaminating agent.*

USP 43 ⟨1208⟩ STERILITY TESTING—VALIDATION OF ISOLATOR SYSTEMS



*The use of **multiple BIs** at each test location is recommended to more adequately support the process lethality.*

USP 43 ⟨1229.11⟩ Vapor phase sterilization

Unexpected positive BI: case study

If we used **1 BI/location** …

… we might have

- , No growth

or

+, Growth

Single BIs do not allow performing a statistical analysis

Unexpected positive BI: case study

If we used **3 BIs /location** …

… we might have

+ = Growth (yellow)
- = No growth (purple)

Multiples BIs allow performing a statistical analysis

Unexpected positive BI

The use of the **Halvorson-Ziegler equation** and the determination of the **Spore Logarithmic Reduction** (SLR) using the **Stumbo-Murphy-Cochran-Procedure** are essential to understand the real causes of a positive BI and evaluate the obtained reduction of the microbial level.

Most Probable Number Equation

The *Halvorson-Ziegler equation* allows calculating the **Most Probable Number** (MPN) of microorganisms surviving in a positive sample with confirmation of the SLR.

$$\mathbf{MPN = Ln\ (n/r)}$$

where:
ln = natural logarithm function
n = number of replies per point
r = number of negative results per point

SRL Calculation

The correlation between MPN and the initial population of a BI allows determining the **SLR** by the *Stumbo-Murphy-Cochran-Procedure*:

$$\mathbf{SLR = Log_{10}\ N_0 - Log_{10}\ MPN}$$

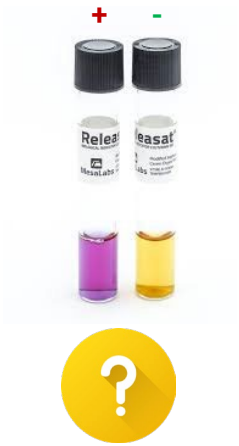
where:
N_0 = initial number of spores in each inoculated substrate
MPN = Ln (n/r)

Stumbo-Murphy-Cochran-Procedure

This calculation is **only possible** when **multiple BIs** are used.

+ = Growth (yellow)
- = No growth (purple)

1 BI/location



Single BIs do not allow to perform a statistical analysis

3 BIs/location

Multiples BIs allow to perform a statistical analysis

Practical example

Three BIs are placed in a specific position only two of them are negative at the end of the decontamination cycle.

MPN = ln (n/r) = ln (3/2) = 0.405

If $N_0 = 2.8 \times 10^6$

SLR = Log_{10} (2.8 x 10^6) - Log_{10} 0.405

SLR = 6.447 - (- 0.393) = **6.840**

Conclusions

- Hydrogen peroxide is a decontaminant, active on surfaces
- Its process is a low-temperature one, useful for heat-sensitive loads
- Its validation includes considering:
  - material compatibility,
  - definition of the targets to achieve,
  - assessing the homogeneity of distribution
  - reaching the safety level required

Thank you for your attention