Introduction to current sterilization methods

Hydrogen peroxide decontamination

Lucia Maita, Technical Customer Support, Fedegari Group

Agenda

Introduction
Decontamination, Gas sterilization & Vapour phase sterilization

Hydrogen peroxide
Chemical properties & Sporicidal Mechanism

Decontamination technologies
Fedegari Isolation technology

Development & Validation of a decontamination process
Normative references & practical case studies

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Decontamination

The removal of microorganisms by **disinfection** or **sterilization**.

Disinfectant—A chemical or physical agent that destroys or removes vegetative forms of harmful microorganisms when applied to a surface.

Sterilant—An agent that destroys all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores. Sterilants are liquid or vapor-phase agents.

USP43 ⟨1072⟩ Disinfectant and Antiseptics

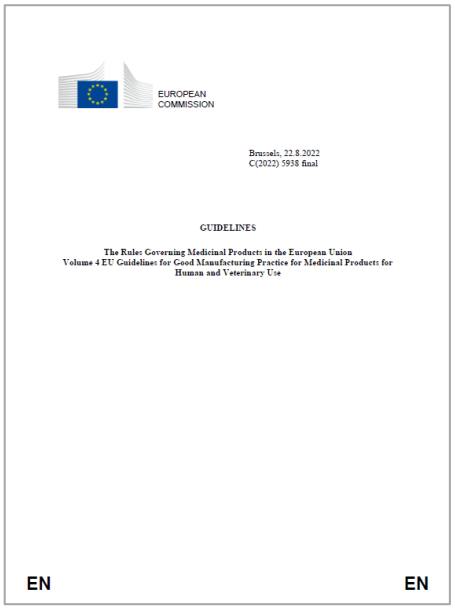
Decontamination

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

USP - 43 ⟨1208⟩ Sterility Testing – Validation of Isolator System

Decontamination

The overall process of **removal or reduction of any contaminants** (chemical, waste, residue or microorganisms) from an area, object, or person. The method of decontamination used **(e.g. cleaning, disinfection, sterilisation)** should be chosen and validated to achieve a level of cleanliness appropriate to the intended use of the item decontaminated. See also Bio-decontamination.


EUROPEAN COMMISSION

Brussels, 22.8.2022
C(2022) 5938 final

GUIDELINES

The Rule: Governing Medicinal Products in the European Union
Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use

EN EN

Bio - Decontamination: A process that eliminates viable bioburden via use of sporicidal chemical agents.

EudraLex Vol. 4 - Annex 1 – Glossary

Decontamination

For materials, equipment, components and ancillary items that are not a direct or indirect product contact part and are **necessary for aseptic processing but cannot be sterilised**, an effective and **validated disinfection and transfer process should be in place**. These items, once disinfected, should be protected to prevent recontamination. These items, and others representing potential routes of contamination, should be included in the environmental monitoring programme.

EudraLex Vol. 4 - Annex 1, Clause 8.49

Gas sterilization (vapour phase sterilization)

Gas sterilization of surfaces may be used for the sterilization of *primary packaging materials*, *equipment* and some *pharmaceuticals*.

European Pharmacopoeia 10.0 – 5.1.1 Methods of preparation of sterile products- Gas sterilisation (vapour phase sterilization)

Sterilizing agent: *physical or chemical entity or combination of entities, having sufficient microsporicidal activity to achieve sterility under specified conditions.*

ISO 11139:2018, 3.288

Sporicidal Vapor Phase Decontamination

The destruction or inactivation of microbial spores using a **vapor** *or* **gaseous agent**.

PDA TR. No. 51, Glossary of Terms

Sterilization

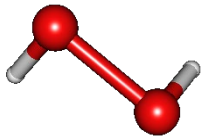
A process used to render a product, environment or pieces of equipment free from viable microorganisms with a specific probability.

PDA TR. No. 51, Glossary of Terms

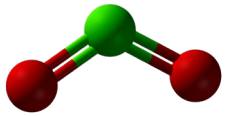
Vapor Phase Sterilization

Sterilization can be accomplished using *sporicidal agents suspended in air* (i.e., vapor). Sterilizing agents that operate in this fashion include *hydrogen peroxide* (H_2O_2), *peracetic acid* (CH_3CO_3CH), *formaldehyde* (CH_2O), and *glutaraldehyde* [$CH_2(CH_2CHO)_2$] in aqueous solution.

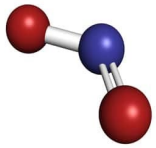
USP - 43 ⟨1229 .11⟩ Vapor phase sterilization



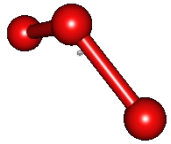
Hydrogen Peroxide
H_2O_2



Chlorine dioxide
ClO_2



Nitrogen dioxide
NO_2



Ozone
O_3

The Resistance of Some Clinically Important Microorganisms to Chemical Disinfectants

(Listed in Order of Decreasing Resistance)

Type of Microorganisms	Examples
Bacterial spores	*Bacillus subtilis* and *Clostridium sporogenes*
Mycobacteria	*Mycobacterium tuberculosis*
Nonlipid-coated viruses	Poliovirus and rhinovirus
Fungal spores and vegetative molds and yeast	*Trichophyton*, *Cryptococcus*, and *Candida* spp.
Vegetative bacteria	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella* spp.
Lipid-coated viruses	Herpes simplex virus, hepatitis B virus, and human immunodeficiency virus

USP43 ⟨1072⟩ Disinfectant and Antiseptics

General Classification of Antiseptics, Disinfectants, and Sporicidal Agents

Chemical Entity	Classification	Example
Aldehydes	Sporicidal agent	2% Glutaraldehyde
Alcohols	General purpose disinfectant, antiseptic, antiviral agent	70% Isopropyl alcohol, 70% alcohol
Chlorine and sodium hypochlorite	Sporicidal agent	0.5% Sodium hypochlorite
Phenolics	General purpose disinfectant	500 µg per g Chlorocresol, 500 µg per g chloroxylenol
Ozone	Sporicidal agent	8% Gas by weight
Hydrogen peroxide	Vapor phase sterilant, liquid sporicidal agent, antiseptic	4 µg per g H_2O_2 vapor, 10%–25% solution, 3% solution
Substituted diguanides	Antiseptic agent	0.5% Chlorhexidine gluconate
Peracetic acid	Liquid sterilant, vapor phase sterilant	0.2% Peracetic acid, 1 µg per g peracetic acid
Ethylene oxide	Vapor-phase sterilant	600 µg per g Ethylene oxide
Quaternary ammonium compounds	General purpose disinfectant, antiseptic	Concentration dependent on application, Benzalkonium chloride
β-Propiolactone	Sporicidal agent	100 µg per g β-Propiolactone

USP43 ⟨1072⟩ Disinfectants and antiseptics

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Sporicidal process

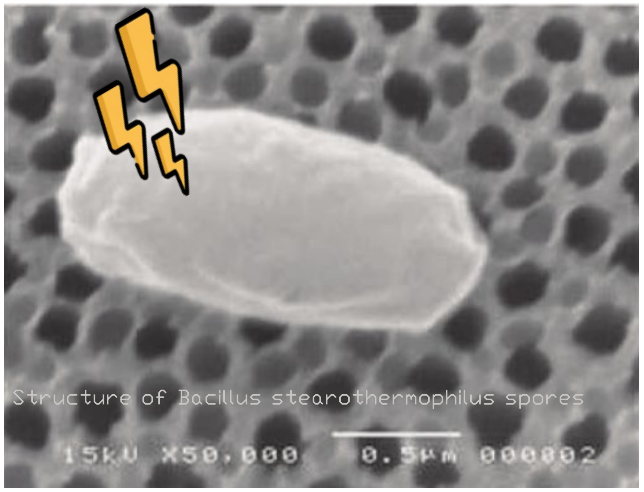
A gaseous, vapour or liquid treatment applied to **surfaces**, using an agent that is recognised as capable of killing bacterial and fungal spores.

….. 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

Sporicidal Mechanisms

- **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.

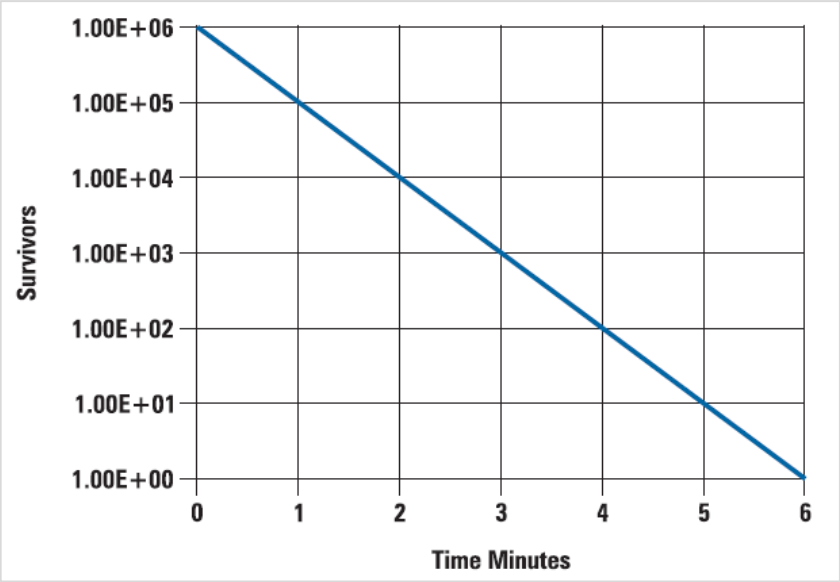
- **Alkylation of macromolecules within the microorganism**

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



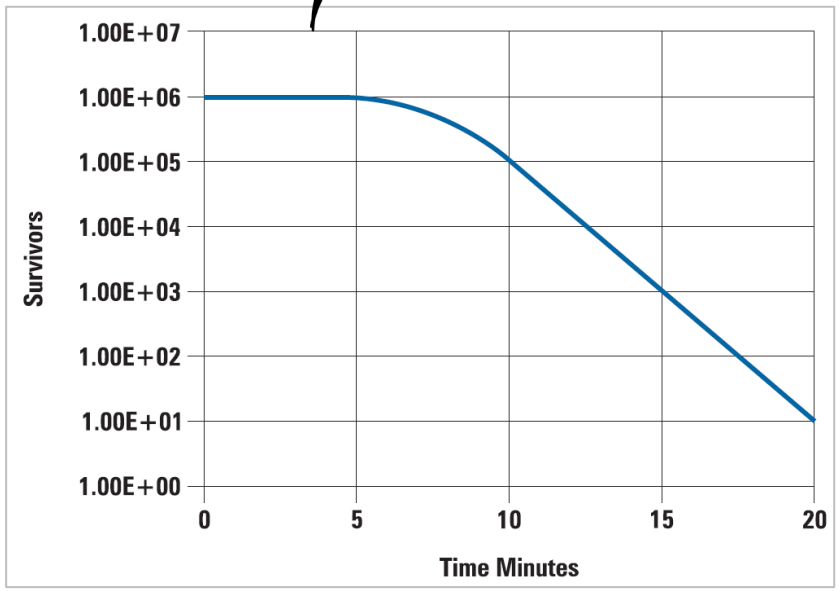
Idealized Plot of Death Kinetics_PDA TR N. 51

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



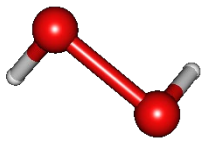
Typical Survival Curve - PDA TR N. 51

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

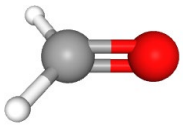
If the process relies on condensation, the concentration and mass of the liquid layer will vary during the cycle.

Common sporicidal decontamination agents

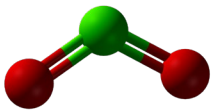
- Hydrogen peroxide vapor
- Peracetic acid vapor
- Chlorine dioxide gas
- Formaldehyde vapor
- Hydrogen peroxide and peracetic acid vapor in combination



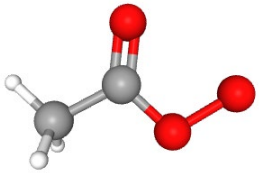
Hydrogen Peroxide
H_2O_2



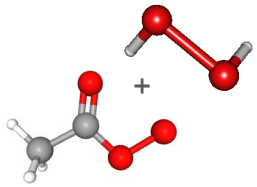
Formaldehyde
CH_2O



Chlorine dioxide
ClO_2



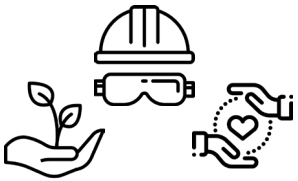
Peracetic acid
$C_2H_4O_3$



Hydrogen Peroxide +
Peracetic acid

Biocide selection

Material compatibility

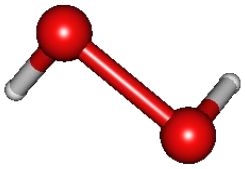


Environment, health and safety

Site production experience

Validation

Hydrogen Peroxide – H_2O_2



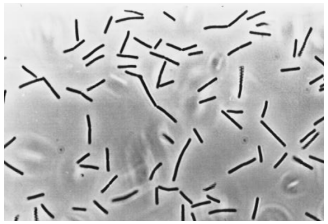
PubChem

Hydrogen Peroxide is a peroxide and **oxidizing agent** with **disinfectant**, **antiviral** and **anti-bacterial** activities. Upon rinsing and gargling or topical application, hydrogen peroxide exerts its **oxidizing activity** and produces *free radicals which leads to oxidative damage to proteins and membrane lipids*. This may inactivate and destroy pathogens and may prevent spreading of infection.

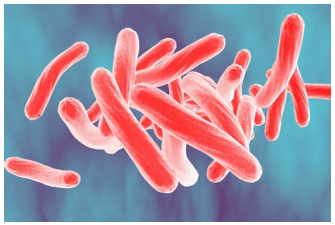
Lethality Mechanism of H_2O_2

Hydrogen peroxide is particularly effective against a **wide range of microorganisms**, including *bacteria*, *bacterial spores*, *fungi*, and *viruses*.

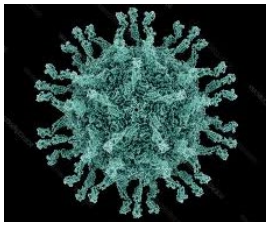
PDA Technical Report No. 51



Bacterial spores
B. subtilis

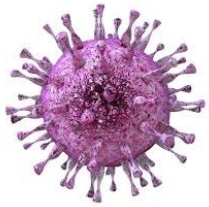


Mycobacteria
M. tuberculosis



Non lipid or
small viruses
polio virus

Fungi
Candida specie



Enveloped virus
herpes

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Bio - Decontamination: *A process that eliminates viable bioburden via use of sporicidal chemical agents.*

EudraLex Vol. 4 - Annex 1 – Glossary

H_2O_2 Decontamination: when?

- **Heat-sensitive materials** (including electronic devices) that should be transferred between classified areas (class C,D → class A, B) in order to minimize the risk of contamination

- **Surface of aseptic processing room** (e.g. cleanroom)

- **Aseptic processing system** (e.g. isolators)

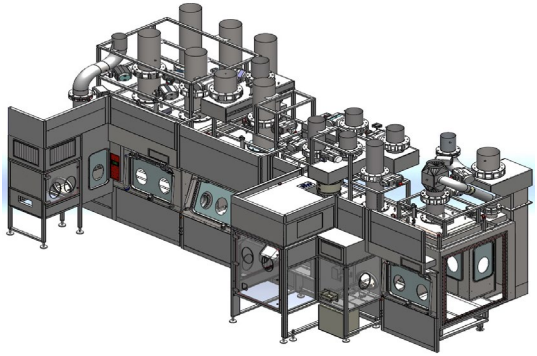
Fedegari Isolator technology

FCDV

Fedegari Cabinet Decontamination Vapor

FCTS

Fedegari Sterility Test Isolator



Customizable solutions

Flexible Filling Line Isolator

Fedegari Bio-Decontamination Unit- FCDV

Applications	Bio-pharmaceutical
Loads	Heat sensitive
Processes	Bio-decontamination

DESIGN & TECHNICAL FEATURES

- Chamber: stainless steel (AISI 316L) polished (Ra <0.4 µm)
- Doors: stainless steel (AISI 316L) with a central glass panel
- Door's gaskets: 100% air-tight guaranteed
- Direct control of H_2O_2 in the chamber (control loop)
- Great flexibility: FCDV can be used as a pass-through hatch aired with synchronized doors and pressure control

COMPLIANT TO

European directives (2014/30/EU – electromagnetic compatibility (EMC), 2014/35/EU – Low tension equipment (LVD), 2006/95/EC – Safety of machinery (MD)), **European standards (**EN 55011, EN IEC 61000-4-2, EN IEC 61000-4-4, EN IEC 60204-1), **FDA** (compliance for non-metallic component in contact with process fluids), **GMP, GAMP5, 21 CFR Part 210, 211 e 11, UL 508A, NFPA-79, ASME BPE**

Fedegari Sterility Test Isolator- FCTS

Applications	Bio-pharmaceutical
Loads	Powders, Heat sensitive
Processes	Bio-decontamination, Sterility testing

DESIGN & TECHNICAL FEATURES

- Chamber: stainless steel (AISI 316L) polished (Ra <0.4 µm)
- Door's gaskets: 100% air-tight guaranteed
- Direct control of H_2O_2 in the chamber (control loop)
- Internal stainless-steel trolley for material transfer
- Customizable internal storage and racks
- Modular design 100% customizable

COMPLIANT TO

European directives (2014/30/EU – electromagnetic compatibility (EMC), 2014/35/EU – Low tension equipment (LVD), 2006/95/EC – Safety of machinery (MD)), **European standards** (EN 55011, EN IEC 61000-4-2, EN IEC 61000-4-4, EN IEC 60204-1), **FDA**, **GMP**, **GAMP5**, **21 CFR Part 210**, **21111**, **UL 508A**, **NFPA-79**, **ASME BPE**

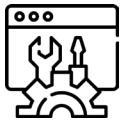
Fedegari Sterility Test Isolator- FCTS

An effective H_2O_2 decontamination: how?

The biocide should reach **all the surfaces** that should be decontaminated at a specific concentration for a pre-determined amount time.

This means that the biocide must have:

- Good and complete **distribution**
- Sufficient contact **time** at specified **concentration**



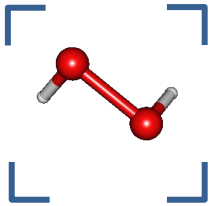
Items exposed to the process should have their surfaces exposed to the greatest extent possible.

… There are several effective approaches to hydrogen peroxide (H_2O_2) injection, including **continuous**, **intermittent**, or **all at once**. Some of the systems utilize an **evacuation or drying step** prior to introduction of the hydrogen peroxide (H_2O_2) to allow for increased concentration without excess condensation. Alternatively, hydrogen peroxide (H_2O_2) can be introduced as a **liquid, followed by target heating**. Following the exposure period, the chamber or target is **aerated** to an acceptable level for further processing of materials and/or personnel exposure (whichever is lowest) prior to opening and removing the sterilized article.

USP 1229.11 – Vapor Phase sterilization

It is essential that the gas is eliminated under conditions that have been previously established as sufficient to ensure that any **residues of gas** or **related transformation by-products** are below concentrations that could give rise to **toxic effect** during product use.

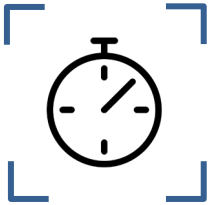
Typical process conditions



400 –1500 ppm H_2O_2 vapor during decontamination

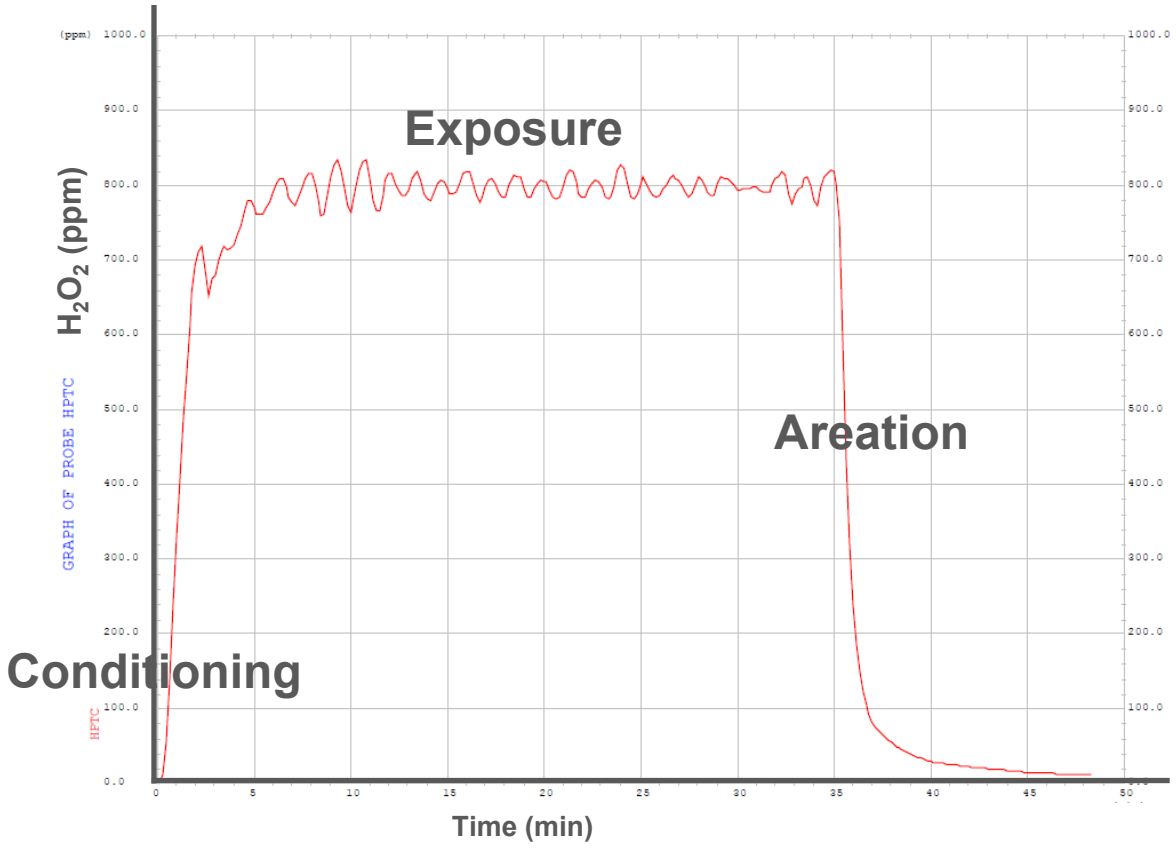
≤ 5 – 90 % Humidity

≤ 25°C Temperature during the process



20 min – 1,5 hours Total cycle time

Decontamination Cycle



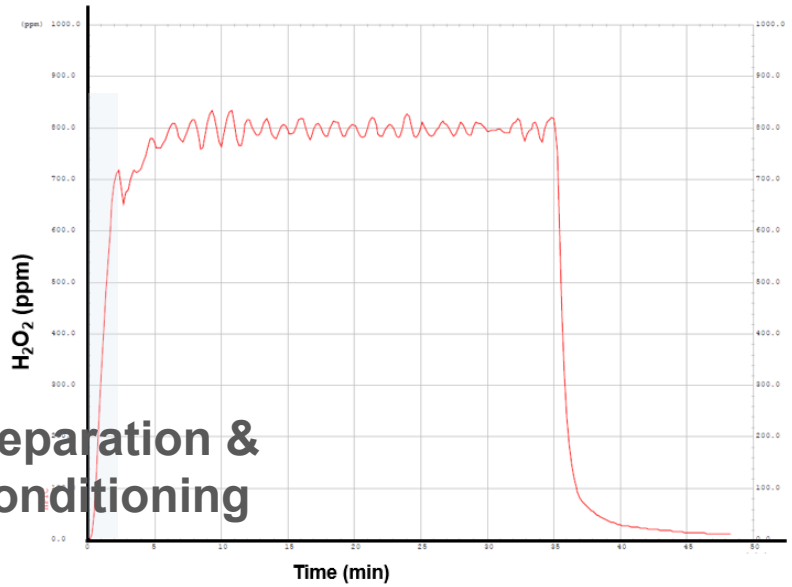
Typical process conditions

Fedegari Thema4 Process Controller is responsible for the control of machine hardware and cycles.

Allows the user to define cycle parameters:

- Relative Humidity (RH) Set Point
- Temperature Set Point
- Decontamination Time
- H_2O_2 Concentration

Preparation & Conditioning Steps

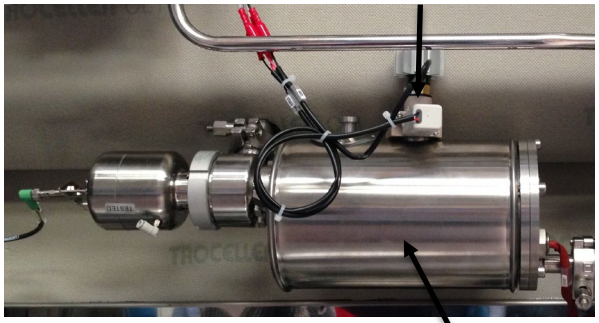


PREPARATION: Achievement of the pre-defined temperature and relative humidity value (set point)

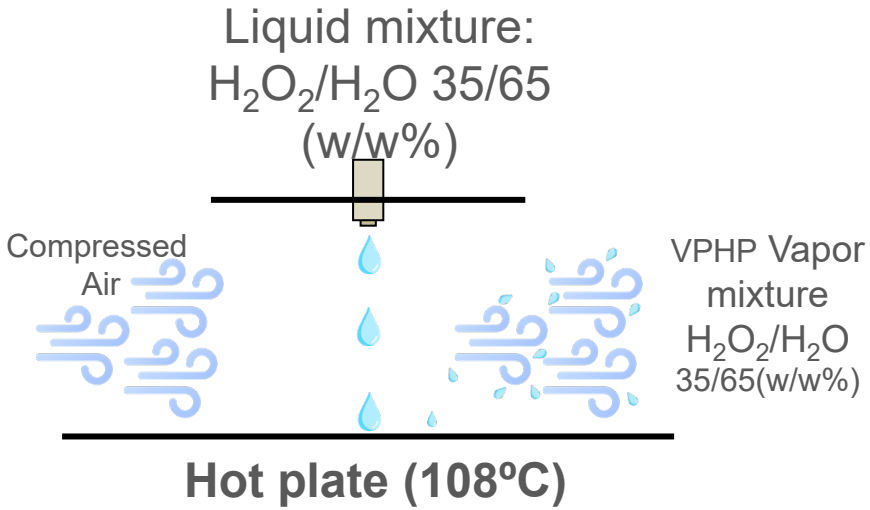
CONDITIONING: VHPH injection at high speed and achievement of the pre-defined Hydrogen peroxide vapor concentration

VPHP Production: Flash vaporization

Reciprocating pump

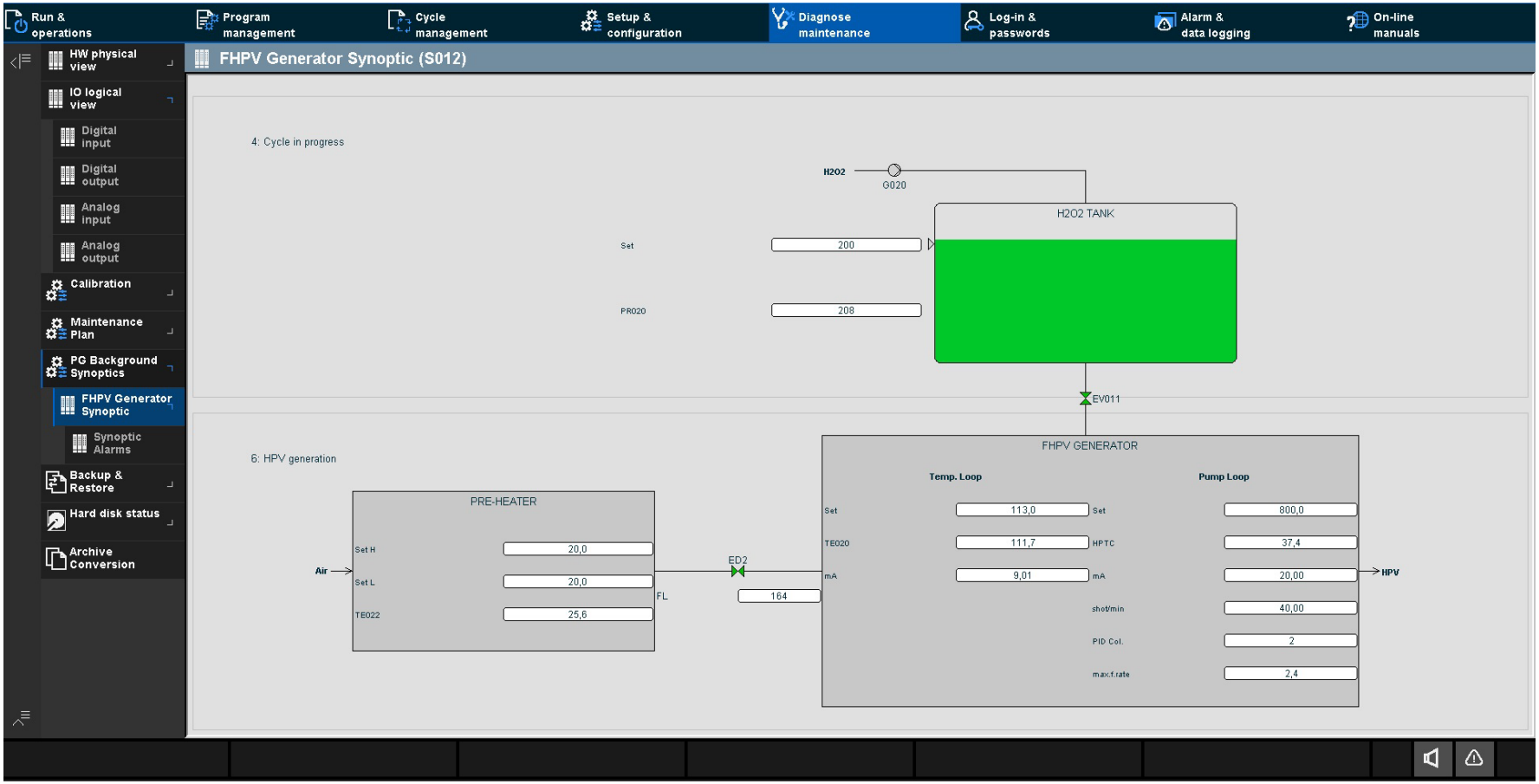


Vaporizer



All the components are vaporized simultaneously

FHPV generator synoptic



H_2O_2 Detection

Dräger

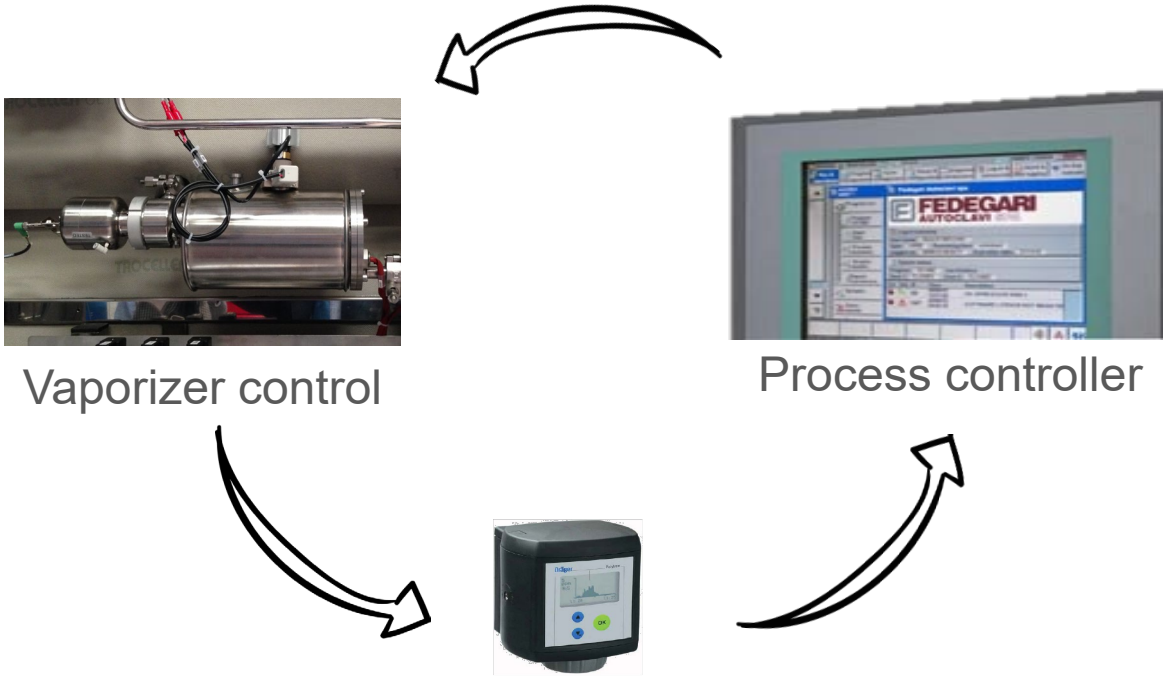
- Electrochemical sensor
- Measurement of H_2O_2 concentration (ppm)
- Low concentration sensor 0.1 – 300 ppm → Intended for **safety level measurement**
- High concentration sensor 100 – 7000 ppm
- Accuracy min 5% max > 20% of reading

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

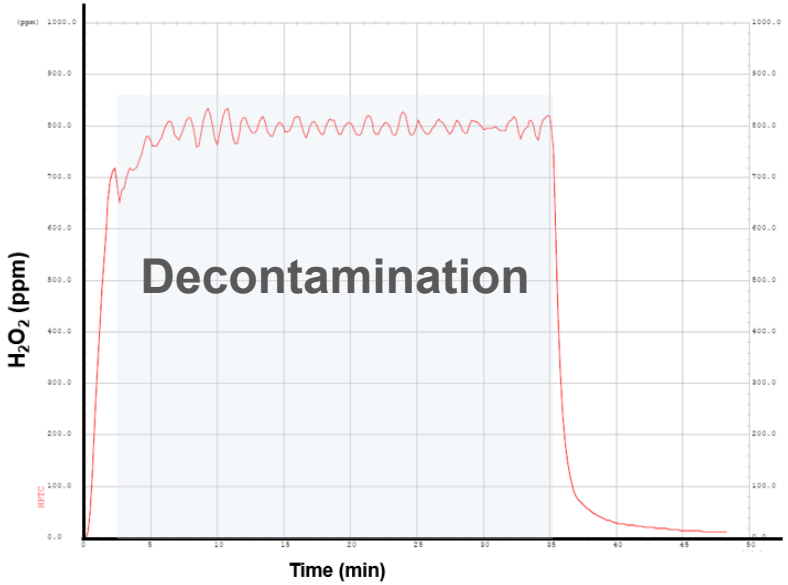
Continuous measuring of H_2O_2 ppm



Fedegari H_2O_2 Concentration Control

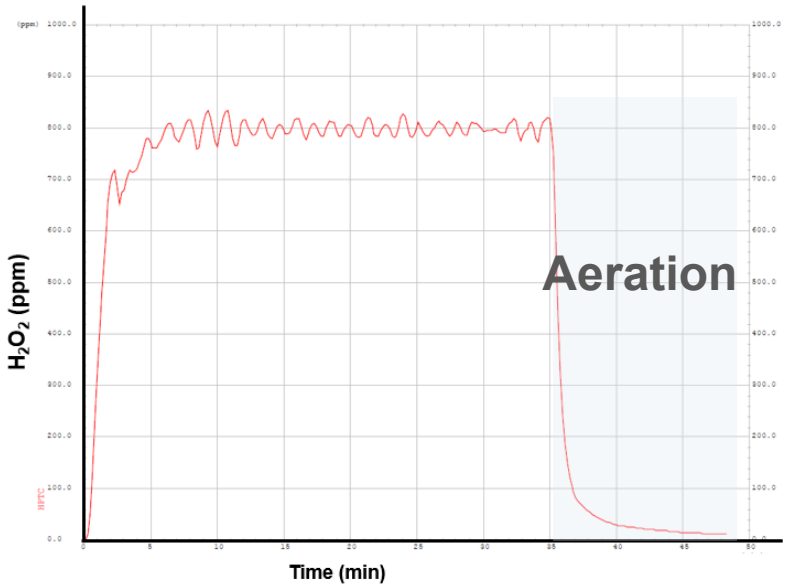
- Process strictly kept **under control**
- Dependent on the **instantaneous concentration of the biocide** (H_2O_2 vapour phase) that is measured in the chamber.
- A change in the H_2O_2 concentration (versus set-point) determines a change in the H_2O_2 injection rate by the generator (**feedback control 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

Aeration step



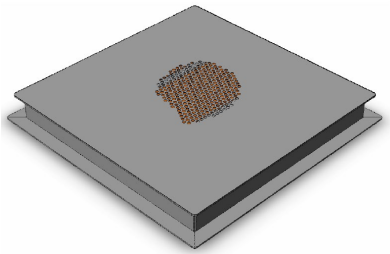
Air injection to replace (by dilution) H_2O_2

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.*

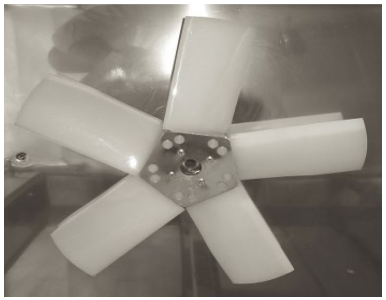
Critical issues during aeration

Adsorptive property of the material subjected to decontamination

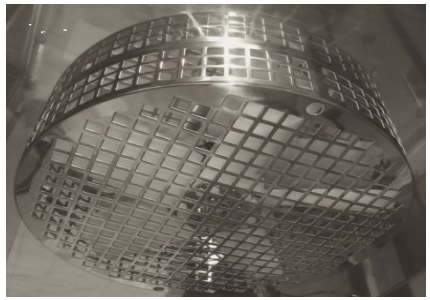
- The higher the H_2O_2 adsorption rate of the materials, the longer the desorption and aeration time;
- Materials with higher adsorption properties can not always be avoided like Tyvek, critical issues with wet loads, paper and nylon;
- The selection of the materials influences the cycle length to the desired residual concentration;
- HEPA filters are typically an example of adsorptive material and have a real impact on the aeration 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

Maintaining process effectiveness Is Temperature a critical factor?

Temperature is strictly linked with the vaporized biocide concentration within the enclosure:

- Low Temperature means a high risk of condensation;
- The higher the Temperature, the higher the desorption of the condensed hydrogen peroxide. The material surface desorption increases the concentration in the vapor phase. However, at very high temperatures, the bioburden inactivation capacity is very inefficient.

To guarantee process repeatability and avoid extra energy consumption it is recommended to keep a stable surrounding environment Temperature.

Agenda

Introduction
Decontamination, Gas sterilization & Vapour phase sterilization

Hydrogen peroxide
Chemical properties & Sporicidal Mechanism

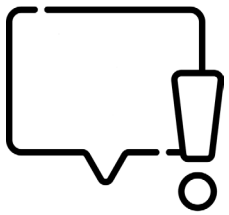
Decontamination technologies
Fedegari Isolation technology

Development & Validation of a decontamination process
Normative references & practical case studies

Focus on 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



Identify the requirements for exposure conditions and process control: e.g. the temperature, humidity. etc

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 Pharmacopoeia 10.0 – 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
- ISO 11139:2018
- PDA TR. No. 51 Biological Indicators for Gas and Vapor-Phase Decontamination Processes: Specification, Manufacture, Control and Use
- PDA TR. No. 51 Design and Validation of Isolator Systems for the Manufacturing and Testing of Health Care Products

Validation

USP 43, <1229.11> Vapor phase sterilization

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.

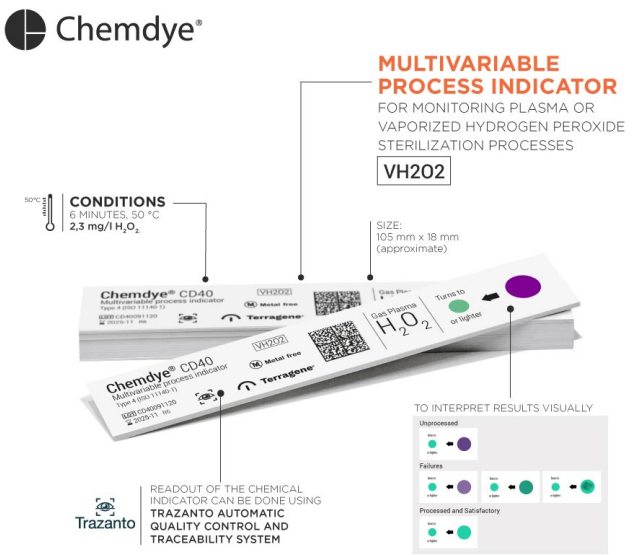
USP 43 ⟨1229.11⟩ Vapor phase sterilization

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:

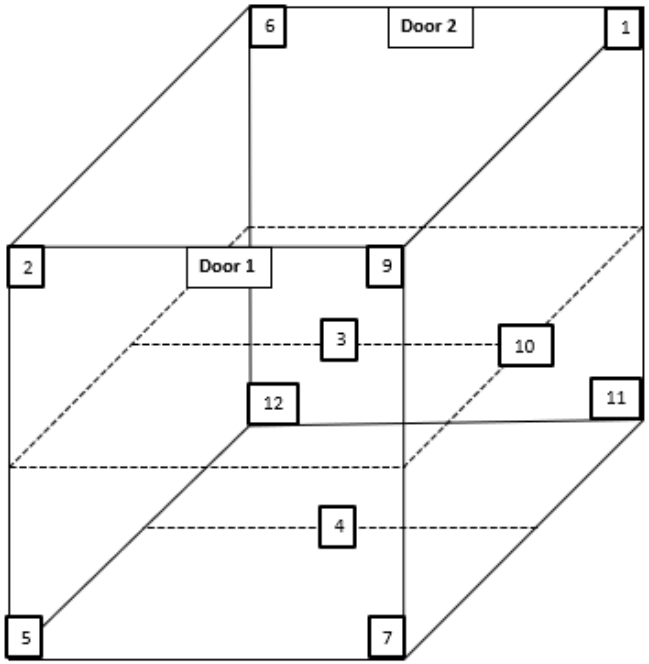
……

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

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 Validation

Current practice is to seek **six log reductions of the biological indicator organism recommended by the manufacturer of the gas generator**. In this document, this is intended to mean that at each point in the isolator the sporicidal process will reduce the survivors by six logs i.e. if there are $2x10^6$ spores in the BI to start with then there will be 2 surviving spores after six log reduction. If there are no survivors, then a six-log reduction is confirmed and there is an additional safety margin the size of which is not known.

PIC/S Recommendation on Isolators Used for Aseptic Processing and Sterility Testing, clause 9.4.13-f),

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.

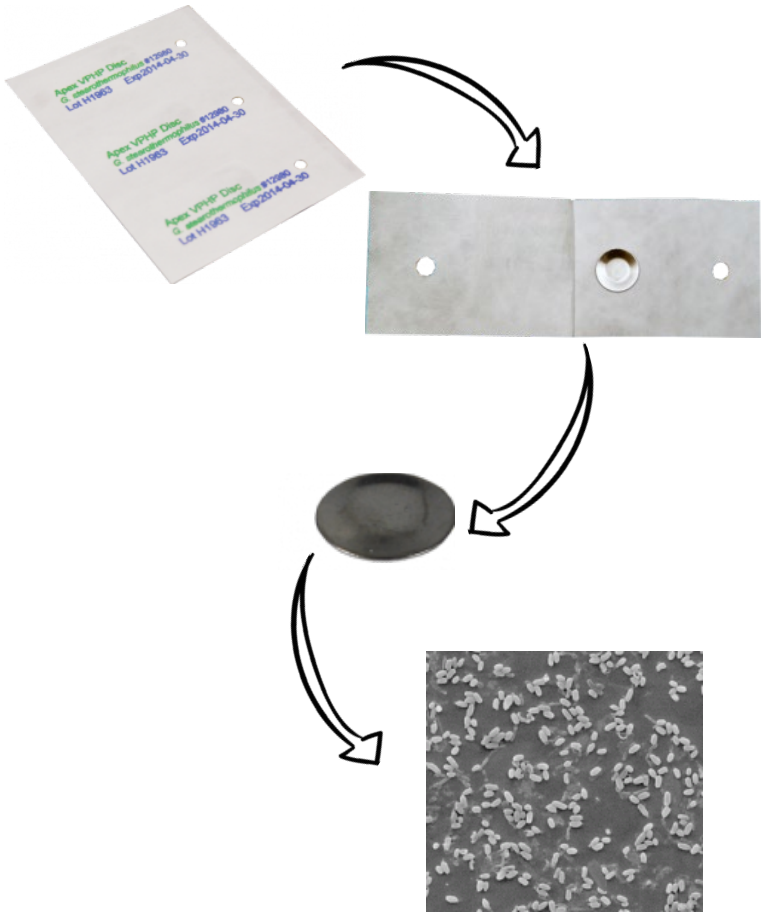
USP 43 ⟨1229.5⟩ Biological Indicators For Sterilization

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.



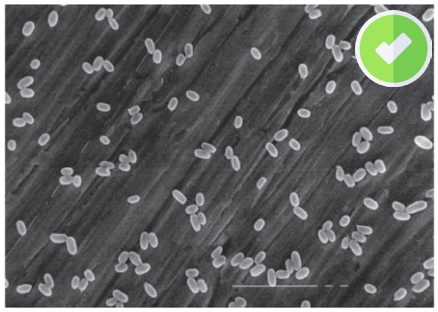
PDA Technical Report No.51

Primary pack characteristics

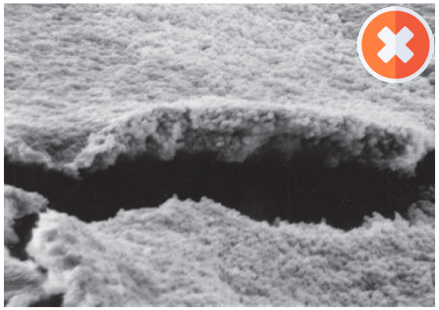
- Provide **little resistance** to the sporicidal vapor-phase decontamination process
- Ensure **containment** of the test organism
- **Protect** the inoculated carrier from contamination **following exposure** to the agent
- **Protect** the inoculated carrier from **damage**
- Bee **free of shedding**
- Be capable of holding all necessary labelling and lot /expiry references
- Be provided with a means to either suspended the BI or tape the BI surface in such a way that is freely exposed to the sporicidal gas
- Be easily opened to minimize the potential cross-contamination when removing spore carriers for placement in growth media
- Maintain the integrity of the inoculated carrier during transport and storage

PDA TR No. 51

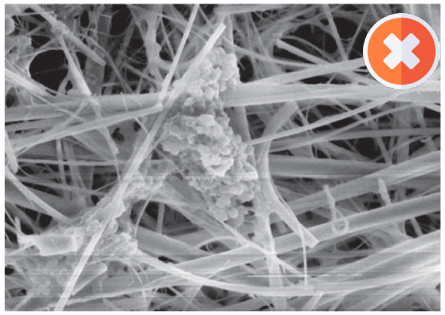
Carrier for spores



Clean spores on a stainless steel carrier



Encrustation of the spore layer on a stainless steel carrier



Clumped spores on paper carrier

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.

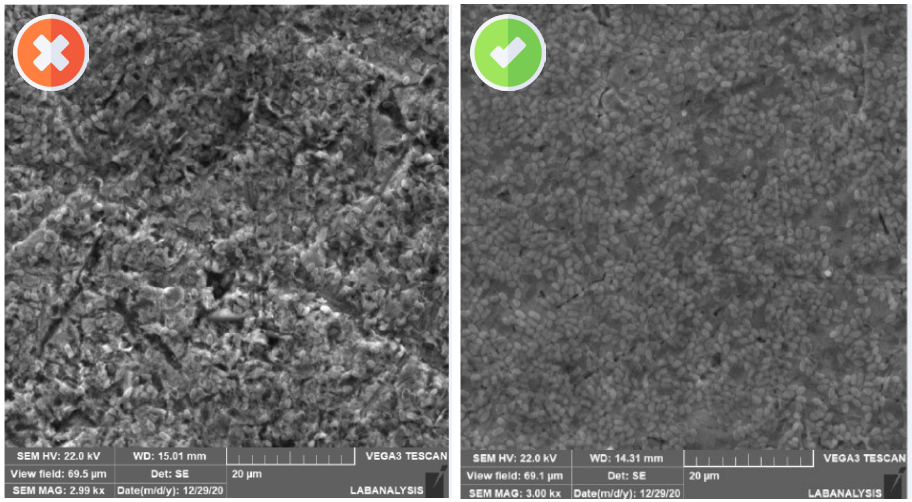
USP 43 ⟨1229.5⟩ Biological Indicators For Sterilization

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.

Particular attention is given to areas that pose problems relative to the concentration of the agent. A larger number of BIs may be required in isolators that are heavily loaded with equipment and materials. The ability of the process to reproducibly deliver a greater than three-log kill is confirmed in three consecutive validation studies.

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

BIs & H_2O_2 Process Validation

- As H_2O_2 vapor has poor penetration abilities, it is crucial to select a BI that has been designed for use in surface decontamination processes.

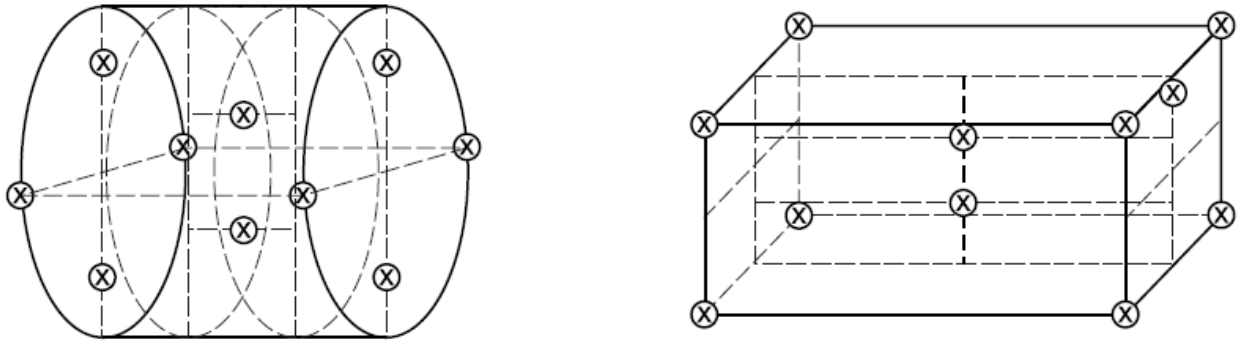
- Currently there are **no standards for the manufacture and qualification of these BIs**

ISO 11138-6 *Sterilization of health care products - Biological indicators Part 6: Biological indicators for hydrogen peroxide sterilization processes*
Status: Under development

- **PDA Technical Report No. 51** provides guidance on "*Biological Indicators for Gas and Vapor – Phase Decontamination Processes: Specification, Manufacture, Control and Use*".

BIs & H_2O_2 Process Validation

The BIs should be evenly **distributed throughout the camber and the load**



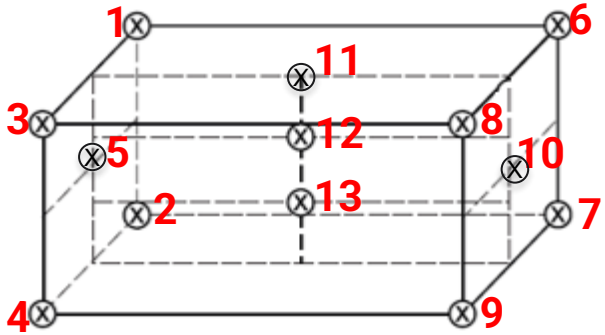
Key

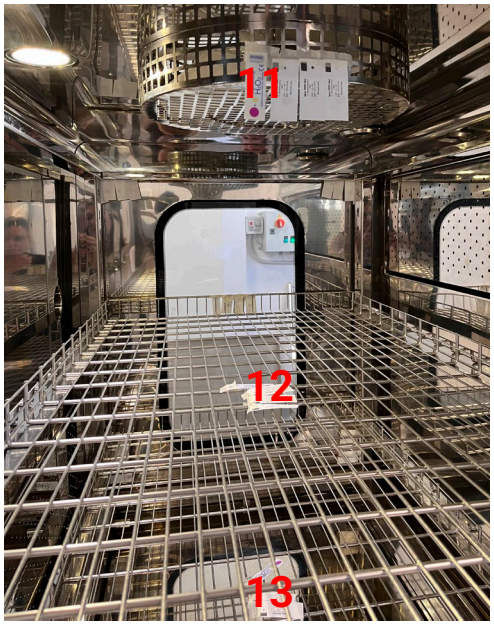
X locations for BIs, PCDs or temperature sensors

NOTE The diagram shows examples for locations in typical chamber usable space. Different chamber sizes can require more or fewer locations however a similar distribution pattern can be used.

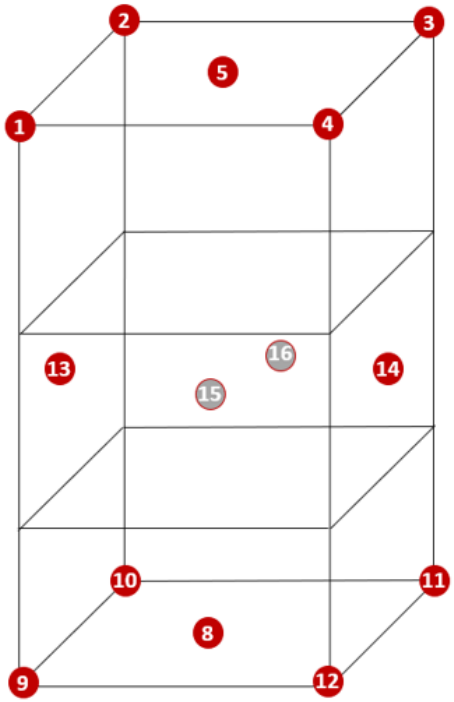
ISO 22442 – Annex K (informative) Recommended validation test procedures

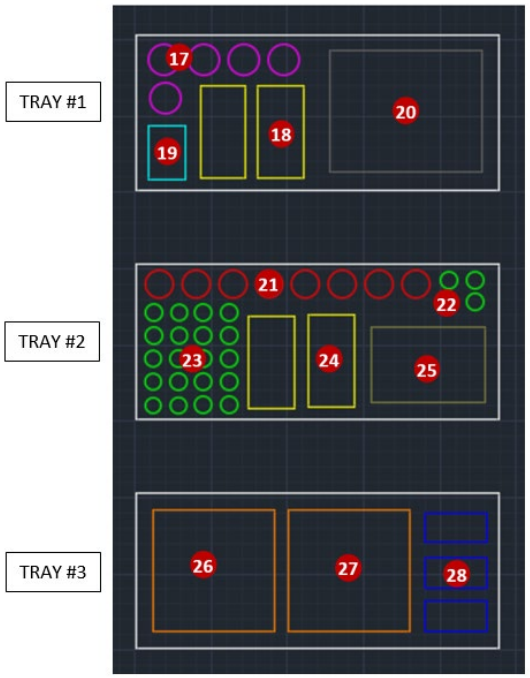
Practical example: CIs-BIs chamber mapping





Chamber & Load: CIs-BIs location





Tray #	Material Description	Quantity
1	Bottle with sanitizer	5
	Tank with sanitizer	1
	Sterile disposable covers	2
	Sterile waste bags	1
2	Petri plates 55 mm	24
	Petri plates 90 mm	8
	Sterile disposable covers	2
	ICR – Swab bag	1
3	Sterile wipes box	4
	Sterile marker	3

BI placement & handling

Proper BI Placement During VHP Decontamination Cycles - Kurt McCauley Spore News MesaLabs Volume 9, No. 5

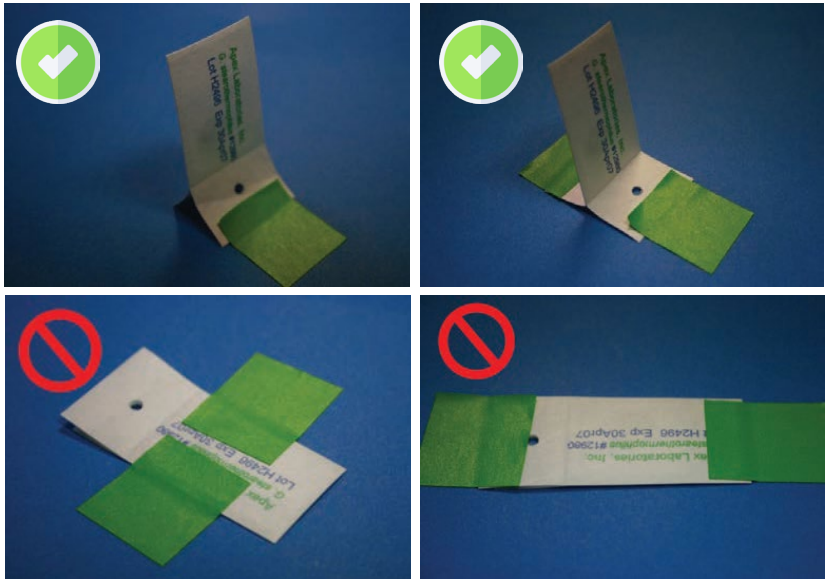
The printed side should always face towards the vapor flow, and at no time should it be obstructed. Ideally, the BI should be positioned so that the flow of vapor can pass by both sides of the envelope

A hole has been provided in the envelope so the BI can be hung from a fixture on the wall, ceiling or other structure. BI units can be hung individually or in multiples.

BI placement & handling

Proper BI Placement During VHP Decontamination Cycles - Kurt McCauley Spore News MesaLabs Volume 9, No. 5

Place the tape on the peel flaps, do not cover the spore location



BI placement & handling

Proper BI Placement During VHP Decontamination Cycles - Kurt McCauley Spore News MesaLabs Volume 9, No. 5

Placing marks on the pocket of the envelope should be avoided. Certain types of ink may also catalyze hydrogen peroxide and should be avoided completely.

BI placement & handling

Proper BI Placement During VHP Decontamination Cycles - Kurt McCauley Spore News MesaLabs Volume 9, No. 5

Due to poor vapor flow, placing BIs into or underneath bottles or other containers is not recommended

Unexpected positive BI: case study

Is the BI fault or our cycle is not the right one?

1. Re-run the cycle
2. BIs properly produced, stored and placed

Unexpected positive Bis

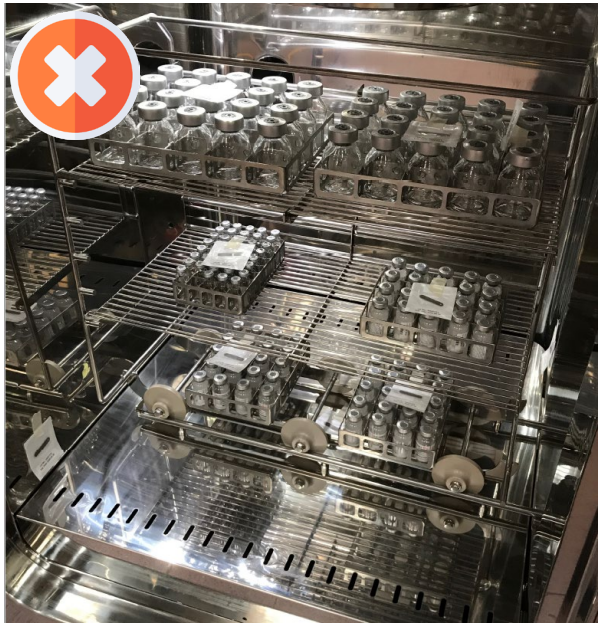
Despite the growth of two BIs at that location, we can still prove that a 6 SLR was achieved at that specific test location

This SLR value is what guidelines and/or rules require about decontamination

Unexpected positive BI: case study

*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



Triplicate BIs allow evaluating decontamination with a **statistical analysis**!

Unexpected positive BI

*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

Triplicate BIs allow evaluating decontamination with a **statistical analysis**!

Unexpected positive BI

If we used **1 BI/location** …

… we might have

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

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**

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 (3/1) = 1.09

If $N_0 = 2.8 \times 10^6$

SLR = Log_{10} (2.8 x 10^6) - Log_{10} 1.09

SLR = 6.447 – 0.0408 = **6.4062**

Unexpected positive BI: case study

Before saying that the cycle has failed you should ask yourself…

- Was the BI correctly manipulated and stored?
- Is the BI not a good one («rogue» BI)?
- What is the microbiological result that I need (SLR)?
- Did we routinely observe multiple positive BIs at multiple locations?

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**.

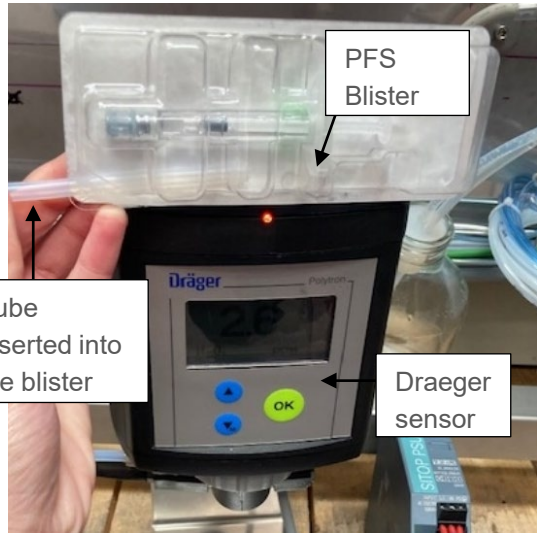
USP 43 ⟨1208⟩ Sterility Testing—Validation of Isolator Systems

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.

Product H_2O_2 residual concentration

Measuring the H_2O_2 concentration in the product



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

Materials compatibility

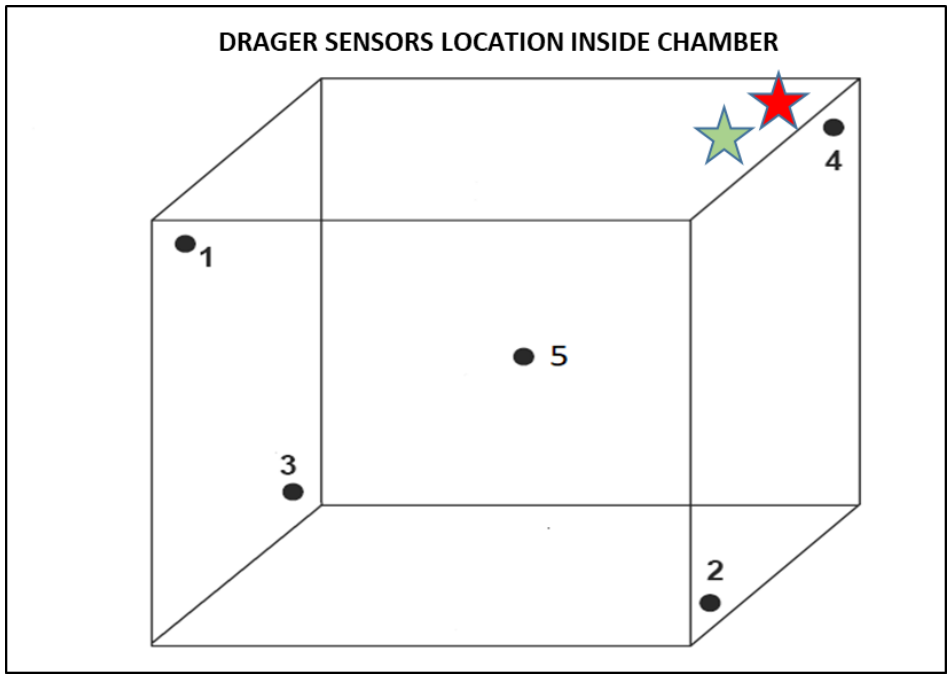
- **Deterioration of elasticity-** or strength flexibility, visible damages
- **Absorption** – Degassing time
- **Penetration** – Product damage
- **Microbiological effectiveness** – Surface finish

Conclusions

- Hydrogen peroxide is a decontaminant, active on surfaces
- Its process is a low-temperature one, useful for heat-sensitive loads
- It might provide a SAL 10^{-6} only on surfaces
- 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

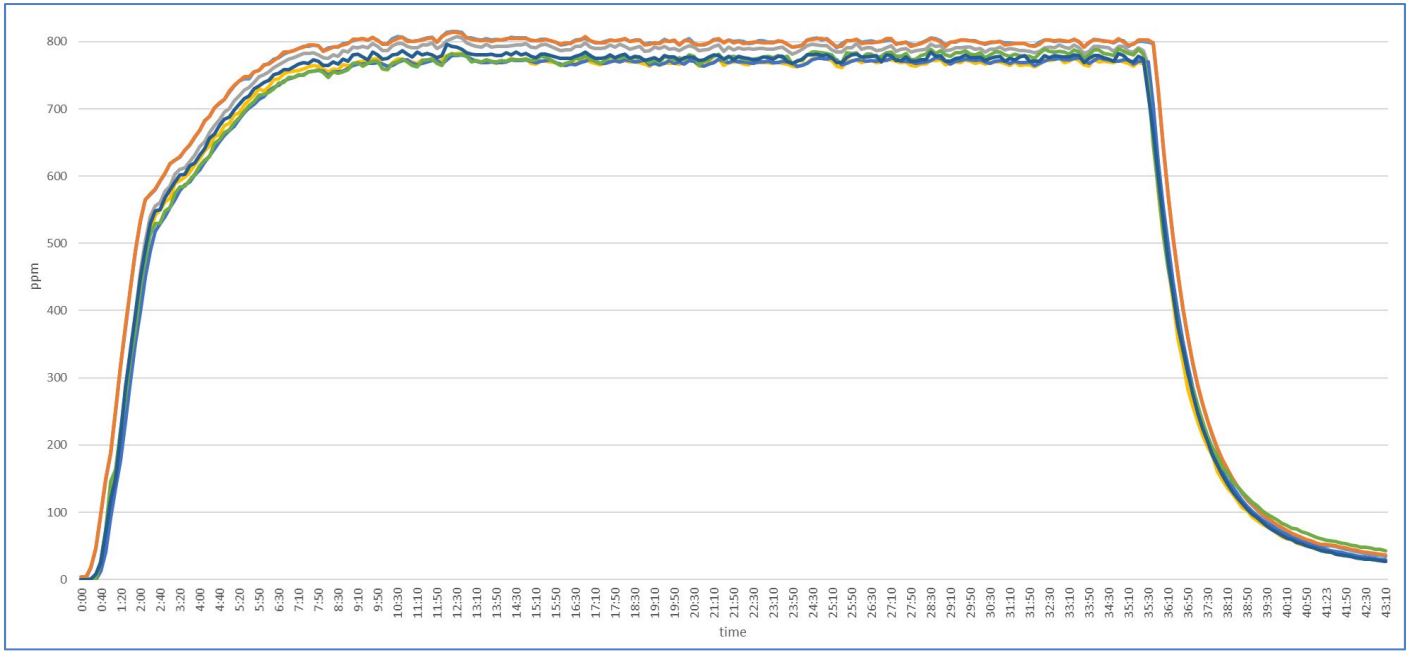
Vaporized H_2O_2 distribution



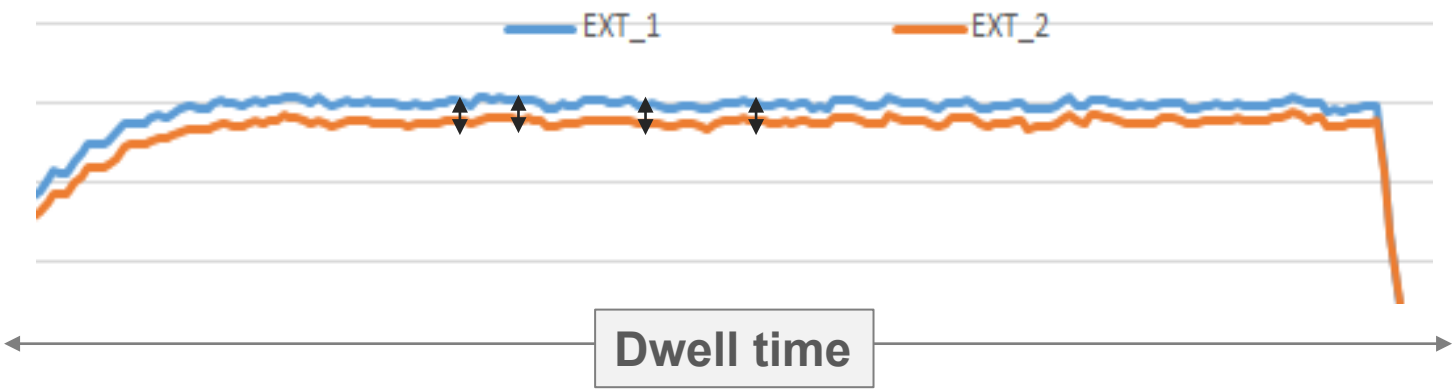
EXT 1 and EXT 2 are located in the ceiling of the chamber; LOG1, 2, 3, 4, 5, 6, inside the chamber.

EXT1 controls the process

Vaporized H_2O_2 empty chamber distribution

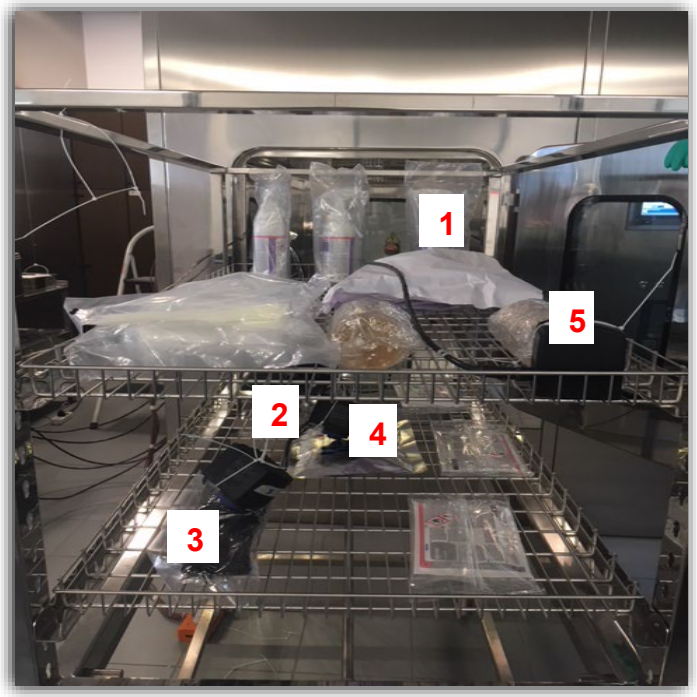


Vaporized H_2O_2 distribution



For each time interval during the decontamination *phase*, we calculate the max difference between the sensors considered

Load and sensors



Several items with different materials

1 – Plastic bag for Klercide
2 – Stainless steel box
3 – Stoppers bag
4 – Aluminium bag
5 – Petri Plates

Dräger sensors (LOG1, 2, 3, 4, 5) are located next to the sample to investigate; EXT1 and EXT2 are located in the ceiling

Vaporized H_2O_2 distribution