Hydrogen peroxide decontamination

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Fedegari group

Hydrogen peroxide session: program of the day

OVERVIEW OF THE MAIN TOPICS TREATED

PRACTICAL SESSION

- *DRY CYCLE*
- *HYDROGEN PEROXIDE MEASUREMENT*

Hydrogen peroxide session: main topics

- Hydrogen peroxide definition
- Regulation
- Application fields
- Decontamination target
- Decontamination technologies:

  VPHP (dry or wet cycle), DRY FOG
- Sporicidal Concentration
- Materials
- Packaging Integrity verification

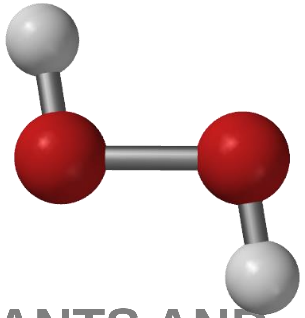
- Sensors
- Safety
- Catalyzer
- Example of dry cycle
- Hydrogen peroxide mapping
- Biological indicators and D-value
- SLR calculation

Hydrogen peroxide definition

PubChem

Hydrogen peroxide is a strong oxidizing agent used in aqueous solution as a ripening agent, bleach, and topical anti-infective. It is relatively unstable and solutions deteriorate over time unless stabilized by the addition of acetanilide or similar organic materials.

Hydrogen peroxide classification



USP NF 2021, General Chapter ⟨1072⟩ - DISINFECTANTS AND ANTISEPTICS

Chemical Entity	Classification	Example
Hydrogen peroxide	Vapor phase sterilant, liquid sporicidal agent, antiseptic	4 µg per g H_2O_2 vapor, 10%–25% solution, 3% solution

Hydrogen peroxide: European regulation

European Medicines Agency (EMA)

Eudralex Volume 4, EU GMP, Annex 1, Manufacture of Sterile medicinal products, draft

5.34 Fumigation or vapour disinfection of clean areas such as Vapour Hydrogen Peroxide (VHP) may be useful for reducing microbiological contamination in inaccessible places.

5.19 For open, positive pressure isolators or closed isolators with decontamination by a sporicidal agent, the surrounding area should correspond to a minimum of grade D.

Hydrogen peroxide: US regulation

USP - NF 2021, General Chapter ⟨1208⟩ STERILITY TESTING; VALIDATION OF ISOLATOR SYSTEMS

Among the chemicals that have been used to treat isolators are peracetic acid, chlorine dioxide, ozone, and hydrogen peroxide; each has different requirements for exposure conditions and process control.

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 rooms (ex cleanroom) and of aseptic processing systems (ex. isolators)

Decontamination: when?

Decontamination unit (Pass Box)

Class
C, D

Class
A, B

Clean
room

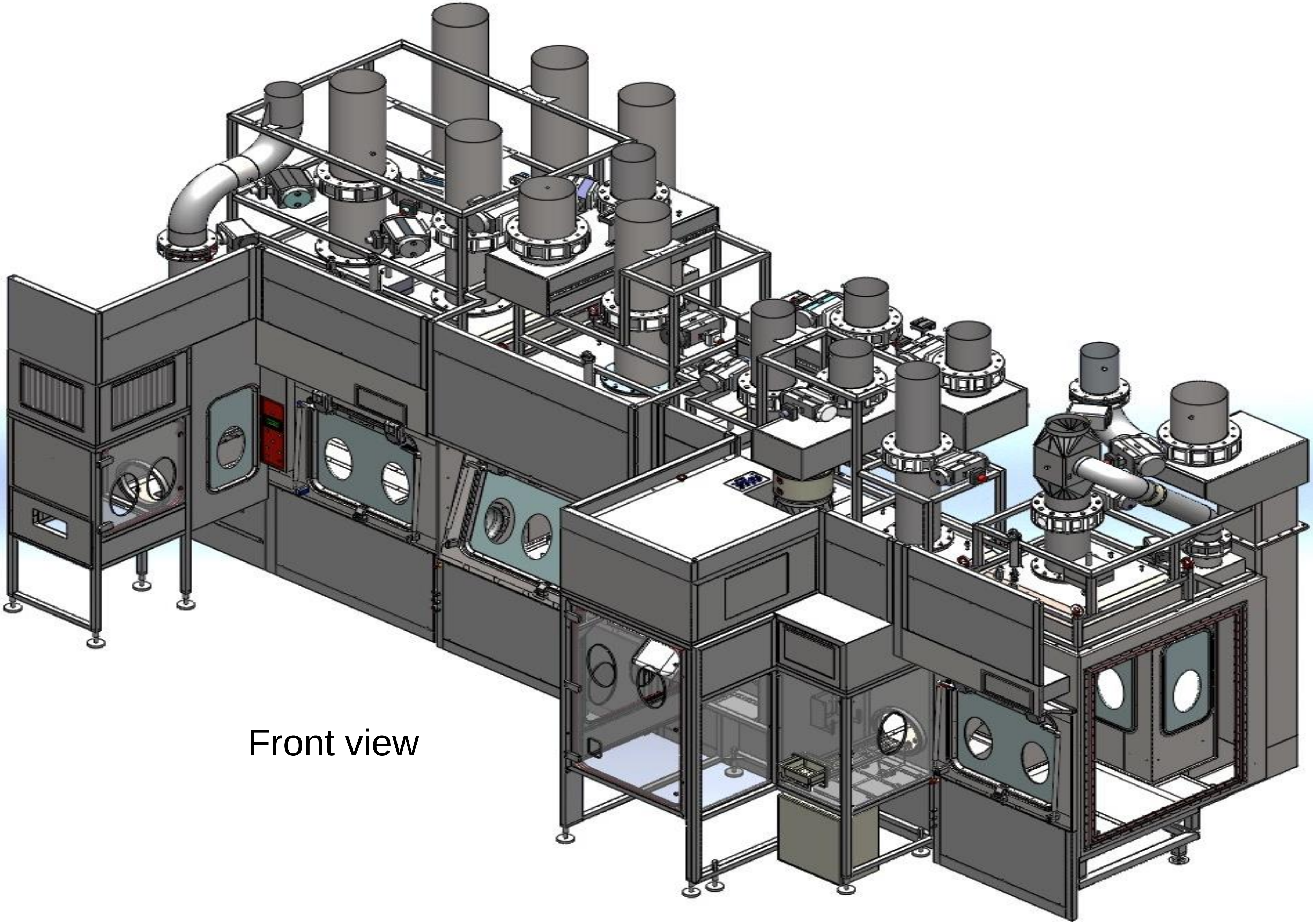
✓ Heat sensitive product

✓ Waste

Decontamination: when?

An isolator at…

…….Fedegari FAT area



Front view

Decontamination technologies

The most widespread technologies

- Vapor phase hydrogen peroxide

  WET CYCLE

  DRY CYCLE

- Dry fog

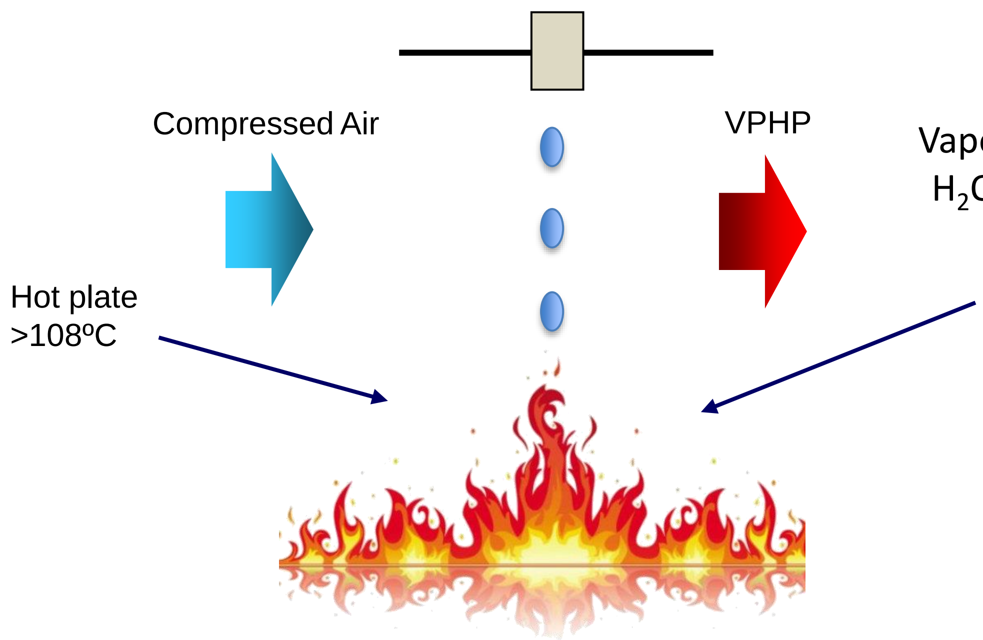
VAPOR PHASE HYDROGEN PEROXIDE

Vapor Phase Hydrogen Peroxide (VPHP):

how is it produced?

VPHP Production: Flash Vaporization

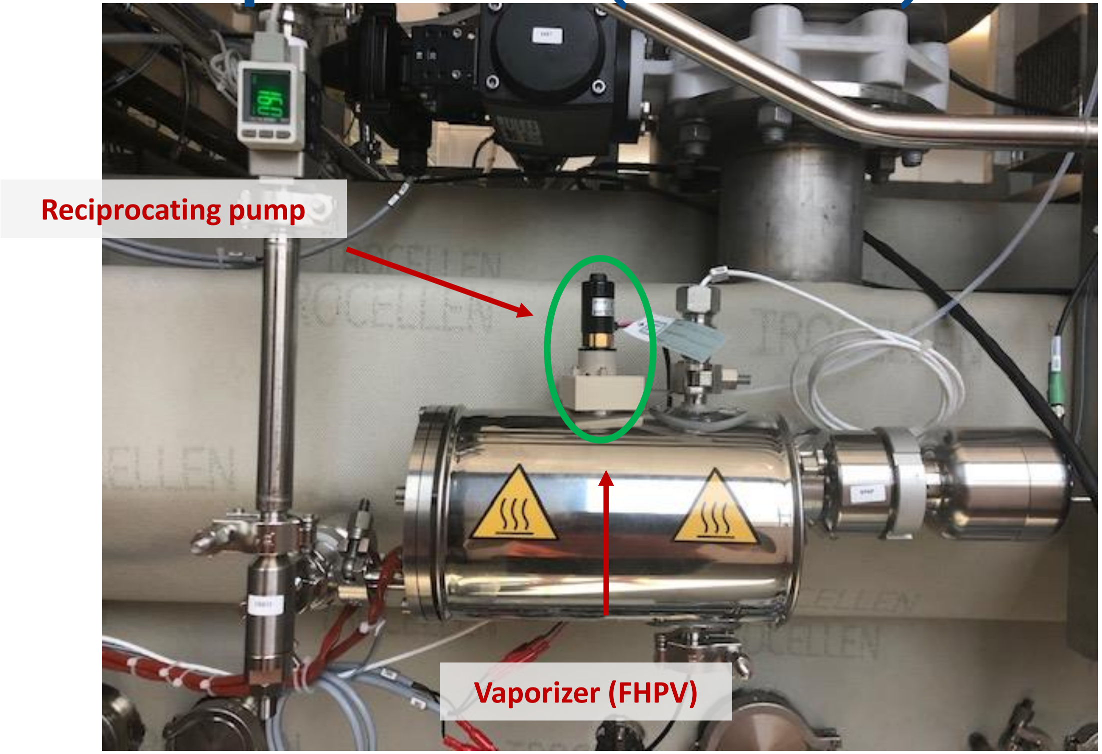
Liquid mixture:
H_2O_2/H_2O 35/65 (w/w%)



Vapor mixture:
H_2O_2/H_2O 35/65 (w/w%)

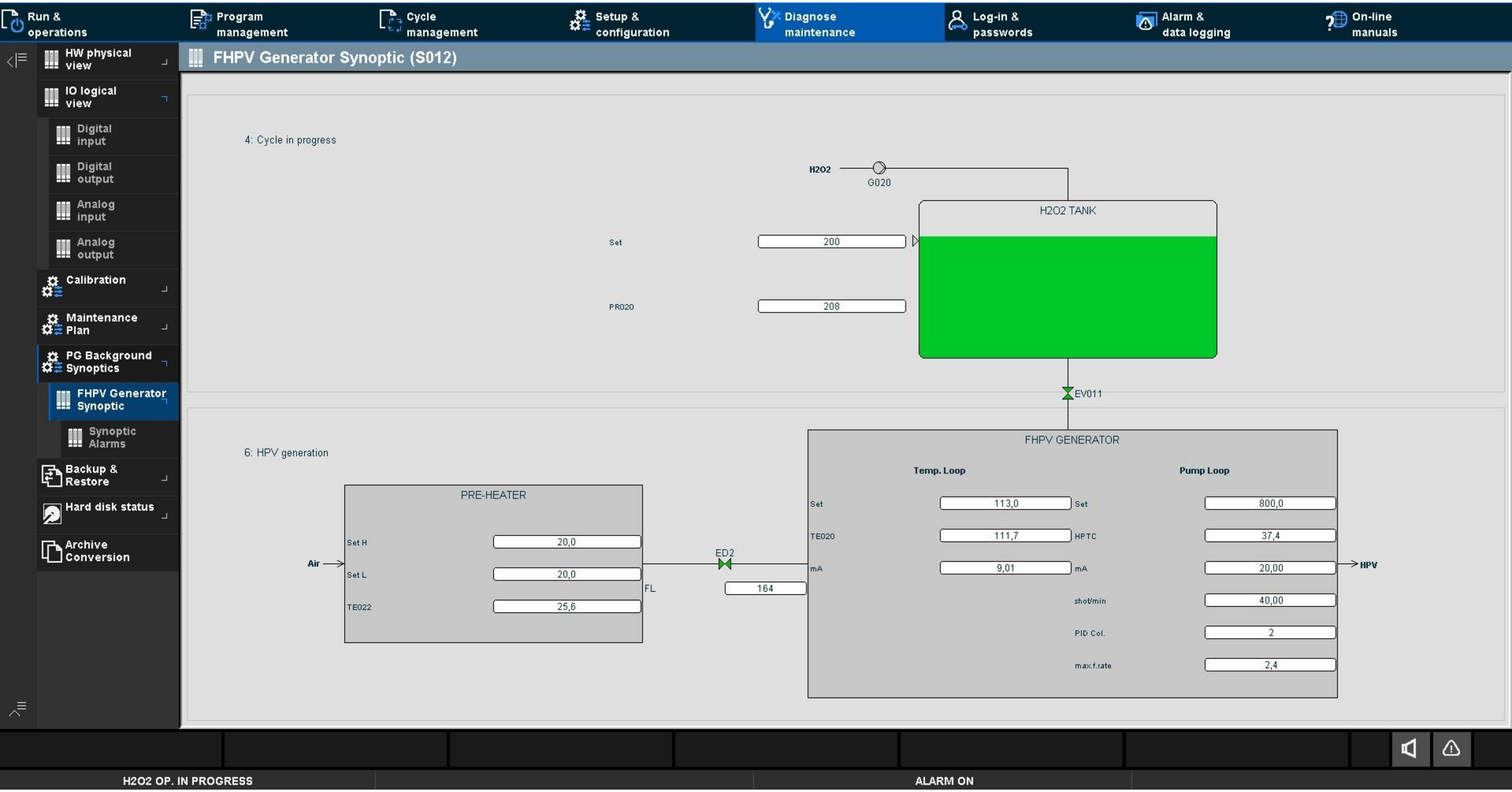
All the **components** are **vaporized simultaneously**

Fedegari Hydrogen Peroxide Vaporizer (FHPV)



It produces vaporized hydrogen peroxide from the H_2O_2/H_2O liquid mixture

FHPV generator synoptic



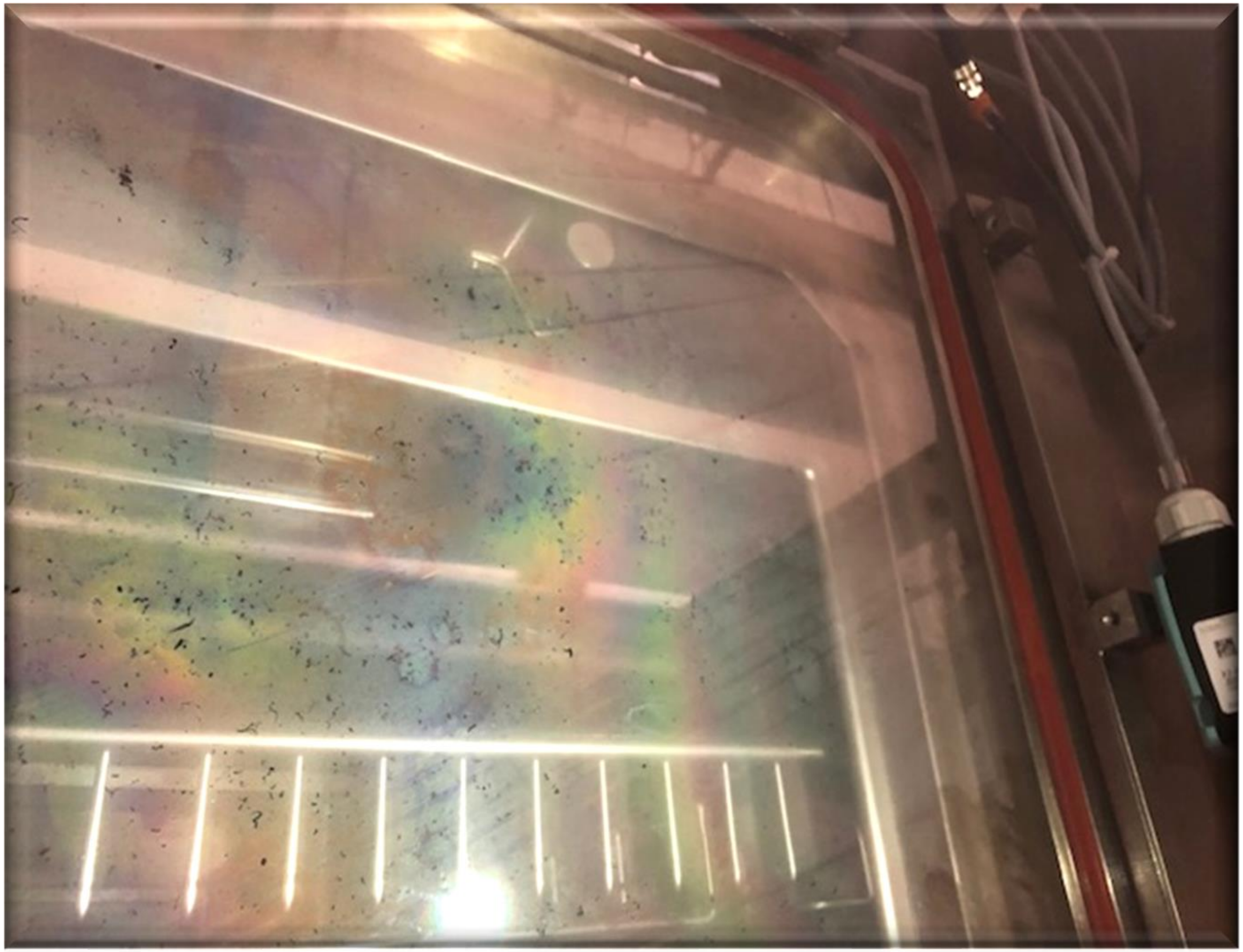
VPHP: wet and dry cycle

Wet cycle

- *effective in a short time lapse*
- more penetrating
- wet load
- *long cycle*
- more agressive on materials
- concentration not well controlled

Dry cycle

- *effective in a longer time lapse*
- less penetrating
- dry load
- *shorter cycle*
- less agressive on materials
- concentration, well controlled

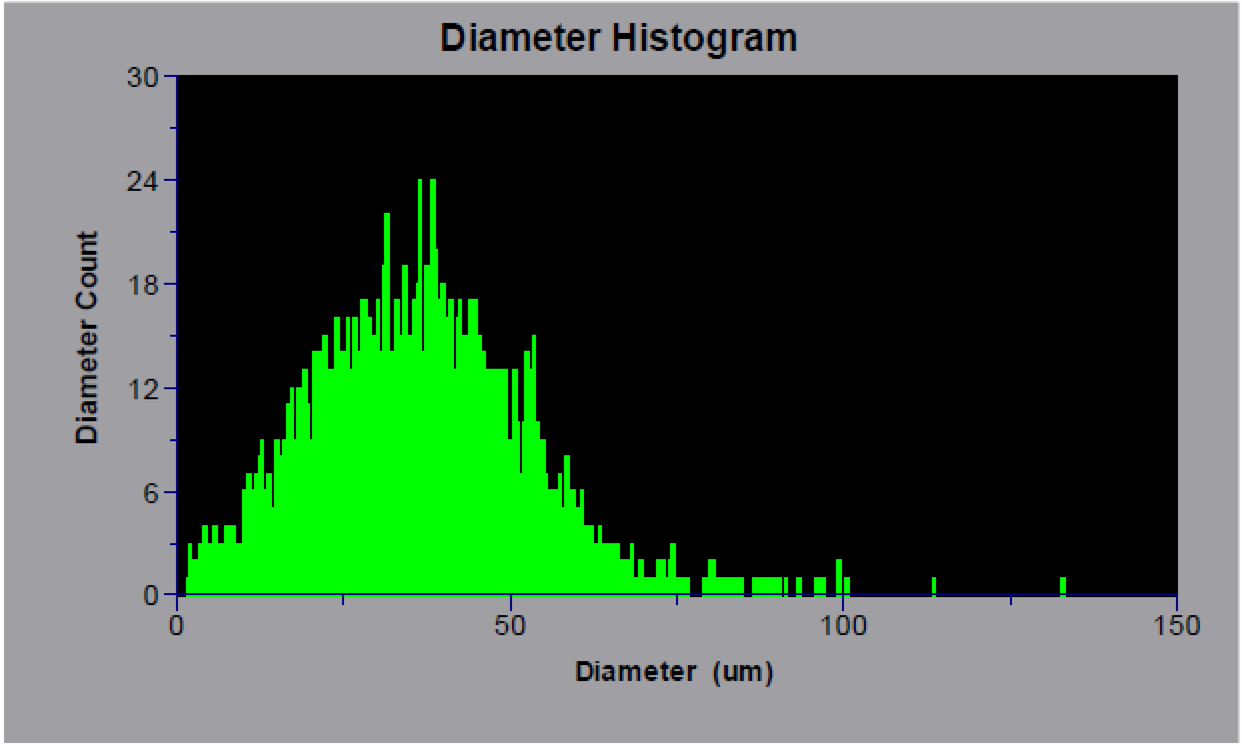


Wet cycle
visible condensation to naked eye

Dry fog

- Penetrating
- Control based on reading RH/ injecting grams
- No reliable concentration control

Dry fog



Droplets dimension distribution

Hydrogen peroxide concentration

«STANDARD» PERCENTAGE

35%

SAFETY DATA SHEET
Hydrogen Peroxide 35% Durox® LRA

SDS # : 7722-84-1-35-27
Revision date: 2015-05-08
Format: NA
Version 1

PeroxyChem

1. PRODUCT AND COMPANY IDENTIFICATION

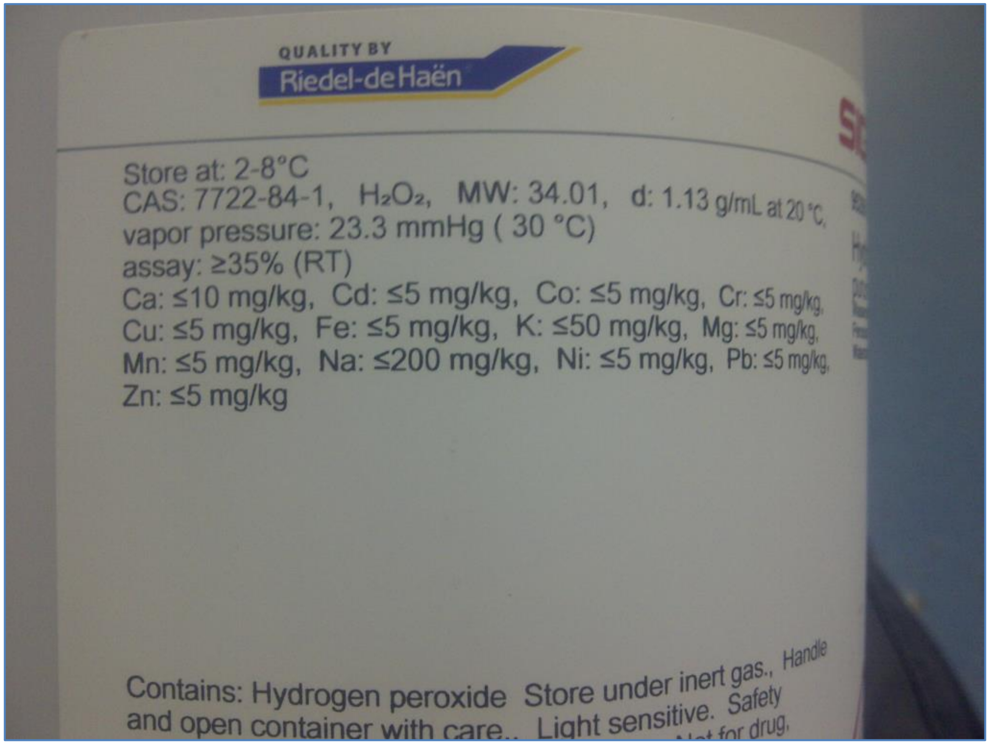
Product Identifier

Product Name	Hydrogen Peroxide 35% Durox® LRA

Other means of identification

CAS-No	7722-84-1

Hydrogen peroxide concentration



Hydrogen peroxide concentration

SUMMARY

On the test item "Metallic device in VHP system", analyses have been performed for the verification of the possible presence of residues. In particular, the presence of typical inorganic H_2O_2 stabilizers were investigated.

In fact the device underwent:

- Determination of silicon/silica (performed on a washing aqueous solution)
- Determination of phosphates, nitrates, sulphates (performed on a washing aqueous solution)

INTRODUCTION

On behalf of FEDEGARI AUTOCLAVI SpA has been performed a study for the verification of the possible presence of residues on the test item.

Hydrogen peroxide residues

4. **SILICA**

Silicon detected using ICP technique (see 3. Silicon paragraph) is silicon dissolved in the solution. Presumably all the silicon detected with this technique is related to the presence of dissolved silicates in the washing solution. Metallic silicon is not detectable not being dissolved.
The results obtained for silicon will then be processed so as to express the content of silicates in solution expressed as silica equivalent.

RESULTS
All the results are related to the analytes present in the washing solution (400ml).

1. **NITRATES, SULPHATES and PHOSPHATES**

Nitrates (mg/L)	Sulphates (mg/L)	Phosphates (mg/L)
5.47	21.34	74.82

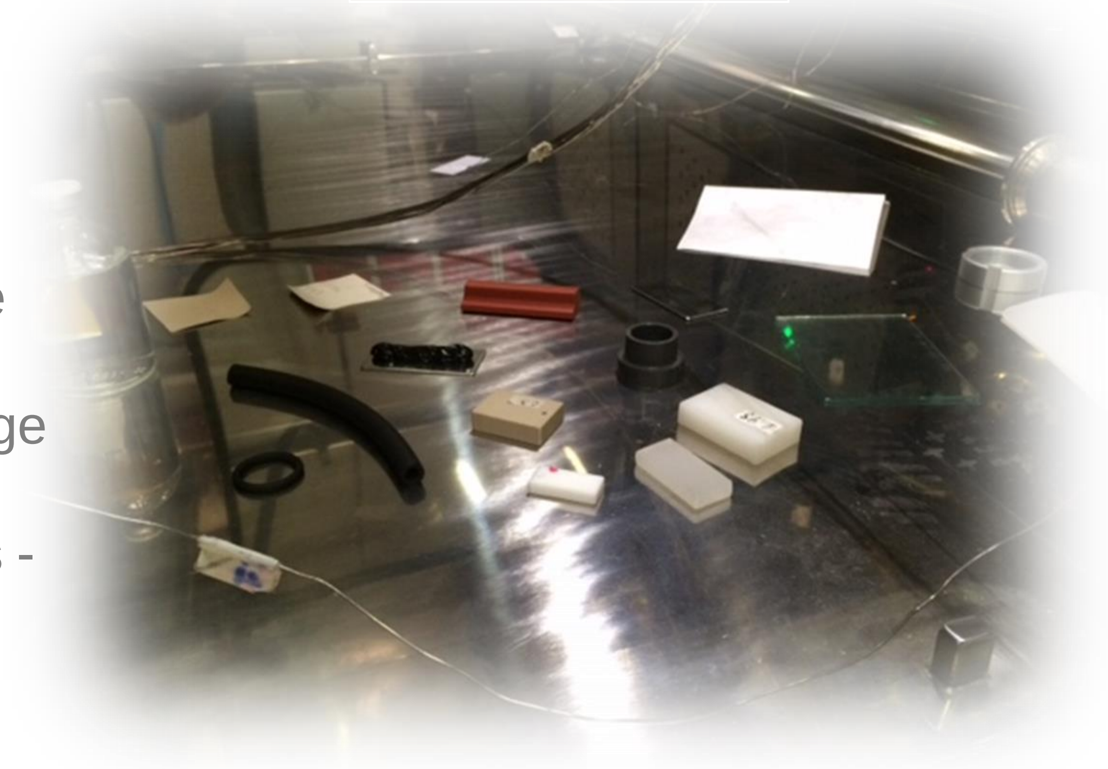
2. **SILICON and SILICA**

Silicon (mg/L)	Silica (mg/L)
0.157	0.336

Materials compatibility

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

MACHINE PARTS



Materials compatibility

EXAMPLES OF CRITICAL LOADS

Package integrity verification

USP – NF 2021, GENERAL CHAPTER ⟨1208⟩ STERILITY TESTING; VALIDATION OF ISOLATOR SYSTEMS: *PACKAGE INTEGRITY VERIFICATION*

Some materials are adversely affected which by decontaminating agents, can result in inhibition of microbial growth. Of concern is the penetration of decontaminating agents into product containers.

FALSE NEGATIVES

Material compatibility: SEM investigation A case study

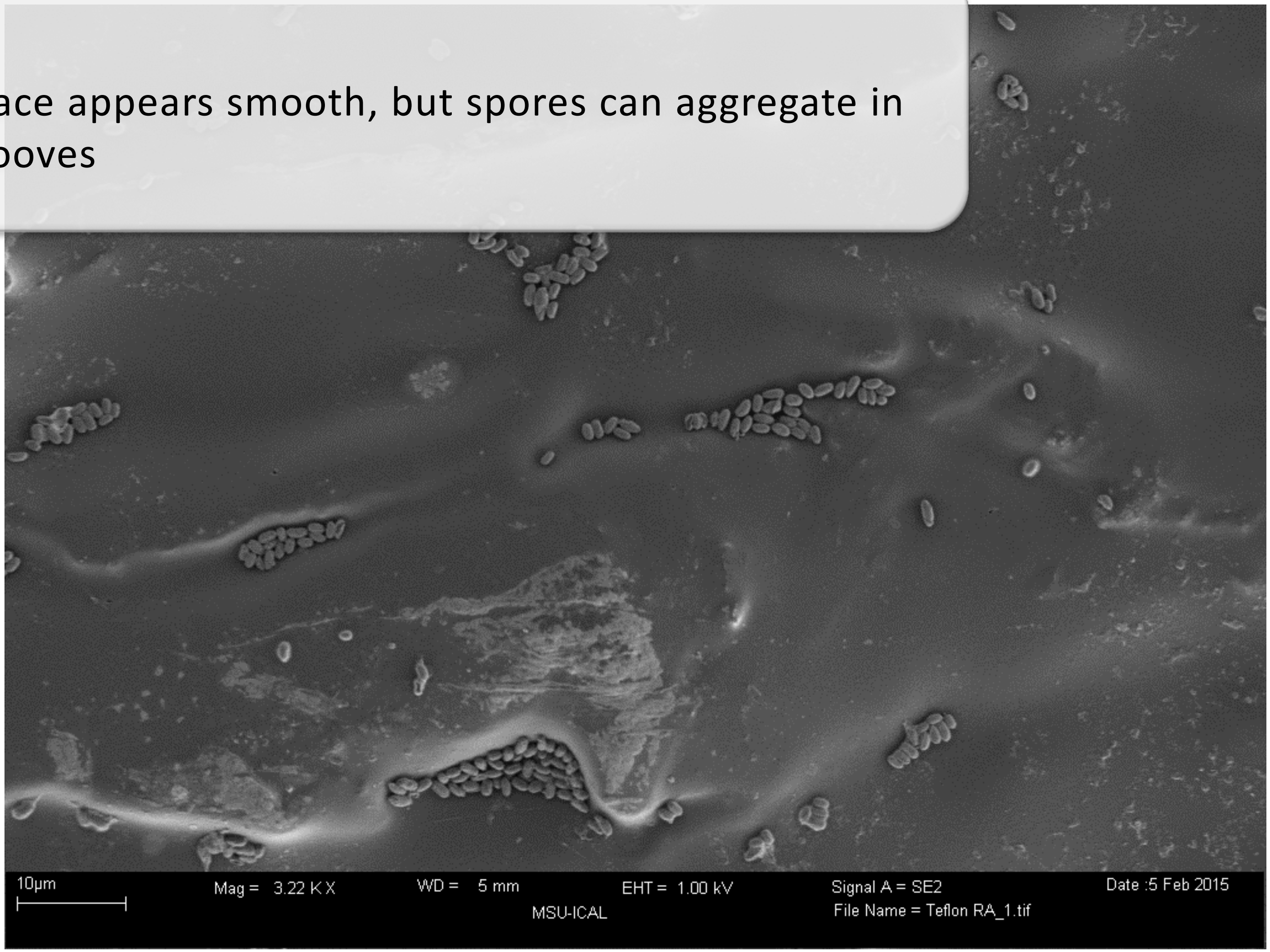
Different materials were inoculated with 10^6 *Geobacillus Stearothermophilus* spores and analyzed by scanning electron microscope (SEM)

Spore monolayers → **Good substrate** for decontamination

Spore clusters
Spores in grooves or cavities → **Bad substrate** for decontamination

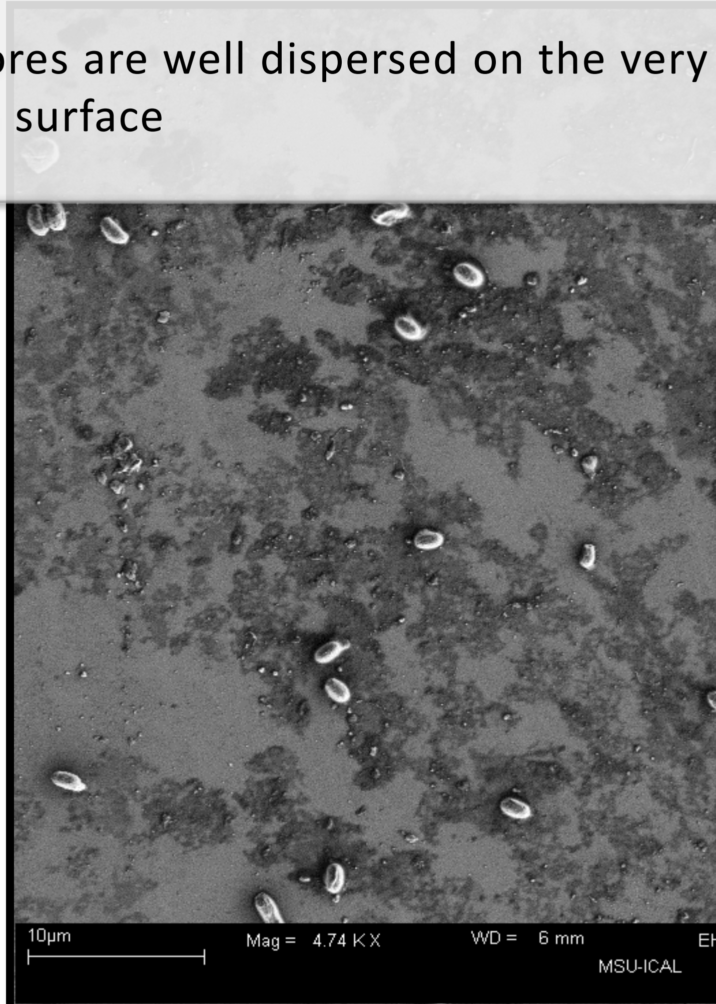
Teflon

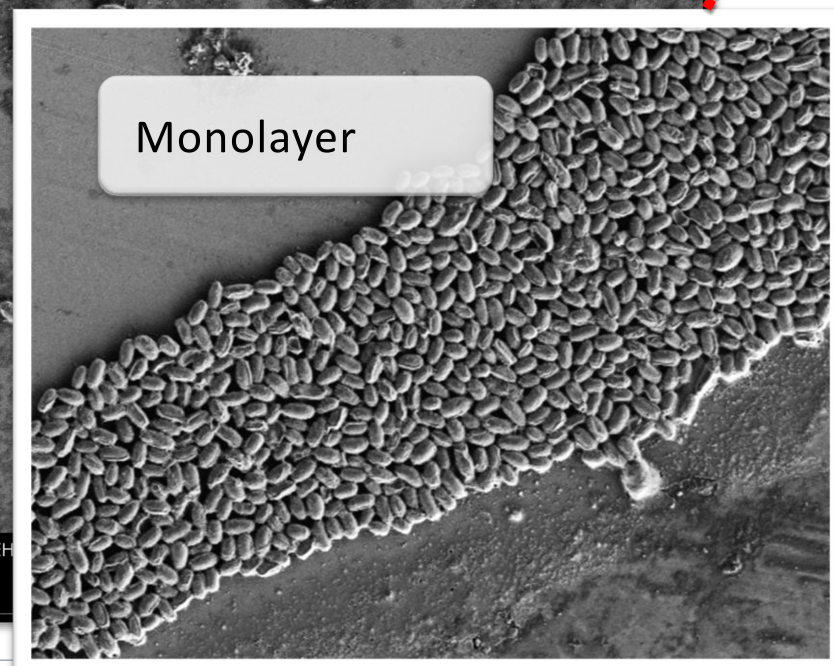
The surface appears smooth, but spores can aggregate in some grooves



Glass

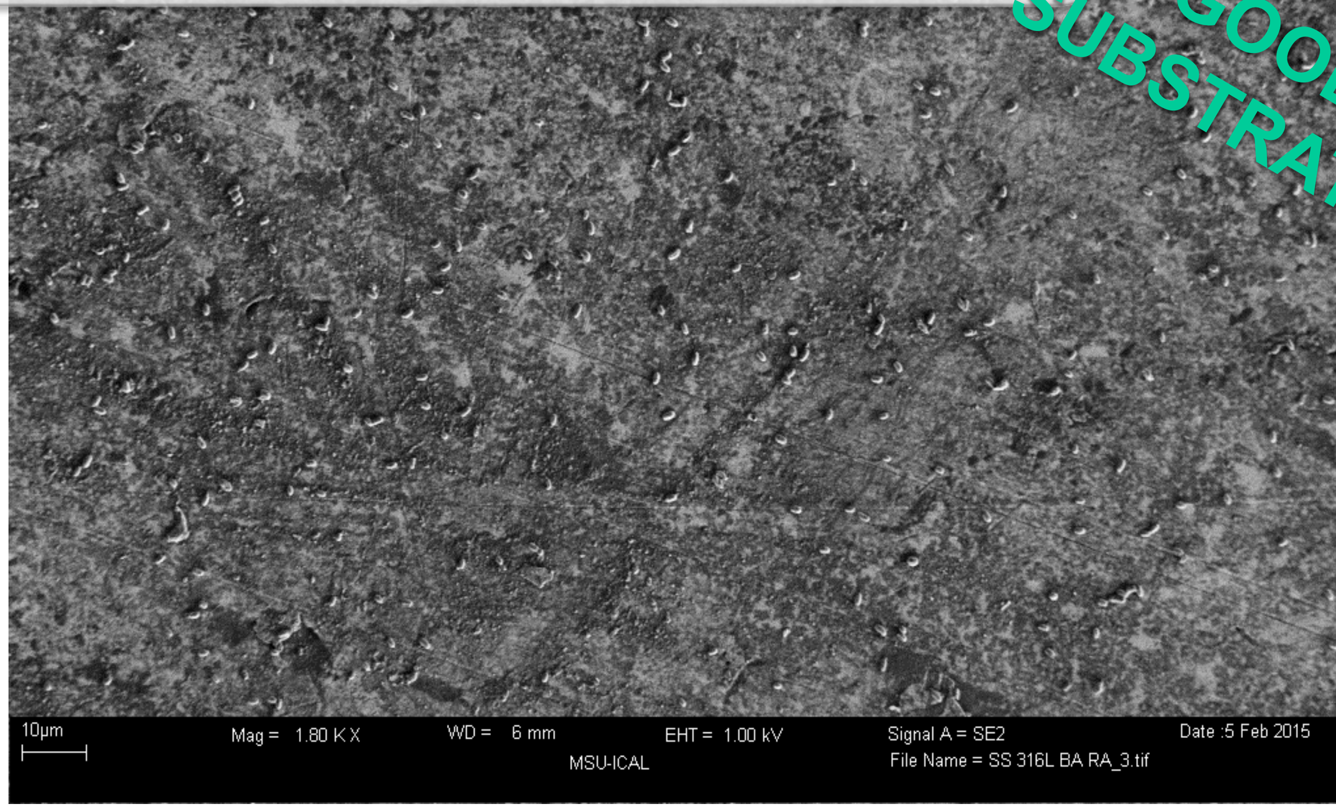
The spores are well dispersed on the very smooth surface





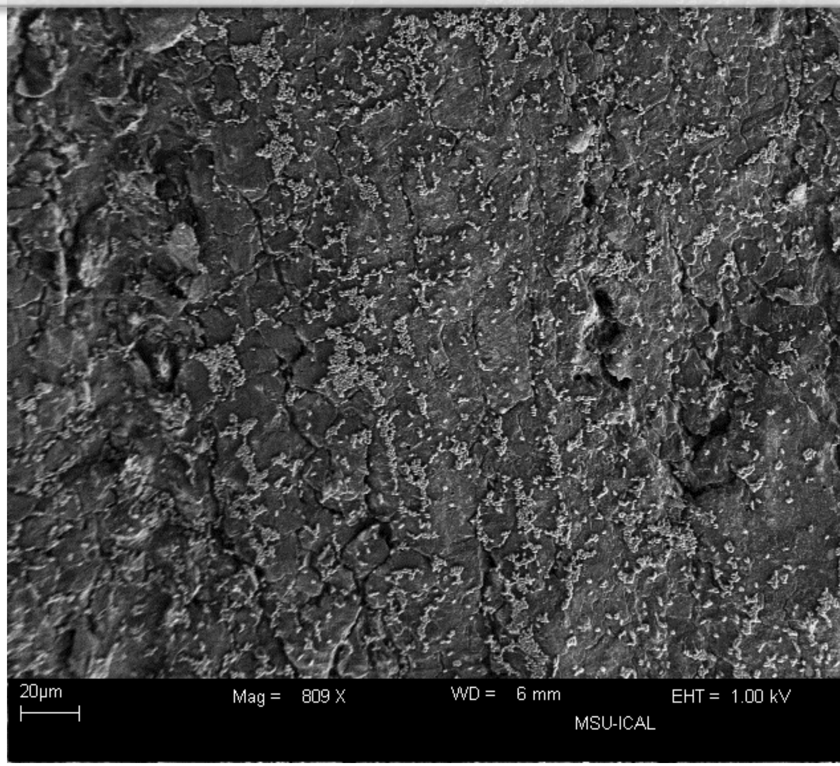
Stainless steel 316 L

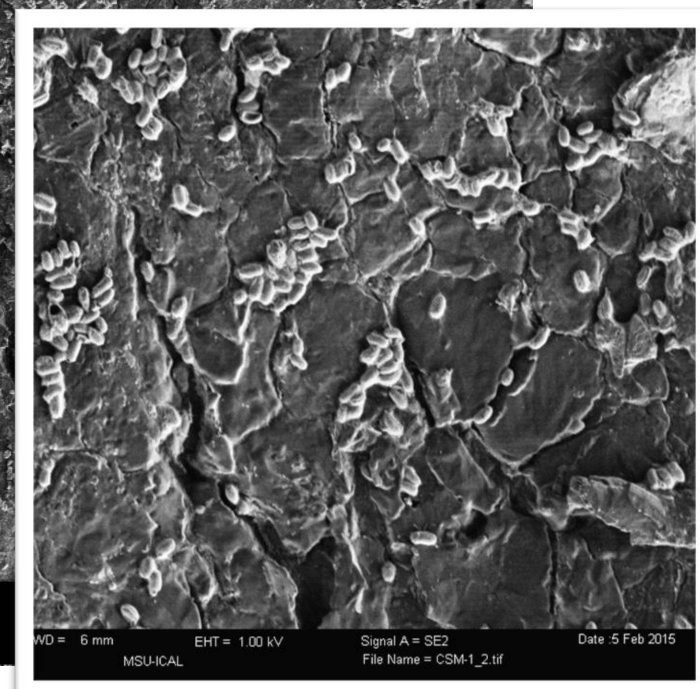
The spores show a good dispersion on the relatively smooth surface



CSM (Hypalon™)

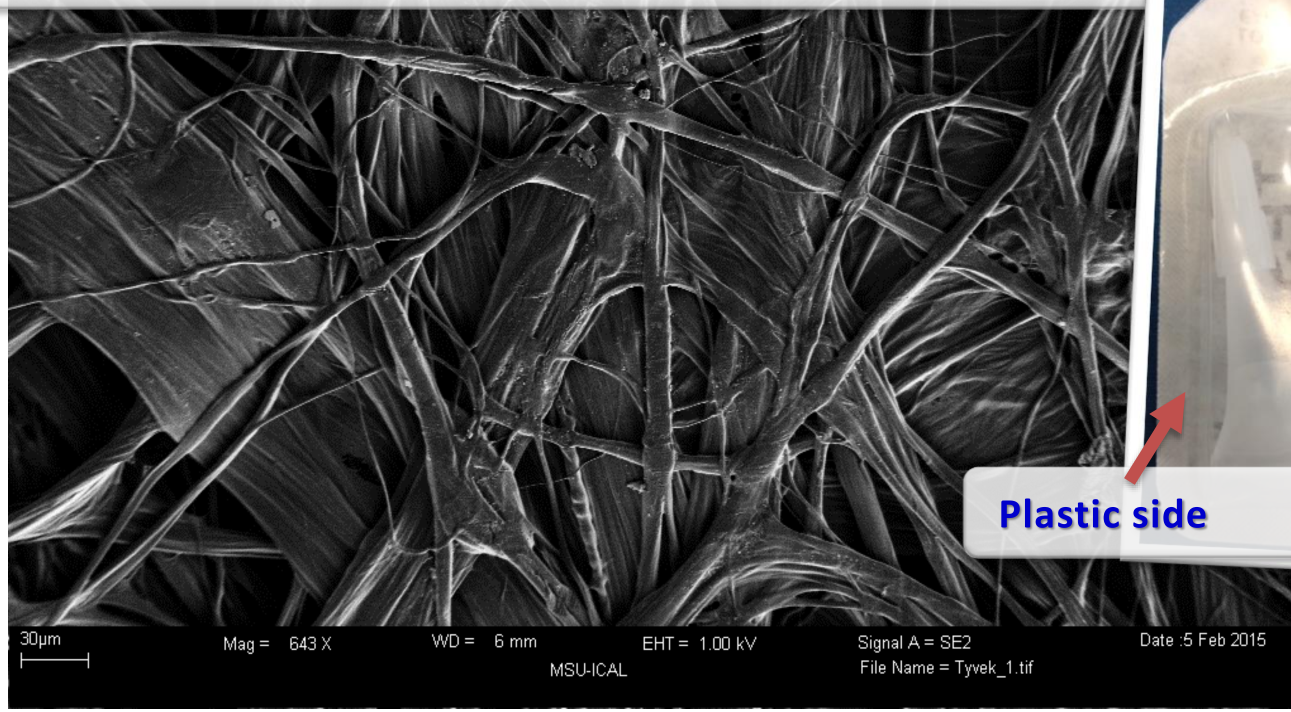
The spores are well dispersed, but they show slight clumping in some areas (relatively smooth surface)





Tyvek®

The spores are not visible, it is likely that they fell into cavities. However H_2O_2 vapors can easily penetrate through it.



Hydrogen peroxide detection

Dräger

Electrochemical sensor

Measuring electrode: $H_2O_2 \longrightarrow O_2 + 2\,H^+ + 2\,e^-$

Counter electrode: $1/2\,O_2 + 2\,H^+ + 2\,e^- \longrightarrow H_2O$

Hydrogen peroxide detection

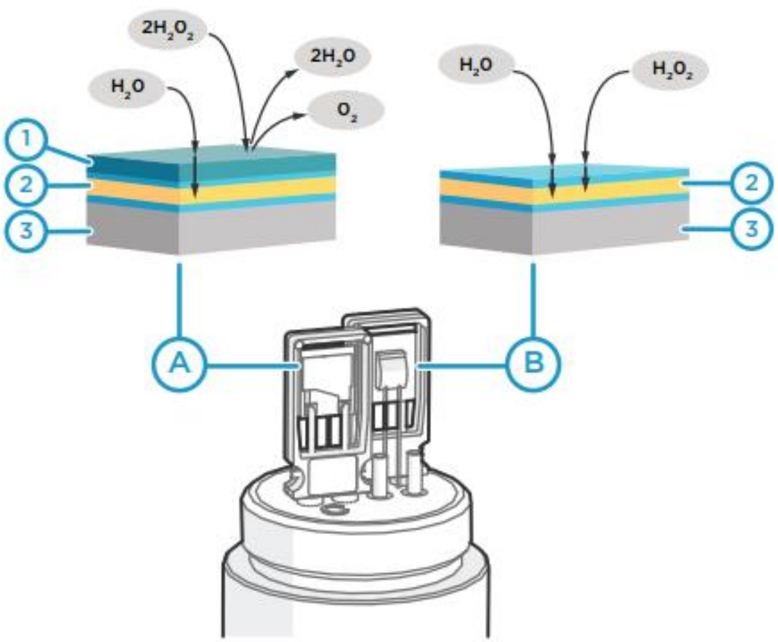


Figure 2 Operating principle of PEROXCAP measurement

- A HUMICAP sensor with a catalytic layer (under the probe filter). This sensor only senses water vapor.
- B HUMICAP sensor without a catalytic layer (under the probe filter). This sensor senses the air mixture with both hydrogen peroxide vapor and water vapor.
- 1 Catalytic layer over the thin film polymer. This layer catalyzes hydrogen peroxide into water and oxygen and prevents it from entering the sensing polymer.
- 2 Thin film polymer between two electrodes.
- 3 Alumina substrate.

VAISALA

Hydrogen peroxide detection

Cavity ring-down spectroscopy (CRDS)

Lower Detection Limit: < 3ppb

PDA Parenteral Drug Association

Decontamination cycle: its structure

The decontamination process with VPHP (vaporized phase hydrogen peroxide) can be executed with two different decontamination mechanisms: dry and wet. *The difference between the two processes lies in the concentration of VPHP in the chamber during the injection phase:*

DRY

- for the dry cycle, the concentration of H_2O_2 is below the dew point,

WET

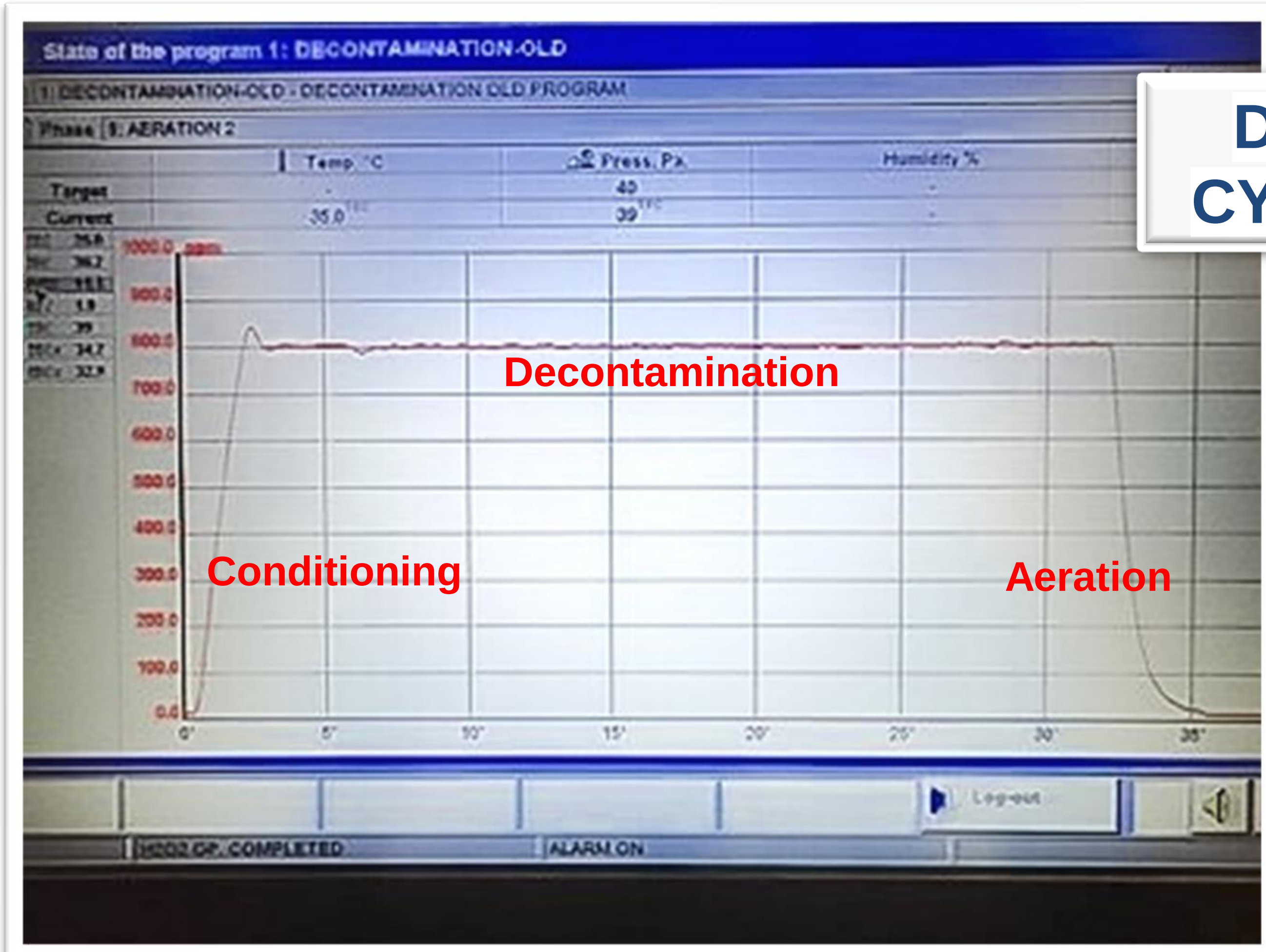
- in the wet cycle, a quantity of vaporised hydrogen peroxide is injected to saturate the air in the chamber.

Decontamination cycle: its structure

Both cycles are generally constituted by four phases:

- Preparation
- Conditioning
- Decontamination
- Aeration

Decontamination cycle: its structure

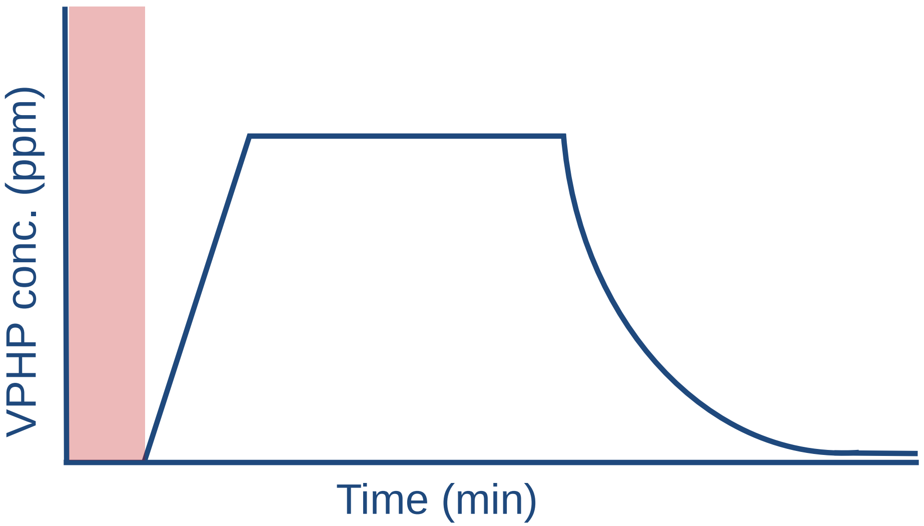


DRY CYCLE

Decontamination cycle: its structure

1. Preparation

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

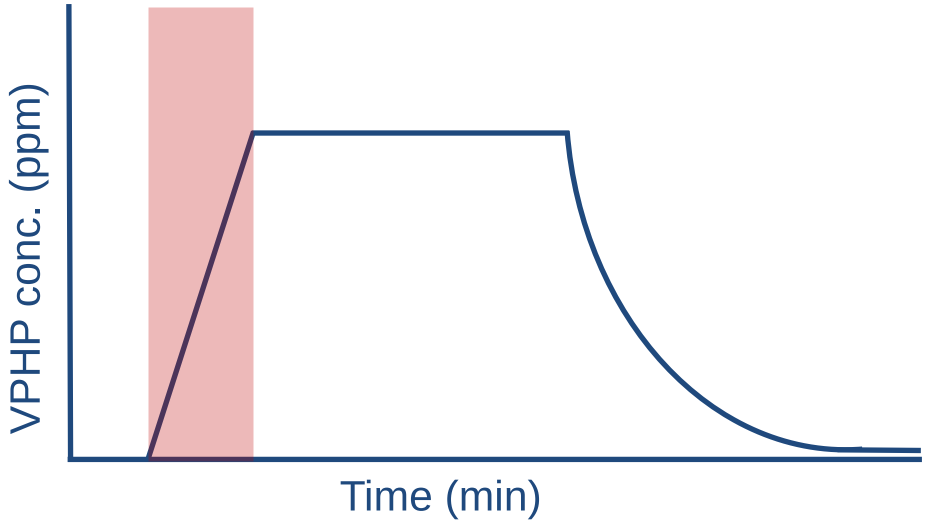


Diagram

Decontamination cycle: its structure

2. Conditioning

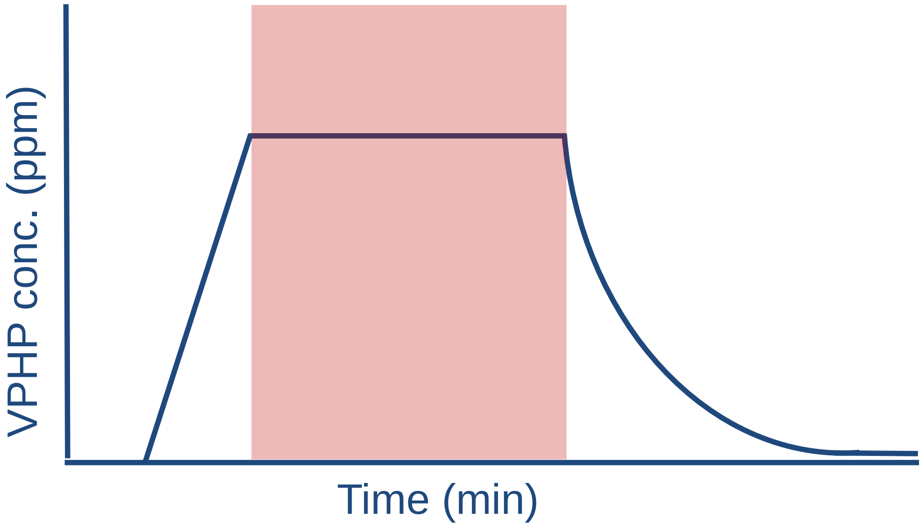
- ✓ VPHP injection at a high speed
- ✓ Achievement of the pre-defined VPHP concentration



Decontamination cycle: its structure

3. Decontamination (dwell time)

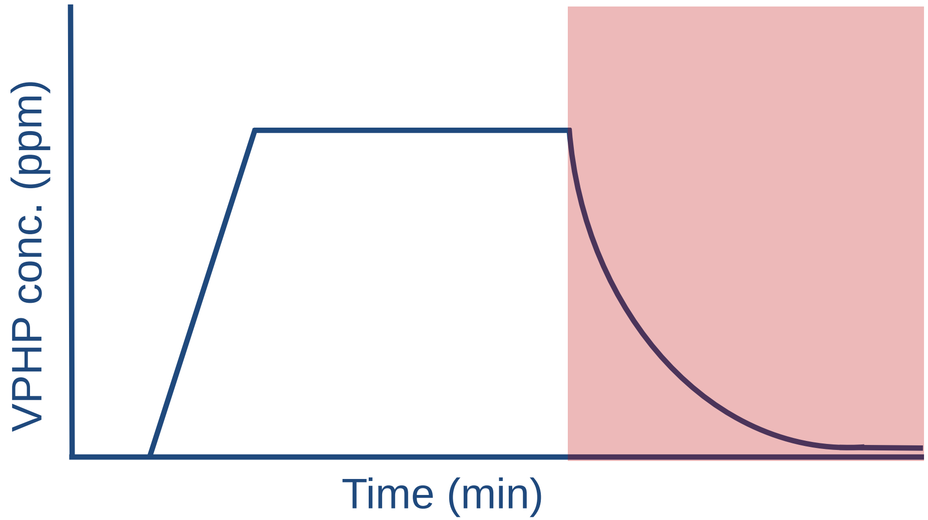
- ✓ VPHP injection at a reduced rate
- ✓ VPHP concentration is maintained constant for a pre-defined time



Decontamination cycle: its structure

4. Aeration

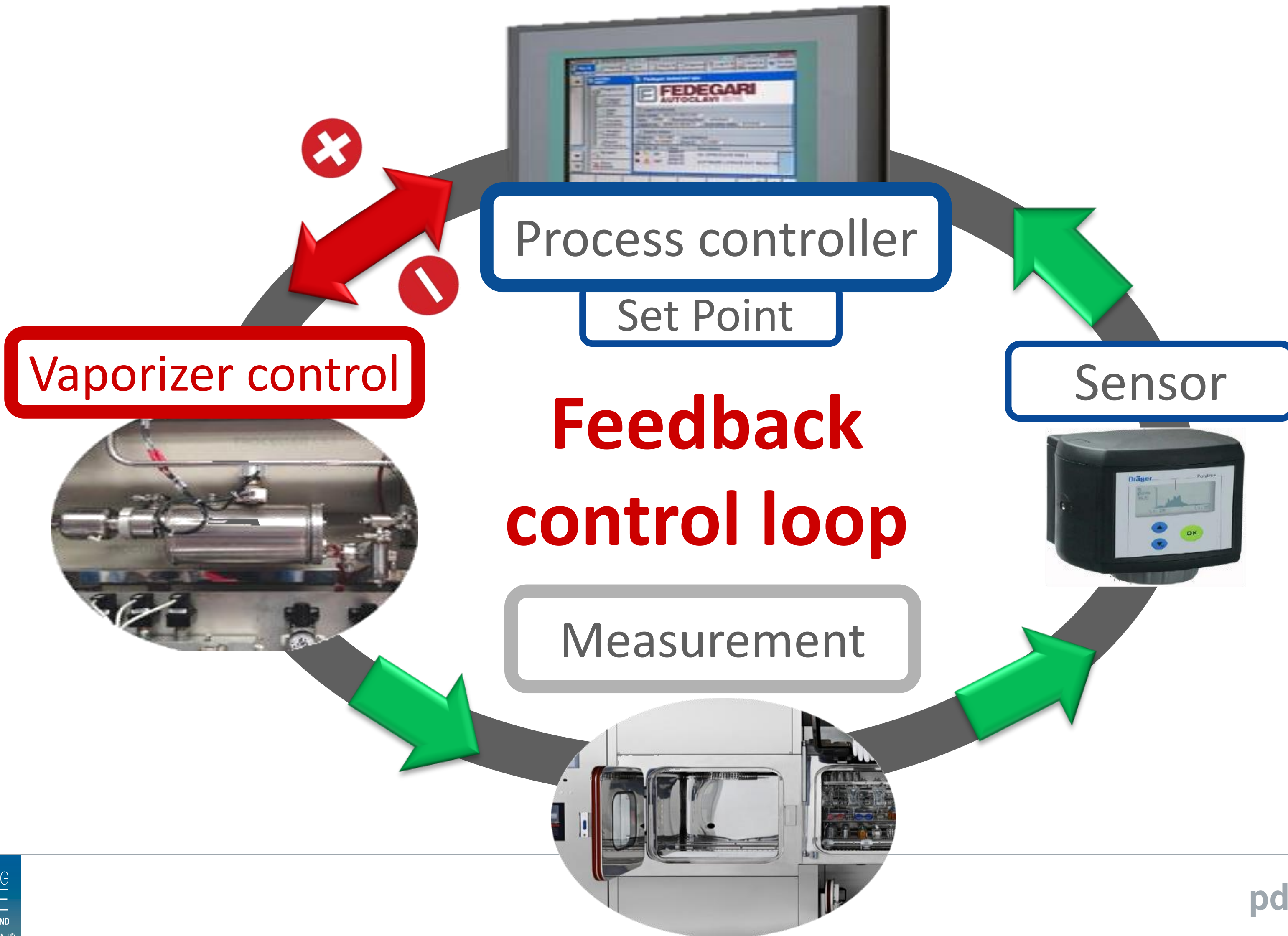
- ✓ Air injection to replace (by dilution) H_2O_2



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

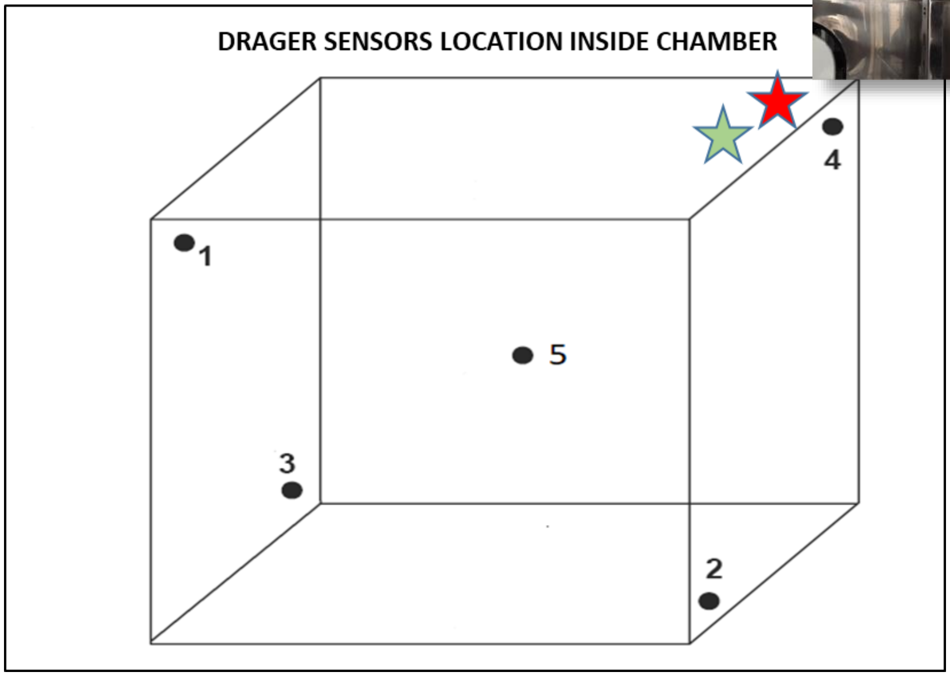
- ✓ The time depends on both air exchange rate and H_2O_2 desorption from the decontaminated material (↑ temperature: ↑ v_{des})

Biocide concentration: our approach



Test - Dräger sensors location

Empty chamber



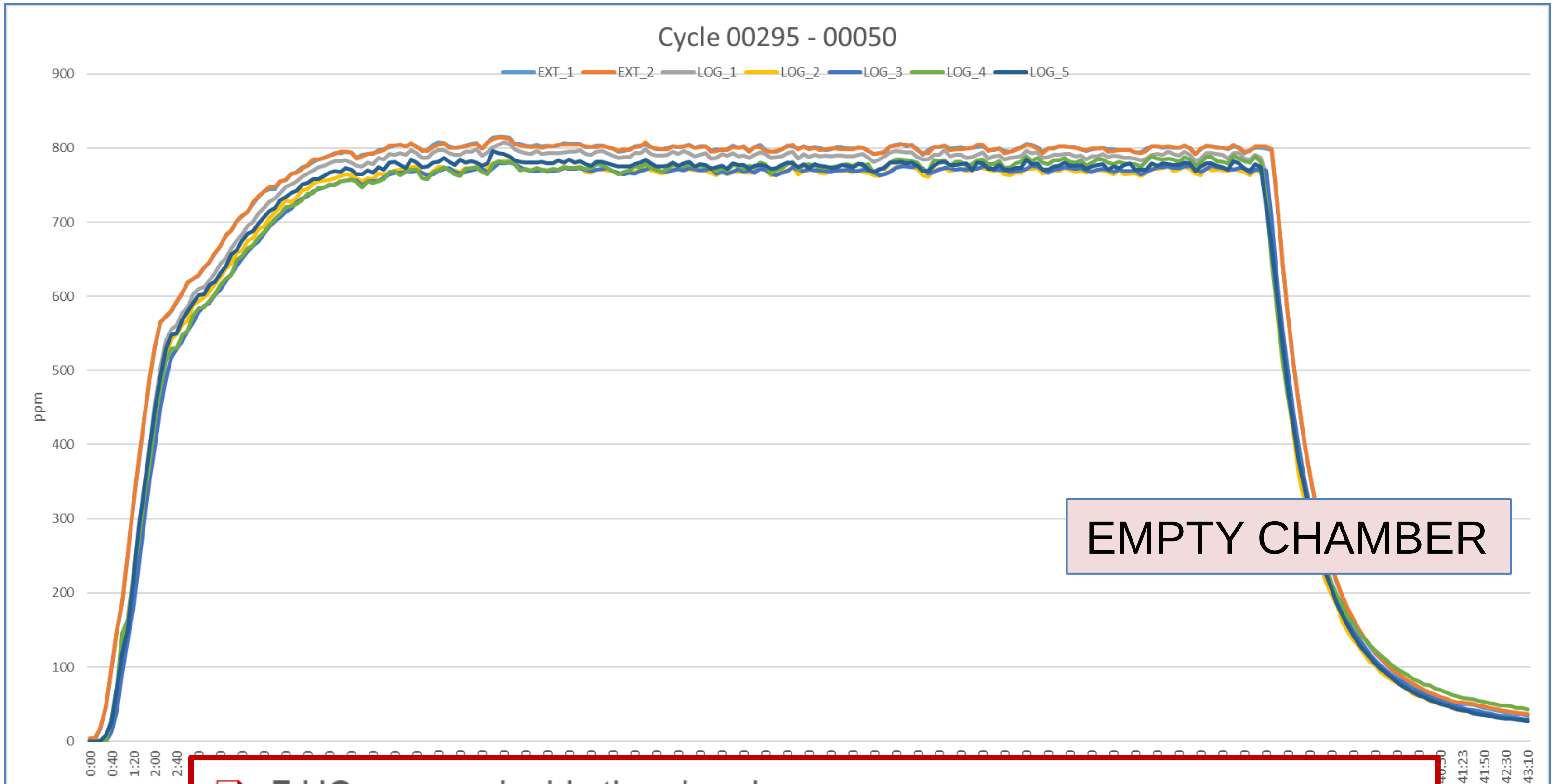
EXT 1

EXT 2

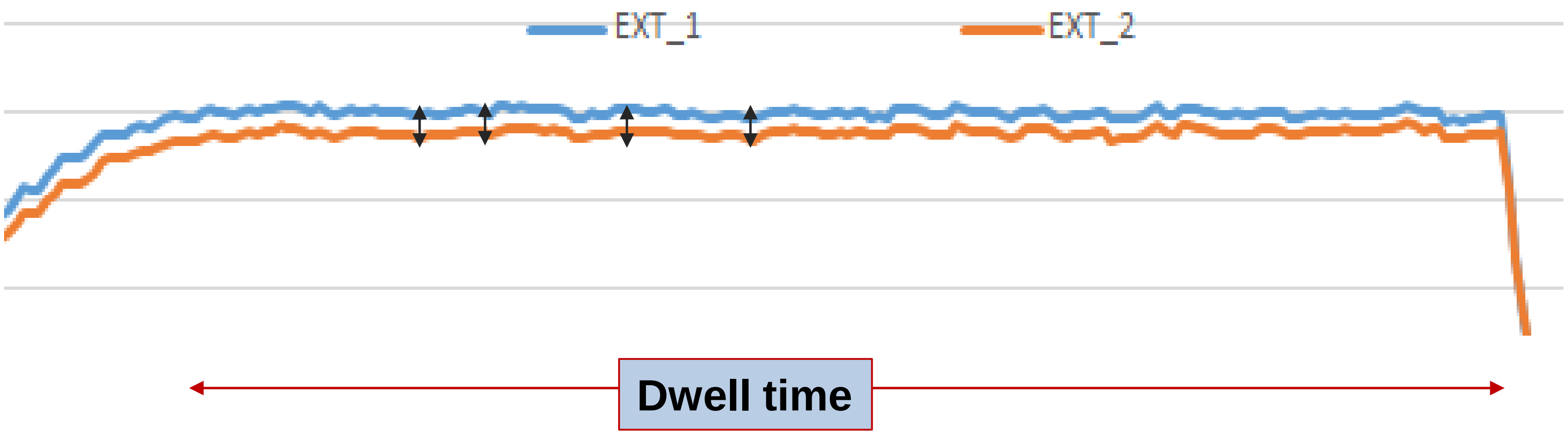
EXT 1 and EXT 2 are located in the ceiling of the chamber; LOG1, 2, 3, 4, 5, 6, inside the chamber.

<u>EXT1 controls the process</u>

Hydrogen peroxide distribution

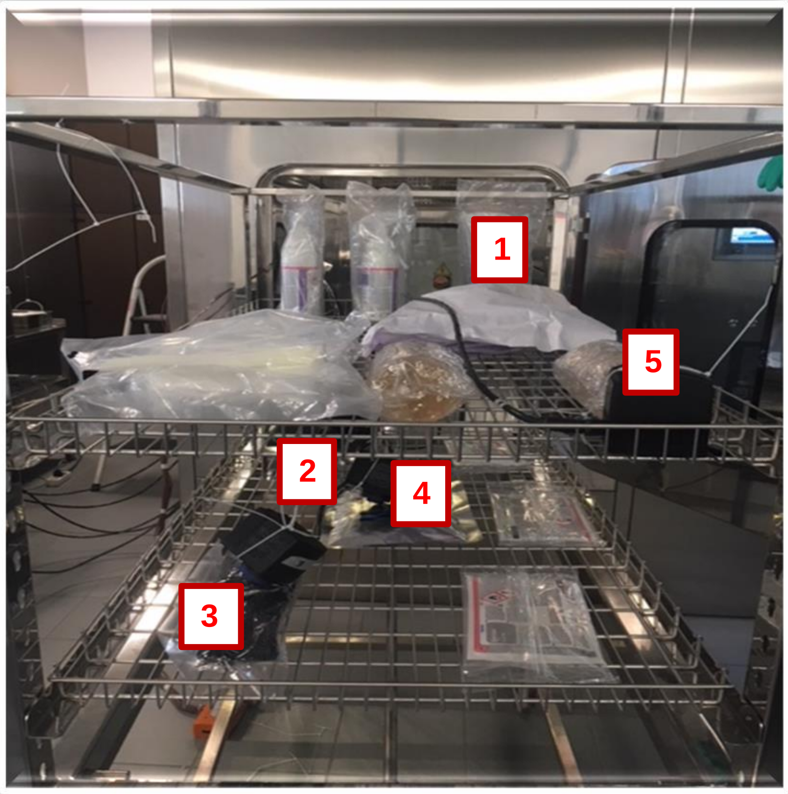


- 7 HC sensors inside the chamber
- A cycle at 800 ppm, 30 minutes, Hydrogen Peroxide 35% V/V (to titrate)
- The data profiles are collected and analyzed



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

Load and sensors

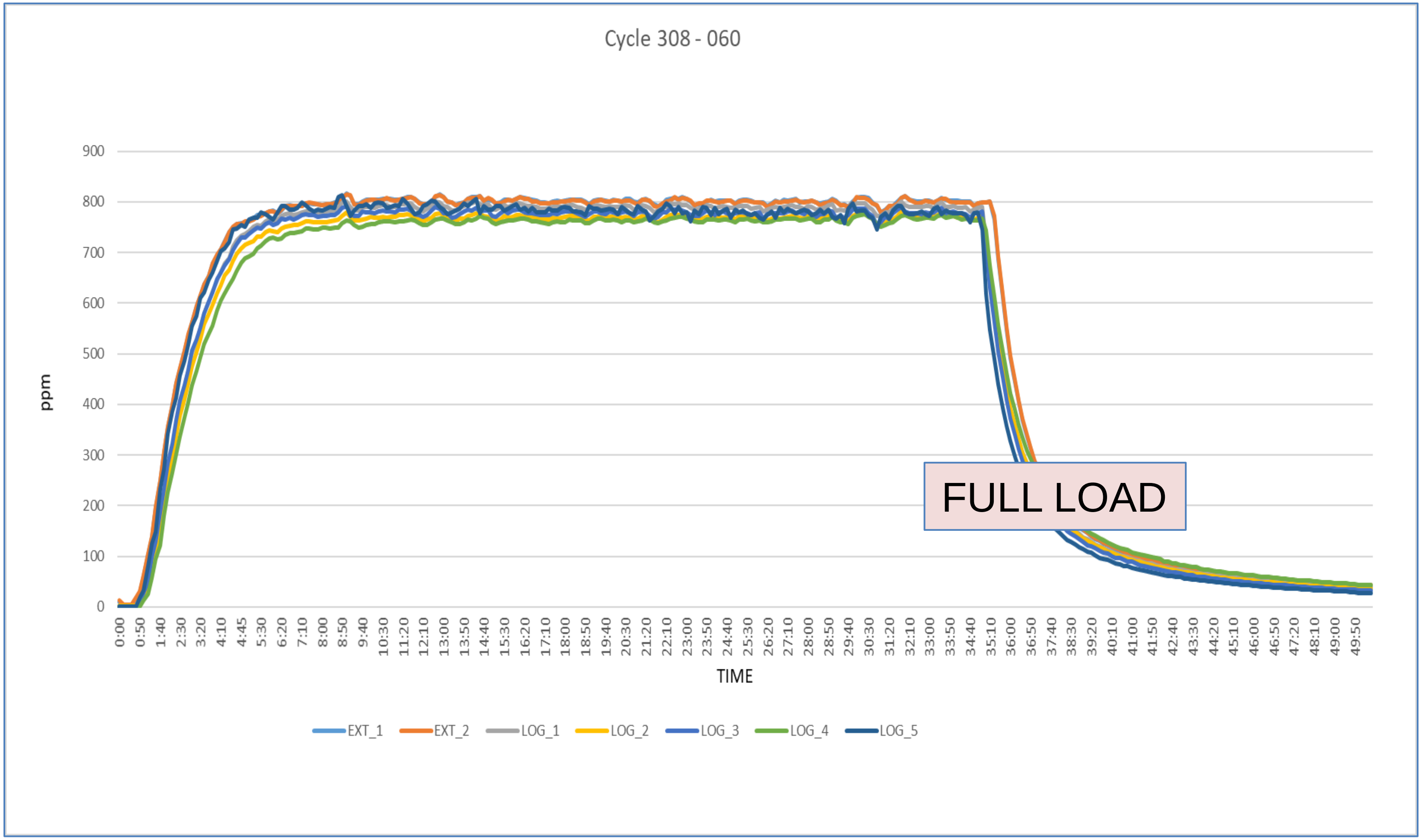


Several items with different materials

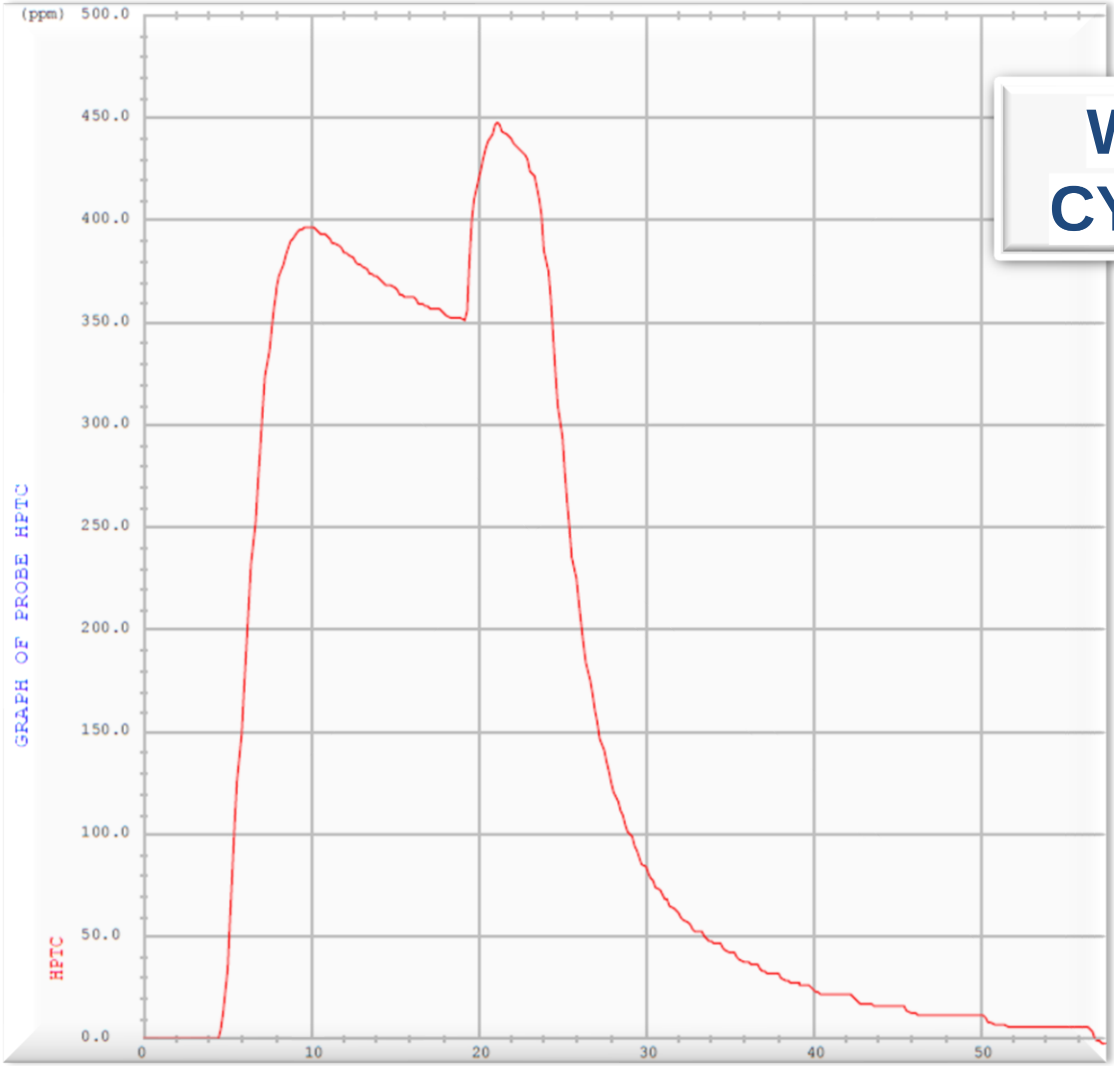
1 – Plastic bag for Klercide
2 – Stainless steel box
3 – Stoppers bag
4 – Al bag
5 – Petri Plates

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

Hydrogen peroxide distribution



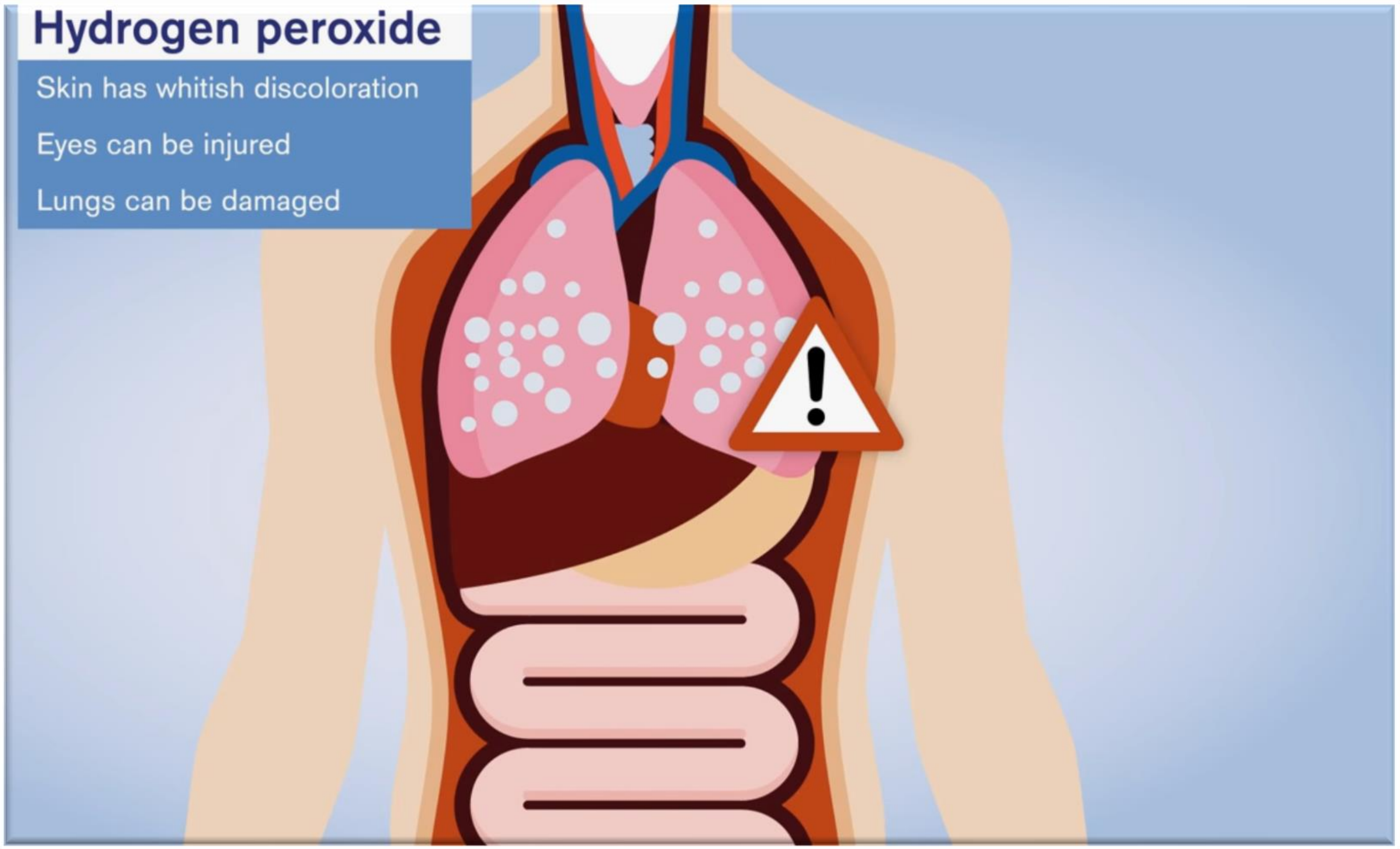
ppm



WET CYCLE

time

Health and Safety

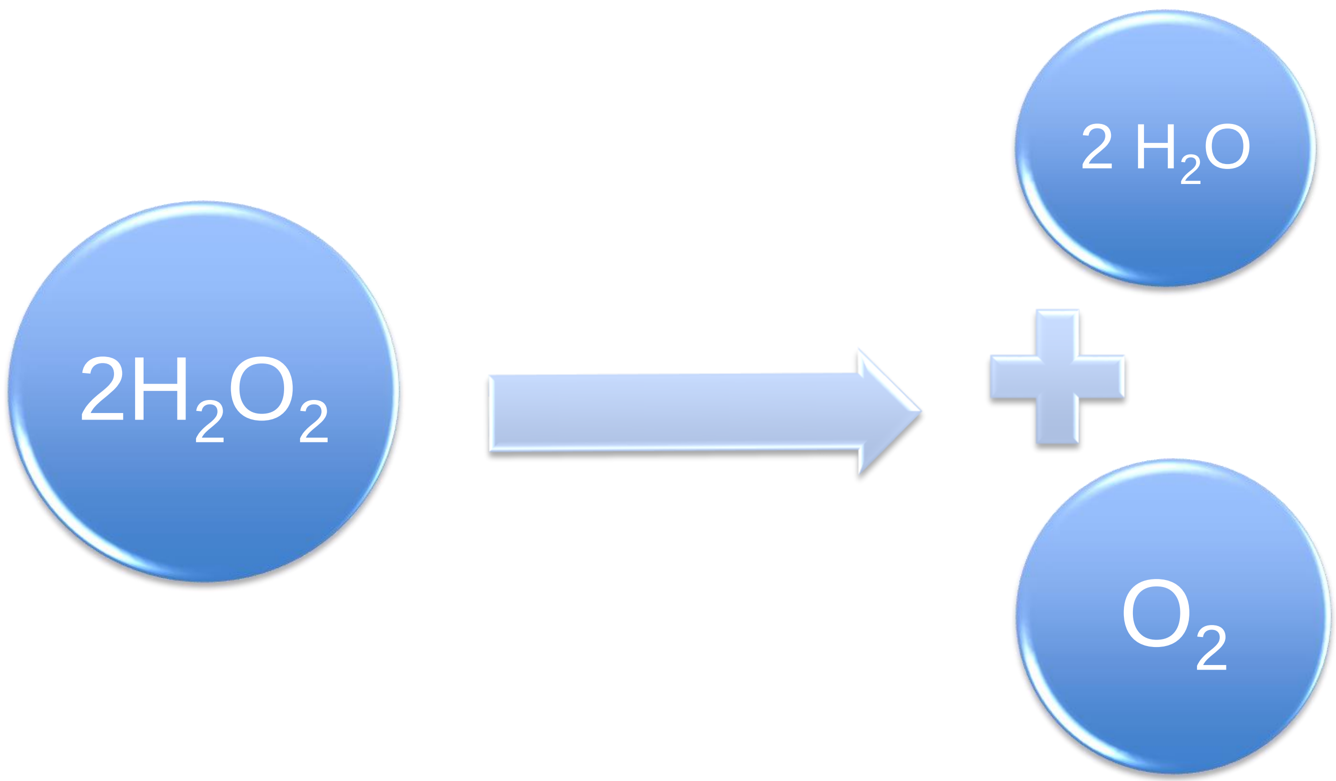


Safety

1 ppm is the TLV (Threshold Limit Value), TWA (Time-Weighted Average) declared by OSHA

TLVs® are not standards. They are guidelines designed for use by industrial hygienists in making decisions regarding safe levels of exposure to various chemical substances and physical agents found in the workplace.

Hydrogen peroxide degradation



Hydrogen peroxide degradation

Different choices on the market

- an «absorption bed» with heaters
  to trap the molecule and to increase its degradation rate

- a catalyst applied on a carrier material, high conctat surface
  to degrade the molecule

PDA Parenteral Drug Association

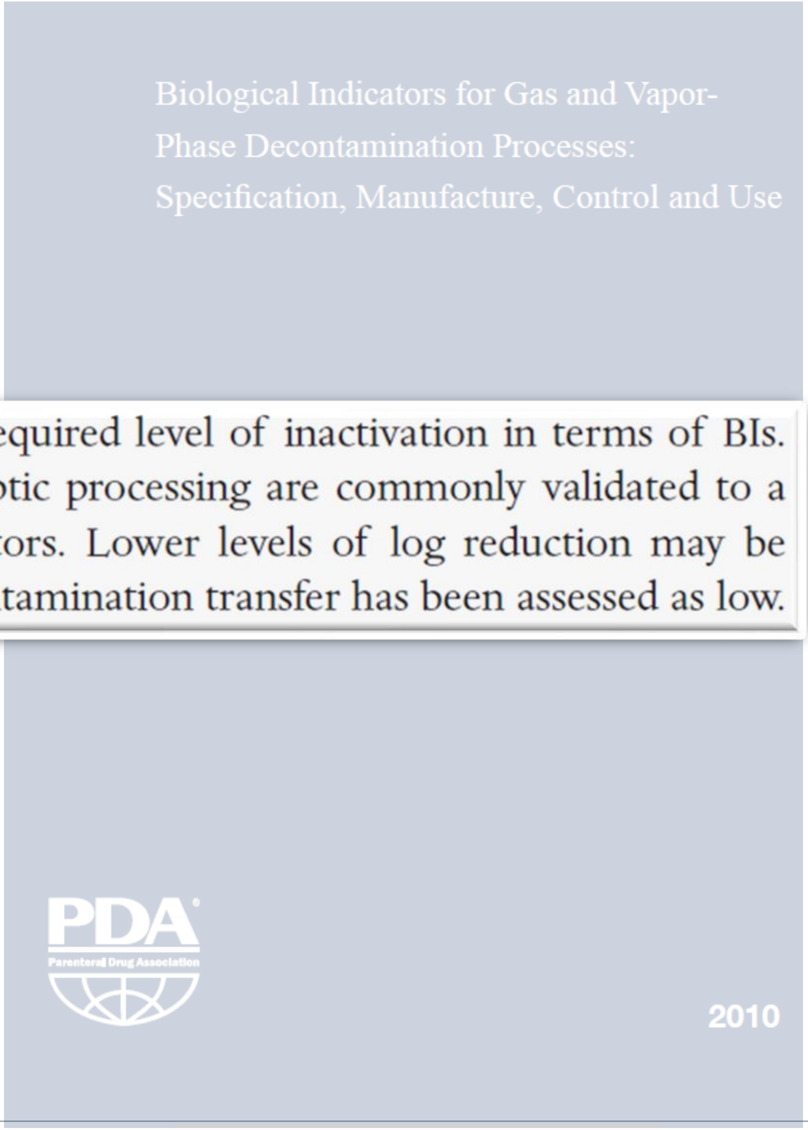
SLR achieved

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 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. 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 → **6 SLR**

SLR achieved


Biological Indicators for Gas and Vapor-Phase Decontamination Processes: Specification, Manufacture, Control and Use

PDA Parenteral Drug Association

2010

Cycle development starts with the definition of the required level of inactivation in terms of BIs. Sporicidal gassing cycles for critical areas used in aseptic processing are commonly validated to a minimum of 6-log reduction using biological indicators. Lower levels of log reduction may be acceptable in areas or on surfaces where risk of biocontamination transfer has been assessed as low.

Biological indicators (BIs)

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

Biological Indicators

Vapor Phase Sterilization

The biphasic nature of these materials precludes the accurate determination of specific lethal conditions (for establishment of *D* values, see *Vapor Phase Sterilization* ⟨1229.11⟩). BIs using either *G. stearothermophilus* or *B. atrophaeus* have been utilized in the evaluation of these processes.

USP – NF 2021, ⟨1229.5⟩ BIOLOGICAL INDICATORS FOR STERILIZATION

Biological Indicators

Certificate of Analysis

Apex Biological Indicator (Reorder # HMV-091)
for Gaseous Hydrogen Peroxide

Lot #: **H0955**
Manufacture: 2015 April 07 Expiration: 2016 January 31
Indicator: *Geobacillus stearothermophilus* 12980[1]
Mean population: 2.5 x 10^6 CFU per stainless steel carrier[2]
Storage conditions: 2 - 8°C; less than 50% RH; move to ambient conditions ≥ 1 hr before use.
Shipping conditions: Ambient temperatures; cold pack and desiccant may be used to moderate conditions during shipping.

Resistance Characteristics:

D-value[3]: 1.0 minutes in 2mg/L gaseous H_2O_2

D-value is reproducible only when exposed and cultured under identical conditions used to obtain results reported here. MPN method used. Units are manufactured in compliance with Mesa Laboratory, Bozeman Manufacturing Facility's quality standards and ISO 11138-1 guidelines and all appropriate subsections.

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

Incubate at 55 – 60°C for 7 days. The recommended growth medium is Soybean Casein Digest Medium (SCDM), Tryptic Soy Broth (TSB) or Mesa Releasat Medium (PM/100).

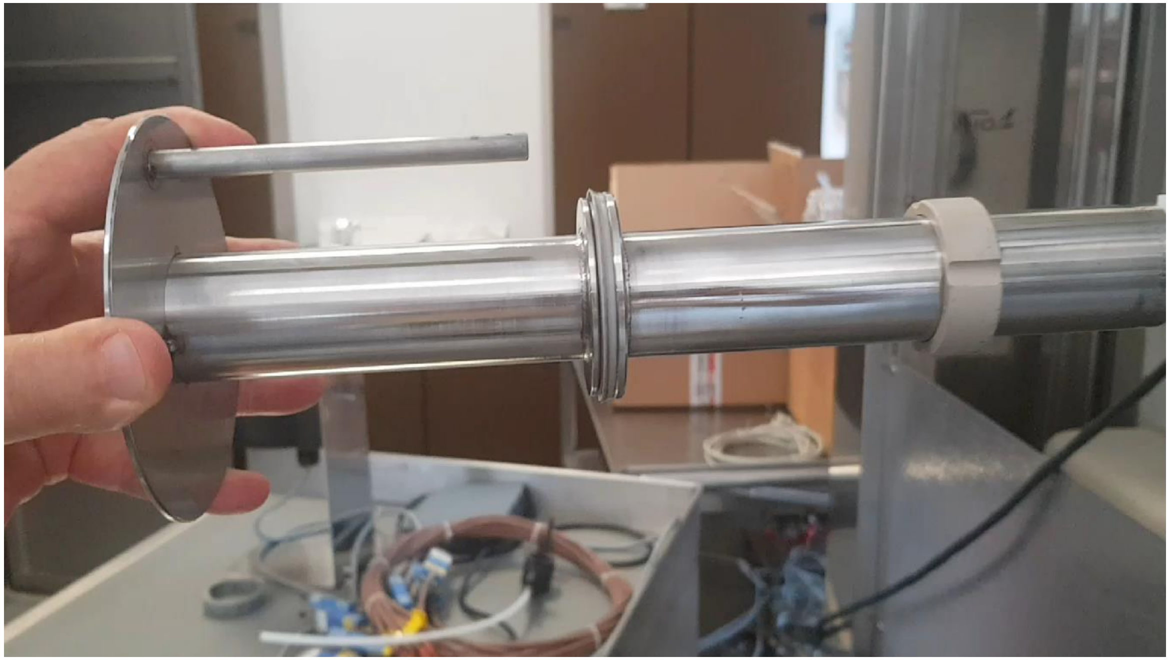
D-value determination

ISO 18742: Sterilization of health care products - Biological and chemical indicators - Test equipment

Resistometer

Test equipment designed to create defined combinations of the physical and/or chemical variables of a sterilization process.

Fedegari VPHP BIER



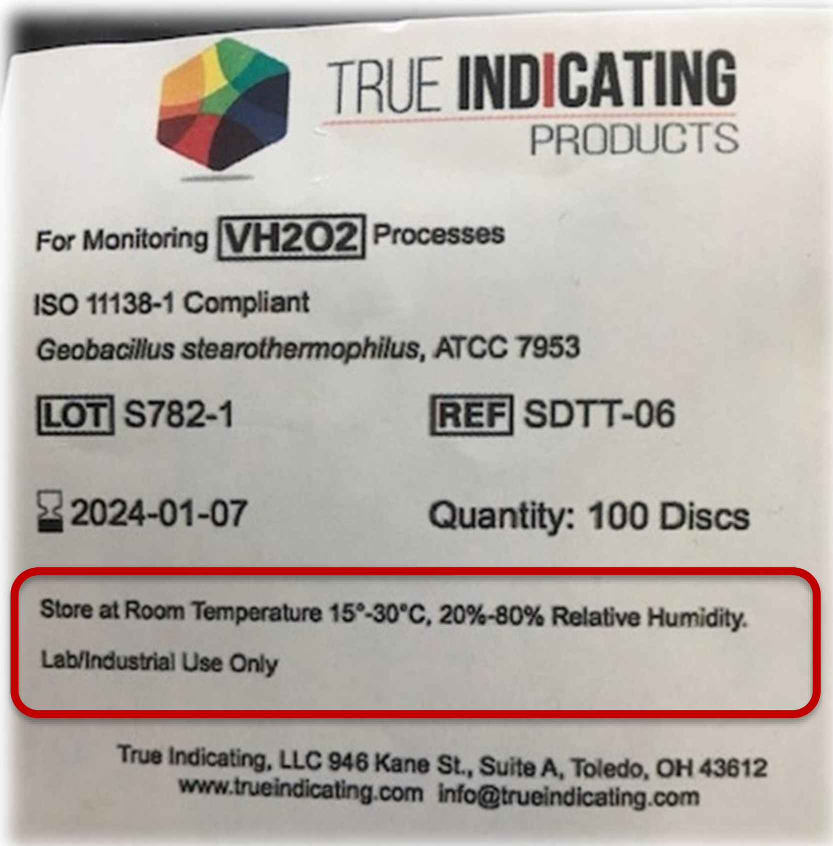
BI storage

- Refrigerate at **2÷8° C**
- **RH < 50%** (insert a desiccant pouch inside the bag where they are kept)

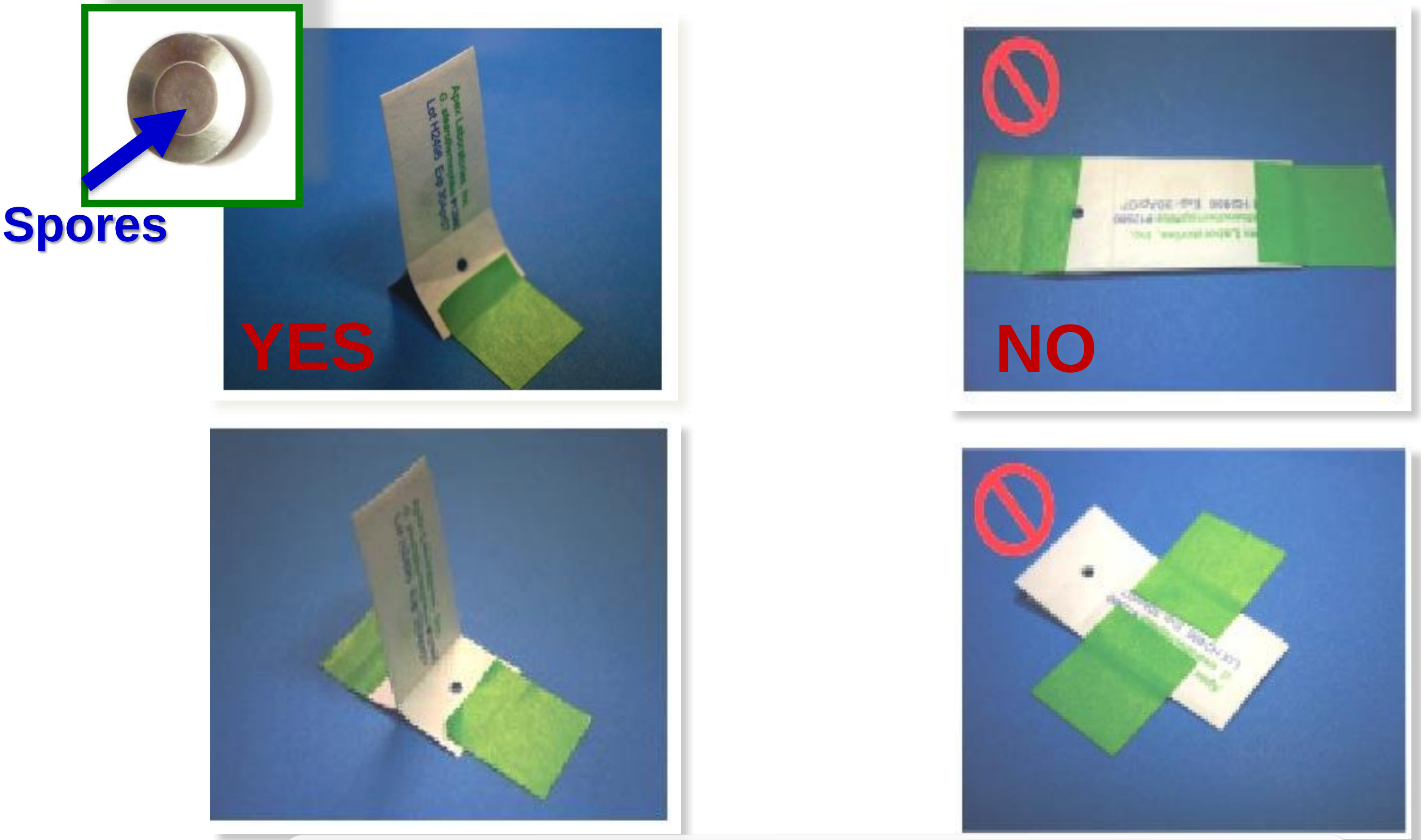
MesaLabs

Move to ambient conditions ≥ 1h before use

BI storage


TRUE INDICATING
PRODUCTS
For Monitoring VH2O2 Processes
ISO 11138-1 Compliant
Geobacillus stearothermophilus, ATCC 7953
LOT S782-1
REF SDTT-06
2024-01-07
Quantity: 100 Discs
Store at Room Temperature 15°-30°C, 20%-80% Relative Humidity.
Lab/Industrial Use Only
True Indicating, LLC 946 Kane St., Suite A, Toledo, OH 43612
www.trueindicating.com info@trueindicating.com

BI placement & handling



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

BI placement & handling

- Do not use adhesive tapes or inks that absorb or catalyze hydrogen peroxide degradation
- Do not write on the spore location

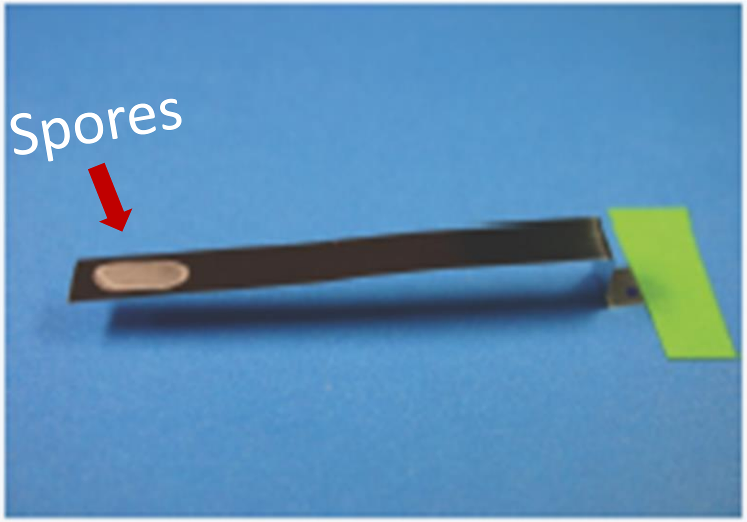
BI placement & handling

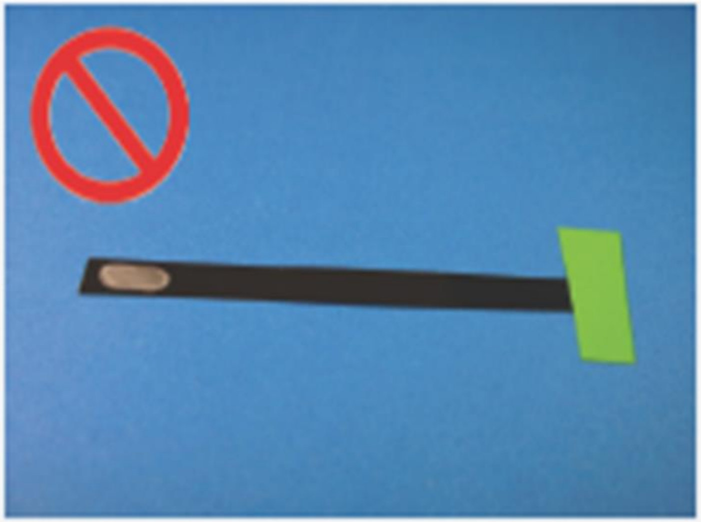
Do not place the BI into or under a container

BI placement & handling

«Naked» BIs: spores are inoculated on a stainless steel ribbon not wrapped

YES **NO**





Unexpected positive Bis: a case study

Is the BI fault or

our cycle is not a right one?

Unexpected positive Bis: a case study

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

Unexpected positive Bis: a case study

- **VPHP** has a poor penetrating capability: it is a **surface decontaminating agent**

- «…**Quality control** of **BIs** for **sporicidal vapor-phase processes is imperative,** since minor changes in the manufacture, storage, and presentation of the BI may affect its sensitivity to the decontaminating agent…»

«PDA TR.51, «Biological Indicators for Gas and Vapor-Phase Decontamination Processes: Specification, Manufacture, Control and Use

Unexpected positive Bis: a case study

RE-RUN A CYCLE WITH MULTIPLE BIs

Unexpected positive Bis: a case study

Triplicate BIs at the «**worst case» locations** allow to evaluate the situation with a **statistical analysis**

Unexpected positive Bis: a case study

If we used **one BI/location**, we might have:

BI (+)

BI (–)

If we used **3 BIs/location**, we might have

(+ + +)

(- - -)

(- - +) , (- + +)

Single BIs do **not** allow to perform a **statistical analysis**

Unexpected positive BIs: a case study

Halvorson-Ziegler equation:

Most Probable Number of surviving spores

Number of BIs/location

$$MPN = \ln\left(\frac{n}{r}\right)$$

Number of negative BIs/location

- **Applicable** only with **multiple BIs/location**
- It allows to calculate the **average number** of **surviving spores** per **BI**

Unexpected positive Bis: a case study

Example: after running a VPHP cycle we observed two positive and one negative BIs **(+ + -)** at a specific location

MPN = ln (n/r)

n (number of BIs/location) = 3

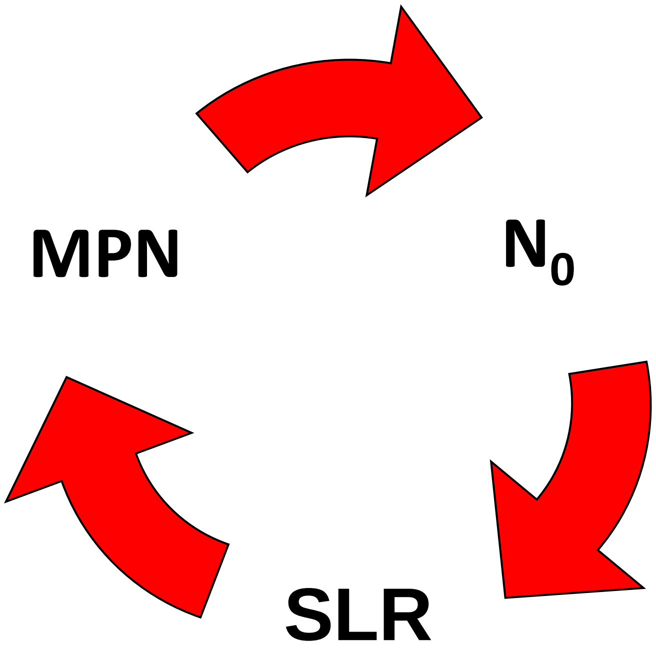
r (negative BIs/location) = 1

MPN = ln (3/1) = **1.099**

On average we have
1.099 survived spores per BIs

Unexpected positive BIs: a case study

There is a link between MPN, the initial population of the used BIs (**N_0**) and the *Spore Log Reduction* (**SLR**) obtained at a specific location



Unexpected positive Bis: a case study

Spore Log Reduction at the specific location where we observed BIs (+ + -):

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

Example:

If spore population per BI = 2.8×10^6

$Log_{10}\ 2.8 \times 10^6 = 6.447$

If (+ + -), MPN = 1.099

Log_{10} MPN = 0.041

SLR = 6.447 – 0.041 = **6.406**

Unexpected positive Bis: a case study

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

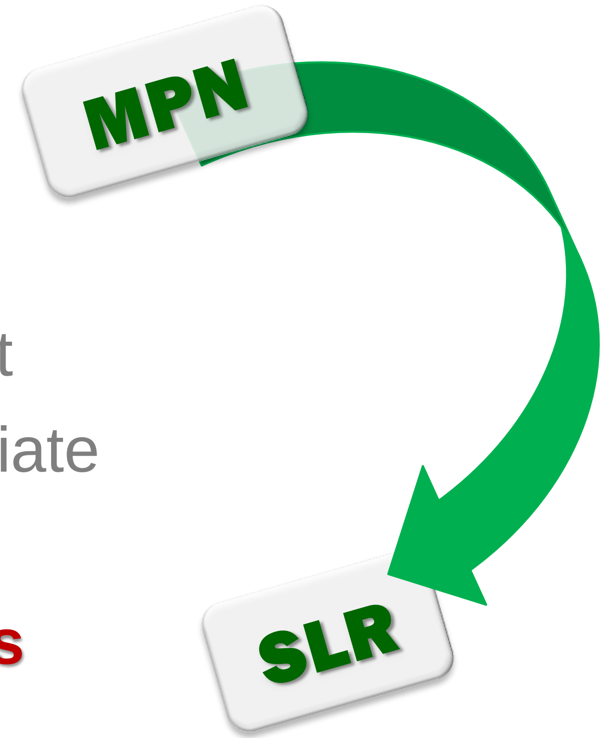
- This SLR value is what **guidelines** and/or **rules require about decontamination**

THE DECONTAMINATION CYCLE WAS SUCCESSFUL !

Unexpected positive Bis: a case study

- This calculation is **ONLY possible** when **replicate BIs** are used.

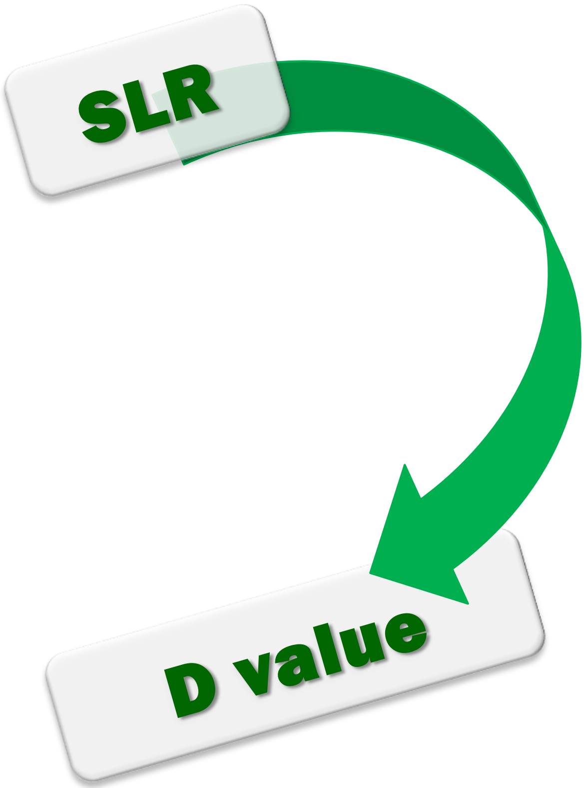
- If 100 BIs were placed at 100 different test locations, it would not be appropriate to perform this calculation as these **100 individual BIs are not replicates** of the others.



Unexpected positive Bis: a case study

D value: time / SLR

Knowing D, how many SLR we have, we can add «x» minutes to reach a SAL 10^{-6}



Before saying that your 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 multiples locations?

Conclusions

- Hydrogen peroxide is a decontaminant, active on surfaces.

- Its process is a low temperature one, useful for heat sensitive loads.

- It migh 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

In the next future….
could it be considered a sterilant?

Thank you for your attention

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