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

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

<table>
<thead>
<tr>
<th>Chemical Entity</th>
<th>Classification</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide</td>
<td>Vapor phase sterilant, liquid sporicidal agent, antiseptic</td>
<td>4 µg per g H₂O₂ vapor, 10%–25% solution, 3% solution</td>
</tr>
</tbody>
</table>
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)

✓ Heat sensitive product
✓ Waste

Class C, D

Class A, B

Clean room
Decontamination: when?
An isolator at...

........Fedegari FAT area
Decontamination technologies

The most widespread technologies

- Vapor phase hydrogen peroxide
- Dry fog
VAPOR PHASE HYDROGEN PEROXIDE

Vapor Phase Hydrogen Peroxide (VPHP): how is it produced?
VPHP Production: Flash Vaporization

Liquid mixture:
\( \text{H}_2\text{O}_2/\text{H}_2\text{O} \ 35/65 \text{ (w/w\%)} \)

Compressed Air

Hot plate
>108°C

VPHP

Vapor mixture:
\( \text{H}_2\text{O}_2/\text{H}_2\text{O} \ 35/65 \text{ (w/w\%)} \)

All the components are vaporized simultaneously
Fedegari Hydrogen Peroxide Vaporizer (FHPV)

It produces vaporized hydrogen peroxide from the H$_2$O$_2$/H$_2$O 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 aggressive on materials
- concentration not well controlled

Dry cycle
- effective in a longer time lapse
- less penetrating
- dry load
- shorter cycle
- less aggressive 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

SAFETY DATA SHEET
Hydrogen Peroxide 35% Durox® LRA

Product Identifier
Product Name: Hydrogen Peroxide 35% Durox® LRA
Other means of identification
CAS-No: 7722-84-1

«STANDARD» PERCENTAGE
35%
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 $\text{H}_2\text{O}_2$ 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**

<table>
<thead>
<tr>
<th>Nitrate (mg/L)</th>
<th>Sulphate (mg/L)</th>
<th>Phosphates (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.47</td>
<td>21.34</td>
<td>74.82</td>
</tr>
</tbody>
</table>

2. **SILICON and SILICA**

<table>
<thead>
<tr>
<th>Silicon (mg/L)</th>
<th>Silica (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.157</td>
<td>0.336</td>
</tr>
</tbody>
</table>
Materials compatibility

- Deterioration of elasticity or strength or flexibility, visible damages
- Absorption – Degassing time
- Penetration – Product damage
- Microbiological effectiveness - Surface finish
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.
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
The surface appears smooth, but spores can aggregate in some grooves.
Glass

The spores are well dispersed on the very smooth surface

VERY GOOD SUBSTRATE!
**Stainless steel 316 L**
The spores show a good dispersion on the relatively smooth surface.
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, \( \text{H}_2\text{O}_2 \) vapors can easily penetrate through it.
Hydrogen peroxide detection

Electrochemical sensor
Measuring electrode: $\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_+ + 2 \text{e}.$
Counter electrode: $\frac{1}{2}\text{O}_2 + 2 \text{H}_+ + 2 \text{e} \rightarrow \text{H}_2\text{O}$
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.
Hydrogen peroxide detection

Cavity ring-down spectroscopy (CRDS)

Lower Detection Limit: < 3ppb
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$_2$O$_2$ 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.
Both cycles are generally constituted by four phases:

- Preparation
- Conditioning
- Decontamination
- Aeration
Decontamination cycle: its structure

- Decontamination
- Conditioning
- Aeration

DRY CYCLE
Decontamination cycle: its structure

1. Preparation

✓ Achievement of the pre-defined temperature and relative humidity value (set point)
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) H₂O₂

✓ H₂O₂ < 1 ppm (TLV/TWA, threshold limit value/time weighted average)

✓ The time depends on both air exchange rate and H₂O₂ desorption from the decontaminated material (↑ temperature: ↑ v_{des})
Biocide concentration: our approach

Feedback control loop

- Sensor
- Measurement
- Process controller
- Set Point
- Vaporizer control

Feedback control loop
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
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

Cycle 308 - 060

TIME

0 10 20 30 40 50 60 70 80 90

ppm

EXT_1  EXT_2  LOG_1  LOG_2  LOG_3  LOG_4  LOG_5

FULL LOAD
WET CYCLE

ppm

time
Hydrogen peroxide

- Skin has whitish discoloration
- Eyes can be injured
- Lungs can be damaged
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

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]
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 contact surface to degrade the molecule
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.
SLR achieved

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)

<table>
<thead>
<tr>
<th>Process</th>
<th>Selected Organism</th>
<th>ATCC Derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peracetic acid</td>
<td><em>Geobacillus stearothermophilus</em></td>
<td>7953 or 12980 (Ph. Eur.)</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td><em>Bacillus atrophaeus</em></td>
<td>9372 (Ph. Eur.)</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td><em>(formerly Bacillus subtilis var. niger)</em></td>
<td></td>
</tr>
<tr>
<td>Peracetic acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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).
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**
Certificate of Analysis

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

Lot #: H0955
Manufacture: 2015 April 02  Expiration: 2016 January 31
Indicator: Geobacillus stearothermophilus 12980\(^{(1)}\)
Mean population: \(2.5 \times 10^6\) CFU per stainless steel carrier\(^{(2)}\)
Storage conditions: 2 - 8°C; less than 50% RH; move to ambient conditions \(\geq 1\) 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\(_2\)O\(_2\)

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 $2 \div 8^\circ$ C
- RH < 50% (insert a desiccant pouch inside the bag where they are kept)

Move to ambient conditions $\geq 1$ h before use
BI storage

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
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

\[
\text{MPN} = \ln\left(\frac{n}{r}\right)
\]

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

Number of BIs/location

Number of negative BIs/location
Unexpected positive Bis: a case study

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

\[ \text{MPN} = \ln \left( \frac{n}{r} \right) \]

\( n \) (number of BIs/location) = 3
\( r \) (negative BIs/location) = 1

\[ \text{MPN} = \ln \left( \frac{3}{1} \right) = 1.099 \]

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 (+ + -):

$$SLR = \log_{10} N_0 - \log_{10} MPN$$

Example:

If spore population per BI = $2.8 \times 10^6$
$$\log_{10} 2.8 \times 10^6 = 6.447$$

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 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
In the next future.... could it be considered a sterilant?
Thank you for your attention

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