Decontamination with H2O2 for aseptic Isolators

ISPE – PDA Conference  Australia
Melbourne
20th September 2019

Richard Denk
Senior Consultant Aseptic Processing & Containment
SKAN AG
Richard.denk@skan.ch

2 Key Factors for Aseptic Processing

- Process well established
- Isolator Technology
- Vaporized Hydrogen Peroxid vH2O2

- Requirements get higher in aseptic processing
- Validation of Cleaning and Disinfection
3 Key Factors for Aseptic Toxic Processing

- Requirements get higher in aseptic processing
- Validation of Cleaning and Disinfection
- New to the BioTech industry

H₂O₂ Decontamination
- Process well established
- Isolator Technology
- Vaporized Hydrogen Peroxide (vH₂O₂)

Operator Protection
- Requirements for high potent substances
- Less Experience with aseptic Isolators

Isolator Process Control

- Equipment Design
- H₂O₂ Cycle Parameter
- Process Control
- Suitability of Biological Indicator
Isolator Air Handling Know-How

Filter System

ISO 8, Grade D/C, Class 100,000

ISO 5, Grade A, Class 100

unidirectional air flow
air velocity 0.45 [m/s] ± 20%
Air Recirculation (for Aseptic-Toxic System)

ISO 8, Grade D/C, Class 100'000

ISO 5, Grad A, Class 100
unidirectional air flow
air velocity 0.45 [m/s] ± 20%

Cartridge HEPA filter system FIPA-FL

no extra space in technical area needed

return air ducts protected with cartridge filters don’t require wash down
Critical Zone

Vaporizer plate for \( \text{H}_2\text{O}_2 \)

Differential pressure indicator

Service covers

Intake air from air handling unit

Recirculation fan

HEPA filter

Diffusor membrane

Unidirectional air flow

RTP

Return air through double door

Critical zone

"Classified working zone"

H2O2 Decontamination Principle

H\(_2\)O\(_2\) Solution

Flash Vaporizer

H\(_2\)O\(_2\) vapour

Carrier gas

HEPA filter

Isolator chamber
# Why Flash Vaporizing?

## Physical Properties of H$_2$O$_2$

<table>
<thead>
<tr>
<th>Property</th>
<th>Hydrogenperoxide</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>34,016</td>
<td>18,016</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>150,2</td>
<td>100,00</td>
</tr>
<tr>
<td>Evaporation Energy</td>
<td>51,66</td>
<td>44,04</td>
</tr>
<tr>
<td>Thermic Capacity of the Steam</td>
<td>42,82</td>
<td>33,62</td>
</tr>
</tbody>
</table>

The important difference of water and hydrogen peroxide for the evaporation technology is the difference in the boiling point due to the hydrogen bonds which are stronger in hydrogen peroxide. As we always use a mixture of hydrogen peroxide and water we need a flash evaporation to avoid the splitting of the mixture in water and hydrogen peroxide during evaporation.

The other important difference is the much lower vapor pressure of the hydrogen peroxide than water vapor. Therefore, hydrogen peroxide vapor will readily condense on the isolator and load surfaces reaching a high concentration. This condensate forms a thin film on the surfaces and is responsible for the rapid bactericidal and sporicidal effect. [2]

[Bässler Hans-Jürgen; 21.08.2018]
H2O2 Decontamination System

H2O2 Evaporation Principle
Phases of Decontamination Cycle

H2O2 Decontamination System

Two Technologies of Decontamination with H2O2
H2O2 Decontamination System

Fastest H$_2$O$_2$ cycles with state-of-the-art SKANFOG® decontamination technology

- Micro-nebulization of H$_2$O$_2$
- An embedded and proven system
- Minimized H$_2$O$_2$ consumption conforming to risk analysis (80% less than VHP)
- Fastest decontamination process (1 hour < 1 ppm)

SKANFOG® Principle
H2O2 Micro Nebulization - SKANFOG

Compressed air

Dual nozzle

H2O2 solution

H2O2 Aerosol

Together always one step ahead

H2O2 Micro Nebulization

Micro nebulization of H2O2 solution into aerosol droplets

Evaporation of aerosol droplets until saturation

Micro-condensation on the surfaces

Inactivation effect

Damage of the Germ

Molecular Structure of H2O2

Droplet size and fast distribution
**H2O2 Micro-Nebulization - Study**

**Room decontamination**

From: P. Vanhecke, V. Sigwarth, C. Moirandat* A Potent and Safe H2O2 Fumigation Approach™ PDA J Pharm Sci and Tech 2012, 66 354-370, Figure 14

---

**Phases of Decontamination Cycle**

Decontamination with H₂O₂ – SKANFOG®

---
**H2O2 Decontamination Parameters**

- **Factor of Influence: Quality of BI**
  - Suitable for H2O2 decontamination
  - Late positives
  - Convenient, suitable resistance

- **Factor of Influence: Design of isolator**
  - Homogeneity of physical parameters
  - Capacity and distribution of H2O2

- **Factor of Influence: Cycle Parameter**
  - achieved inactivation effect
  - Stability of inactivation effect
  - Duration of decontamination process

- **Factor of Influence: Human being**
  - Operator Training
  - Expertise
  - Behavior

---

**Overview of current regulations and standards**

- **PIC/S DEFINITIONS / GLOSSARY**
  - **5.3 Sporidical 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."

  "Current practice is to seek six log reductions of the biological indicator organism recommended by the manufacturer of the gas generator."

- **FDA: ASEPTIC GUIDELINE**

  "Cycles should be developed with an appropriate margin of extra kill to provide confidence in robustness of the decontamination processes. Normally, a four- to six-log reduction can be justified depending on the application. The specific BI spore titer used and the selection of BI placement sites should be justified."

  ➔ ...has to be understood as a total kill of BI inoculated at 10^4 to 10^6 spores / carrier
Overview of current regulations and standards

- FDA: ASEPTIC GUIDELINE

APPENDIX 1: ASEPTIC PROCESSING ISOLATORS

D. Decontamination

Efficacy

The decontamination method should render the inner surfaces of the isolator free of viable microorganisms. Multiple available vapored agents are suitable for achieving decontamination. Process development and validation studies should include a thorough determination of cycle capability. The characteristics of these agents generally preclude the reliable use of traditional methods (e.g., broth inactivation) to determine process efficacy (Ref. 11).


Overview of current regulations and standards

Process Expectations

- Reducing the microbiological contamination
- Decontamination process
- On the inner surfaces of the isolator system
- Sporicidal process
- Achieving a 4 to 6 spore log reduction
  - 6 log reduction ≠ Total Kill of BI inoculated with 10⁶ spores
- No influence from inactivation process on products or tests
Biological Indicator

- Model of microbial reduction
- D-value determination
- Composition of biological indicators

Description of Biological Indicator

- Defined test organism: e.g. Geobacillus stearothermophilus
- Defined initial population: e.g. 1.0 x 10^6 [spores/carrier]
- Carrier material: e.g. stainless steel
- Primary packaging: e.g. Tyvek
- Inactivation method: e.g. gaseous H₂O₂
Basis and Selection of suitable BI as sensor for the inactivation process

Definition D-value
- Statistical calculation based on the ratio of positive / negative BI at different time exposure during a decontamination cycle
- Time in minutes to reduce the population by 1 log step, corresponding to 90% of the initial population
- Example: a D-value of 1.2 represents 1.2min for the population of one BI to be reduced from $10^6$ to $10^5$ spores/carrier

Use
- Information about the BI resistance – higher D-value representing a higher resistance (in reference isolator – characteristic of the BI)
- Information about the decontamination effect of an isolator – lower D-value representing a better decontamination effect (in isolator to be qualified – characteristic of the isolator)
- D-value from a BI lot depends on isolator → not a general statement

Cycle development with SIS 700
### LSKM SIS 700

#### Limited Spearmen Karber Method

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Pos.</th>
<th>Neg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure time min</td>
<td>6.0</td>
<td>9.0</td>
<td>12.0</td>
<td>15.0</td>
<td>21.0</td>
<td>24.0</td>
<td>27.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

+ = Growth  
0 = No growth

---

### Cycle development with SKANFOG®

#### Definition of Total Kill Time (sample)

![Diagram](image_url)

**Principle of SKANFOG®**
**LSKM Fogging process**

**Limited Spearmen Karber Method**

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Pos.</th>
<th>Neg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>0.25</td>
<td>0.50</td>
<td>0.75</td>
<td>1.00</td>
<td>1.25</td>
<td>1.50</td>
<td>1.75</td>
<td>2.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

**Basis and Selection of suitable BI as sensor for the inactivation process**

**Model of Microbial Reduction**

- Initial Population [log-scale]
- Inactivation Time [min]
- D-value [min]
- Survival – Kill Window [min]

**Survival Time [min]**

\[ \geq \text{D-value} \times (\text{log Population} - 2) \]

**Kill Time [min]**

\[ \leq \text{D-value} \times (\text{log Population} + 4) \]

acc. to ISO 14161
Influence of the Decontamination on different surfaces

Stainless steel not polished

Aluminium

Development and Quantification of Decontamination Cycles

Worst Case Study – SIS 700
Development and Quantification of Decontamination Cycles

Chemical Indicator Mapping (optional)

Qualitative evaluation of the homogeneity of the distribution of $\text{H}_2\text{O}_2$ everywhere in the isolator

Evaluation is made through the change of color of chemical indicator $\text{H}_2\text{O}_2$ sensitive

SKANFOG® Fix in Filling Lines

Baxter Halle, filling line

- Qualified 08/2015
- Entire cycle time < 70 min