Biological Indicators and $\text{H}_2\text{O}_2$ Decontamination of Isolator Systems

Volker Sigwarth
Skan AG
Switzerland

Process Development
Biological Indicators and $H_2O_2$ Decontamination

Process Development

- Overview of current Regulations and Standards
- Biological Indicators
- Investigation on Germ reducing Effects
- Method for Cycle Development
- Discussion of Risks, Possibilities, and Experience
Biological Indicators and $H_2O_2$ Decontamination
Process Development

• Research Article

PDA Journal

“Effect of Carrier Materials on the Resistance of Spores of Bacillus Stearothermophilus to gaseous Hydrogen Peroxide”

Volker Sigwarth, Skan AG
Alexandra Stärk, Novartis Pharma AG

PDA Journal, Vol. 57, No.1 January / February 2003
Biological Indicators and $H_2O_2$ Decontamination

Process Development

- **FDA**; GMP Guidance for Industry; Draft August 2003
  
  “Sterile Drug Products Produced by Aseptic Processing”

- **Decontamination Efficacy**

  “Process development and validation studies should include a thorough determination of cycle capability. The characteristics of these agents generally preclude the reliable use of statistical methods (e.g. fractional negative) to determine the process lethality (Ref. 13).”
Biological Indicators and $H_2O_2$ Decontamination
Process Development

- Research Article PDA Journal

“Effect of Carrier Materials on the Resistance of Spores of Bacillus
Stearothermophilus to gaseous Hydrogen Peroxide”

Volker Sigwarth, Skan AG
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- Cited as Reference (13); FDA GMP Guidance for Industry
Overview of current Regulation and Standards

Process Development

- **EN / ISO** 14161 “Guidance for Biological Indicators”
- **USP** 〈1208〉 „Sterility Testing - Validation of Isolator Systems”
  〈55〉 “Biological Indicators - Resistance Performance Test”
  〈1035〉 “Biological Indicators for Sterilization”
- **FDA** “Sterile Drug Produced by Aseptic Processing”
  Appendix 1: “Aseptic Processing Isolators”
- **PIC/S** Isolators Used For Aseptic Processing and Sterility Testing
Overview of current Regulation and Standards
Requirements for Validation

• Definition of Process- **Performance** and **Boundaries**
• Proof of Process- **Performance** within **Boundaries**
• Control of Process- **Parameters** and **Influences**

• **Process Comprehension** for **individual Application**
Biological Indicators and $H_2O_2$ Decontamination

Process Development

- Process Development of an alternative Sterilization Method
Biological Indicators for alternative Sterilization

- $\text{H}_2\text{O}_2$ Decontamination as alternative Sterilization Method
- Model of microbial Reduction Biological Indicators
- D-value Determination Biological Indicators
- Composition of Biological Indicators
  - Test Organism; Initial Population; Carrier Material; Primary Packaging
- Samples commercial available Biological Indicators
Standard Sterilization Methods

- Heat Processes
  - Steam Sterilization
  - Dry Heat Sterilization

- Radiation Processes
  - Gamma Radiation

- described in the Pharmacopoeias

- has to be used if possible

- Process Result
  - „Sterility Assurance Level” SAL

- described Correlation

Physical Parameters ➔ Sterilization Effect
Alternative Sterilization Methods

- Chemical Processes
  - Ethylene Oxide
  - Per Acetic Acid
  - Hydrogen Peroxide, \( \text{H}_2\text{O}_2 \)
  - Ozone
  - Chlorine Dioxide

- Physical Processes
  - Electron Beam
  - Micro Waves
  - UV Beam

- Use only possible if Standard Methods are not applicable
Alternative Sterilization Methods

• One Step *lower* than Standard Sterilization Methods

• Terminology
  - Sanitization
  - Decontamination
  - Inactivation
  - Disinfection

• Process Result „Spore Log Reduction“ *SLR*

• *Based on the Process Expectations* *individually defined*
Alternative Sterilization Methods

$H_2O_2$ Decontamination of Isolator Systems

- Surface Decontamination of the Isolator Chamber
- Vaporizing of aqueous $H_2O_2$ Solution
- Sporicidal Inactivation Process
- widely used in pharmaceutical Industry

- Process Parameters are individually applied
Isolator System

Material: stainless steel, glass
Volume: 1,4 m$^3$ (40 ft$^3$)
# Process Control

## Process

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
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<tbody>
<tr>
<td>Temperature</td>
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<td>Humidity</td>
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<tr>
<td>Air Velocity</td>
<td>m/s</td>
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<tr>
<td>Pressure</td>
<td>Pa</td>
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<tr>
<td>Mass / Balance</td>
<td>g</td>
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## $H_2O_2$ Gas-Concentration

<table>
<thead>
<tr>
<th>Method</th>
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<tr>
<td>Electro-chemical Sensor</td>
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<td>UV-Spectrometer</td>
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<td>IMS-Spectrometer</td>
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<tr>
<td>NIR-Spectrometer</td>
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<td>Wet-chemistry Method</td>
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</table>
Decontamination Cycle

Cycle Phase

Decontamination Effect

1 2 3 4 Cycle Phase

Volker Sigwarth, Skan AG, Switzerland  BI and Isolator Decontamination  February 2006


**Decontamination Cycle**

Phase 1: **Pre-conditioning**

to establish the initial conditions in the chamber
Decontamination Cycle

Phase 2: Conditioning
to establish the decontamination effect
**Decontamination Cycle**

Phase 3: **Decontamination**

to maintain stable decontamination effect
to ensure the total bacterial reduction over time
**Decontamination Cycle**

Phase 4: **Aeration**

to reach minimal residual $\text{H}_2\text{O}_2$ concentration
**Alternative Sterilization Methods**

- No useful Correlation of:

  \[ \text{Physical Parameters} \rightarrow \text{Inactivation Effect} \]

  - Relevant Process Parameters and Values not known
  - Range and Boundaries of Process Values not known
  - Design, Qualification, Routine Use generates Problems
  - Unexpected high Effort for the Qualification of a single Application
Alternative Sterilization Methods

• No useful Correlation of:

\[ \text{Physical Parameters} \rightarrow \text{Inactivation Effect} \]

• The use of alternative Inactivation Methods requires often:

  • \textit{Description of the Inactivation Effect} \textit{directly}
  
  • \textit{Measure of the Inactivation Effect}

  • \textit{by the Use of Biological Indicators}
Microbiological System

Description of Biological Indicator

- defined Test Organism
- defined initial Population
- Carrier Material
- Primary Packaging
- defined Resistance to a specified Inactivation Method
**Microbiological System**

**Resistance Description of Biological Indicator**

- Initial Population of the Test Organisms [CFU/Carrier]
- D-value [min]
- Survival - Kill Window [min]
  - Survival time [min] = D-value \times (\log \text{ Population} - 2)
  - Kill time [min] = D-value \times (\log \text{ Population} + 4)
**Microbiological System**

**Model of Microbial Reduction**

- Initial Population [log-scale]
- Inactivation Time [min]
- Survival Curve
- D-value [min]
- Survival - Kill Window [min]
Microbiological System

Probability Distribution, positive / negative

\[ P(m) = e^{-m} \]

- Probability of negative
- Probability of positive

63% and 37%
**Microbiological System**

**Model of Microbial Reduction**

- Initial Population [log-scale]
- Inactivation Time [min]
- Survival Curve
- D-value [min]
- Survival - Kill Window [min]
**Microbiological System**

D-value of Biological Indicators

- *Measure* of the Inactivation Effect
- *Quantification* achieved Inactivation Effect

**Important**

- D-value Slope of the Survival Curve
- corresponding Model Behavior survival, fractional, kill
Methods of D-value Determination

- Numeration of the residual Population after Inactivation
  Survivor Curve Method \( SCM \)

- Fractional Negative Methods
  Stumbo Murphy Cochran Method \( SMCM \)
  Limited Spearman Karber Method \( LSKM \)

Suitable Method: Selection of Biological Indicators
Description of the Inactivation Effect
**D-value Determination**

**SC Method**

- defined numbers of BI
- defined Exposure Times
- Numeration of residual Population
- Plot of Survivor Curve

- < 50% of initial Population
- > 50 Counts / Carrier
**D-value Determination**

**SC Method**
- Survival Window
- Beginning of fractional Field
- high Lab Effort
- good if *Total Kill* is not required
- Disinfection Testing
- “Last Chance Method”

**No Information about:**
- *Kill Window*
- *Model Behaviour*
**D-value Determination**

**SMCM**

- high Number of BIs
- one Exposure Time; fractional Field
- Relationship of survival / kill
- initial Population
- Calculate D-value

\[
P_n(r) = nC_r \times [P(m)]^r \times [1 - P(m)]^{n-r}
\]
**D-value Determination**

**SMC Method**
- initial Population
- Survival Window
- Beginning of fractional Field
- high statistical Accuracy
- low Lab Effort
- D-value has to be known
- good if *Total Kill* is not required

No Information about:
- *Kill Window*
- *Model Behaviour*

\[
P_n(r) = nC_r \times [P(m)]^r \times [1 - P(m)]^{n-r}
\]
### Stumbo Murphy Cochran Method (SMCM)

**Initial Population** \( N_0: 1.0 \times 10^6 \)

<table>
<thead>
<tr>
<th>exposure</th>
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<td>result</td>
<td>-   -   -   -   +   +   +   +   +   +   +   neg</td>
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</tbody>
</table>

**D-value** = 2.03 [min]

**95% CI D-value** = 2.03 ± 0.05 [min]

+ growth
- no growth
D-value Determination

LSKM

- several Groups of Bioindicators
- different Exposure Times
- observed Model Behavior
- initial Population
- D-value Calculation

\[ P_n(r) = \binom{n}{r} \times [P(m)]^r \times [1 - P(m)]^{n-r} \]
D-value Determination

LSKM

- Kill Window is covered
- Model Behavior is completely shown

\[ P_n(r) = \binom{n}{r} \times [P(m)]^r \times [1 - P(m)]^{n-r} \]
## Limited Spearman Karber Method  LSKM

Initial Population  \( N_0:1.0 \times 10^6 \)

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result

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</table>

D-value  =  2.06 [min]  
95% CI D-value  =  2.06 ± 0.22 [min]

+  growth
-  no growth

Volker Sigwarth, Skan AG, Switzerland  
BI and Isolator Decontamination  
February 2006
Methods of D-value Determination

LSKM

- Determination the *Resistance* of Biological Indicator
- Evaluation of the *complete Model Behaviour*

- Certification of commercial Biological Indicators
- High Effort and Costs
Description of the Inactivation Effect

Selection of Biological Indicators

Description of the Inactivation Effect

- good Estimation of the D-value
- good Understanding of the Model Behavior
- useful and pragmatic Tool
- good Relationship between Information and Costs
### Limited Spearman Karber Method  LSKM

**Initial Population** \( N_0: 1.0 \times 10^6 \)

<table>
<thead>
<tr>
<th>exposure</th>
<th>01</th>
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</table>

**D-value** \[ = 2.06 \text{ [min]} \]

**95% CI D-value** \[ = 2.06 \pm 0.22 \text{ [min]} \]

+  growth
-  no growth

Volker Sigwarth, Skan AG, Switzerland  
BI and Isolator Decontamination  
February 2006
Minimized LSKM, Reactive Pattern

Initial Population \( N_0:1.0 \times 10^6 \)

<table>
<thead>
<tr>
<th>Group</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
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</table>

estimated D-value = 2.0 [min]

- statistical Accuracy lower
- lost of Information in the fractional Field
- good Estimation of the D-value
Description of the Inactivation Effect

Selection of Biological Indicators

Description of the Inactivation Effect

- based on the Application of the minimized LSKM
- achieved Inactivation Effect can be quantified
- Model Behavior of Biological Indicator can be evaluated
- Application is useful and quite easy
- Good Relationship between Information and Costs
Selection of Biological Indicators

- Test Organism
- Initial Population
- Carrier Material
- Primary Packaging

Composition of the Biological Indicator has to reflect the Process Expectations
Process Expectations

$H_2O_2$ Decontamination of Isolator Systems

- Reduction of the microbial Contamination of the Isolator Chamber
- Reduction of the microbial Contamination on Surfaces

No Penetration of the Inactivation Effect

FDA: “Decontamination can be accomplished using a number of vaporizing agents, although these agents possess limited capability to penetrate obstructed or covered surfaces”
Process Expectations

$H_2O_2$ Decontamination of Isolator Systems

- Reduction of the microbial Contamination of the Isolator Chamber
- Reduction of the microbial Contamination on Surfaces

No Penetration of the Inactivation Effect

- Total Kill of a 6 log Population 10 log Reduction
Selection of Bioindicators Reference Isolator

- defined and controlled Reference Isolator
- described and proven $H_2O_2$ Decontamination Cycle
  
  achieved Decontamination Effect
  
  Stability of Decontamination Effect

Comparability of all following data
Selection of Bioindicators

**Test Organism**

*Pharmacopoeia USP* < 1035 >

- Test Organism "highly resistance"
- *vegetative Microbes*
- *Bacteria Spores*

**PIC/S:** “An understanding of the relationship between the resistance of the bioburden and that of the BI should be developed from trials and/or the literature”
### Selection of Bioindicators

#### Test Organism

**Vegetative Microbes**

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>D-value [min]</th>
<th>Carrier Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>&lt; 0.3</td>
<td>Glass</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&lt; 0.3</td>
<td>Glass</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>0.3 – 0.6</td>
<td>Glass</td>
</tr>
<tr>
<td>Acinetobaccter lwofii</td>
<td>0.6</td>
<td>Glass</td>
</tr>
<tr>
<td>Penicillium species</td>
<td>0.3 – 0.6</td>
<td>Glass</td>
</tr>
<tr>
<td>Aspergillus species</td>
<td>0.3</td>
<td>Glass</td>
</tr>
</tbody>
</table>

Method Deviation ± 10%
# Selection of Bioindicators

## Test Organism

### Bacteria Spores

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>D-value [min]</th>
<th>Carrier Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus sphaericus</td>
<td>1.2</td>
<td>Glass</td>
</tr>
<tr>
<td>Bacillus subtilis var. niger</td>
<td>1.3</td>
<td>Glass</td>
</tr>
<tr>
<td>Bacillus stearothermophilus ATCC 12980</td>
<td>1.3</td>
<td>Glass</td>
</tr>
<tr>
<td>Bacillus stearothermophilus ATCC 7953</td>
<td>1.4</td>
<td>Glass</td>
</tr>
</tbody>
</table>

Method Deviation ± 10%
Selection of Bioindicators

- vegetative Microbes  *significant lower Resistance than B. Spores*
- Bacteria Spores  *comparable Resistance*
Selection of Bioindicators  

*Bacillus stearothermophilus*

- generally applied and accepted
- highly stable
- Incubation Temperature 55-60 °C
- Selective against Cross Contamination  
  aseptic Handling

- **ATCC Strain 12980** generally used in the USA
- **ATCC Strain 7953** generally used in Europe
## Biological Indicator for gaseous H$_2$O$_2$

**Testorganism:** B. stearothermophilus  \( \text{ATCC 12980, min 1.0x10}^6 \)

**Carrier / Package:** Stainless steel / Tyvek

**Specified D-value:** 0.9 to 1.8 [min]

### Lot 01

<table>
<thead>
<tr>
<th>exposure time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
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<tbody>
<tr>
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</tbody>
</table>

### Lot 02

<table>
<thead>
<tr>
<th>exposure time [min]</th>
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<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
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<th>pos</th>
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</tr>
</tbody>
</table>

Volker Sigwarth, Skan AG, Switzerland
BI and Isolator Decontamination
February 2006
### Biological Indicator for gaseous H₂O₂

**Testorganism:** B. stearothermophilus ATCC 7953, min 1.0x10⁶

**Carrier / Package:** Stainless steel / Tyvek

**Spezified D-value:** 1.0 to 1.7 [min]

#### Lot 01

<table>
<thead>
<tr>
<th>exposure time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
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</table>

#### Lot 02

<table>
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<tr>
<th>exposure time [min]</th>
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<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>pos</th>
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<tbody>
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</tr>
</tbody>
</table>
Selection of Bioindicators

Test Organism

*Bacillus stearothermophilus*

commercially available Bioindicators with identical Composition

- bigger Differences of Resistance between *single Lots*
- than between *different Strains*
Selection of Bioindicators Population, $N_0$

- Initial Population "total kill" of a 6 log Population ?
- D-value independent of the initial Population
- D-value only depends Resistance of the individual Microbe

Assumptions based on the ideal Model empiric
Initial Population, \( N_0 \)

**Model of microbial Reduction**

- Survival Curve is a *straight Line*
- BI Resistance *independent* of \( N_0 \)
- *individual* Resistance of Microbe defines D-value of BI's
- *Slope* of Survival Curve identically observed
# Selection of Bioindicators

## Population, $N_0$

### Resistance in dependency of Initial Population

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Population, $N_0$ [log Steps]</th>
<th>D-value [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus luteus</td>
<td>$\geq 1.0 \times 10^3$</td>
<td>0.3</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>$\geq 1.0 \times 10^4$</td>
<td>0.3</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>$\geq 1.0 \times 10^5$</td>
<td>0.6</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>$\geq 1.0 \times 10^6$</td>
<td>$&gt; 3.6$</td>
</tr>
</tbody>
</table>

Method Deviation $\pm 10\%$
**Initial Population, $N_0$**

**Model of microbial Reduction**

- Resistance of BI seems to depend on $N_0$

- Survival Curve seems to be not straight

- Model becomes questioned
Population $N_0$  

Model Behavior

Test Organism: B. stearothermophilus  
carrier Material: Glass  

<table>
<thead>
<tr>
<th>Group</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure Time [min]</td>
<td>3.0</td>
<td>6.0</td>
<td>9.0</td>
<td>12.0</td>
<td>15.0</td>
<td>18.0</td>
<td>21.0</td>
<td>24.0</td>
<td>27.0</td>
<td>30.0</td>
<td>+</td>
</tr>
<tr>
<td>Result</td>
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<td>2</td>
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</tr>
</tbody>
</table>

- Initial Population: $1 \times 10^5$  
- estimated D-value: $1.3$ [min]  
- Model behavior: OK
**Population $N_0$   Model Behavior**

**Test Organism:** B. stearothermophilus  
**carrier Material:** Glass  

<table>
<thead>
<tr>
<th>Group</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
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</thead>
<tbody>
<tr>
<td>Exposure time [min]</td>
<td>3.0</td>
<td>6.0</td>
<td>9.0</td>
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</tr>
</tbody>
</table>

- Initial Population: $1 \times 10^6$
- estimated D-value: 1.9 [min]
- Model Behavior: not OK
Selection of Bioindicators Population, $N_0$

By Increasing the Initial Population of BI`s it can be observed

- *Increase in Resistance* higher D-value
- *Artifacts in the Model Behavior* late positive

$H_2O_2$ Decontamination Process seems to be *not confidence*
Selection of Bioindicators  

Dependency of Initial *Population* and *Resistance*

- Vegetative Microbes are relatively *big* forming Agglomerates
- Preparation significantly *difficult* forming Agglomerates
- Suspensions often highly *dirty* forming a Coating

Population, $N_0$
Selection of Bioindicators

Population, $N_0$

Micrococcus luteus 10E6
Selection of Bioindicators

Micrococcus luteus 10E3

Population, $N_0$
Selection of Bioindicators

Population, $N_0$
Selection of Bioindicators \hspace{1cm} Population, \( N_0 \)

Dependency of Initial Population and Resistance

- vegetative Microbes are relatively big forming Agglomerates
- Preparation significantly difficult forming Agglomerates
- Suspensions often highly dirty forming a Coating
- Agglomerates and Coating are not penetrated by \( \text{H}_2\text{O}_2 \)
- inconsistent Results of Bioindicators

The Penetration of the Decontamination Effect of the \( \text{H}_2\text{O}_2 \) Process is

- not ensured
- but also not expected
## Selection of Bioindicators

### Population, $N_0$

#### Dependency of Initial Population and Resistance

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Population, $N_0$ [log Steps]</th>
<th>D-value [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus stearothermophilus</td>
<td>$\geq 1.0 \times 10^2$</td>
<td>1.6</td>
</tr>
<tr>
<td>Bacillus stearothermophilus</td>
<td>$\geq 1.0 \times 10^4$</td>
<td>1.3</td>
</tr>
<tr>
<td>Bacillus stearothermophilus</td>
<td>$\geq 1.0 \times 10^6$</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Method Deviation $\pm 10\%$
Selection of Bioindicators Population, $N_0$
Selection of Bioindicators Population, $N_0$
Selection of Bioindicators

Population, $N_0$
Selection of Bioindicators

Carrier Material

**PIC/S Recommendation on Isolator Technology**

“The carrier type e.g. plastic, paper, metal or other, of the biological indicator organism should be relevant to the materials being gassed or shown to be irrelevant”

**FDA Comments on Isolator Technology**

“Rationale and justification of the use of stainless steel coupons as the challenge carrier for biological indicators”
Selection of Bioindicators

Carrier Material

Isolator Systems

- Stainless Steel: Isolator Chamber, Filling line, dif. Equipment
- Glass: Isolator Windows, div. Product Enclosures
- dif. Metals: e.g. Aluminum

Biological Indicator

- Should reflect the Process
- Should behave according to the Model of microbial Reduction
Effect of Carrier Materials on the Resistance of B. stearothermophilus to gaseous H$_2$O$_2$
## Selection of Bioindicators

### Carrier Material

<table>
<thead>
<tr>
<th>Materials</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>CrNi steel, different qualities:</td>
<td></td>
</tr>
<tr>
<td>1.4301 (304) unpolished</td>
<td>Main chamber material</td>
</tr>
<tr>
<td>1.4301 (304) polished (Ra &lt; 0.8 μm)</td>
<td>Parts of filling line</td>
</tr>
<tr>
<td>1.4435 (316L) unpolished</td>
<td>Steritest pump</td>
</tr>
<tr>
<td>1.4435 (316L) polished (Ra &lt; 0.8 μm)</td>
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<td>Glass</td>
<td>Window and door material</td>
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<td>Media bottles, product units</td>
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<td>Polycarbonate, PC</td>
<td>Window material</td>
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<td>Hypalon</td>
<td>Glove material</td>
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<td>Polyvinylchloride, PVC, soft</td>
<td>Material of glove gauntlets</td>
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<tr>
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<td>Glove ports</td>
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<td>Package of steritest units</td>
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<td>Polypropylene, PP</td>
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<td>HEPA-filter pad</td>
<td>HEPA-filter</td>
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</table>
Carrier Material; Glass

Roughness
• P-P: 137 nm
• RMS: 3 nm
• $R_a$: 1 nm

Wettability
• high
## Carrier Material, Model Behavior

### Test Organism:
B. stearothermophilus

### Initial Population:
> 1.0 x 10^6

### Carrier Material:
Glass

### Results Table

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<tr>
<th>Group</th>
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<th>02</th>
<th>03</th>
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</table>

- estimated D-value: 1.2 [min]
- Model Behavior: OK
**Carrier Material; stainless steel 1.4435, not polished**

### Roughness

- P-P: 817 nm
- RMS: 82 nm
- $R_a$: 57 nm

### Wettability

- high
# Carrier Material, Model Behavior

**Test Organism:** B. stearothermophilus  
**Initial Population:** > 1.0 x 10^6  
**Carrier Material:** Stainless Steel, not polished

<table>
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- estimated D-value: **1.3 [min]**  
- Model Behavior: **OK**
Carrier Material; stainless steel 1.4435, polished

Roughness
- P-P: 1064 nm
- RMS: 165 nm
- $R_a$: 127 nm

Wettability
- high
Carrier Material, Model Behavior

Test Organism: B. stearothermophilus
Initial Population: > 1.0 \times 10^6
Carrier Material: Stainless Steel, polished

+ growth
- no growth

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<tr>
<th>Group</th>
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</table>

- estimated D-value: 0.9 [min]
- Model Behavior: OK
Carrier Material; PTFE, Teflon

Roughness
• P-P: 1266 nm
• RMS: 180 nm
• Rₐ: 138 nm

Wettability
• low
**Carrier Material, Model Behavior**

**Test Organism:** B. stearothermophilus  
**Initial Population:** > 1.0 x 10^6  
**Carrier Material:** PTFE

+ growth  
- no growth

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<th>Group</th>
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</table>

- estimated D-value: 1.6 [min]  
- Model Behavior: OK
Carrier Material, Hypalon

Roughness

- P-P: 2114 nm
- RMS: 350 nm
- $R_a$: 286 nm

Wettability

- low
### Carrier Material, Model Behavior

- **Test Organism:** B. stearothermophilus
- **Initial Population:** > $1.0 \times 10^6$
- **Carrier Material:** Hypalon

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</table>

- Estimated $D$-value: 3.0 [min]
- Model Behavior: OK
Carrier Material, Aluminium; commercial Sample

Roughness
• P-P: 1322 nm
• RMS: 188 nm
• $R_a$: 145 nm

Wettability
• high
Carrier Material, Model Behavior

Test Organism: B. stearothermophilus
Initial Population: > 1.0 x 10⁶
Carrier Material: Aluminum, anodized

<table>
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<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
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</table>

- estimated D-value: 8.4 [min]
- Model Behavior: not OK
**Carrier Material**,  Aluminium; Air Sampler

**Roughness**
- P-P: 1436 nm
- RMS: 290 nm
- $R_a$: 236 nm

**Wettability**
- high
## Carrier Material, Model Behavior

<table>
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</table>

- estimated D-value: 7.9 [min]
- Model Behavior: OK
Carrier Material, Aluminium; Filling Line

Roughness
- P-P: 1478 nm
- RMS: 132 nm
- Rₐ: 94 nm

Wettability
- high
Carrier Material, Model Behavior

Test Organism: B. stearothermophilus
Initial Population: > 1.0 x 10³
Carrier Material: Aluminum, anodized

+ growth
- no growth

<table>
<thead>
<tr>
<th>Group</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
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- estimated D-value: > 33.7 [min]
- Model Behavior: not able to be evaluated
## Selection of Bioindicators

### Carrier Material of BI

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<th>Carrier Material of BI</th>
<th>D-value Estimations [mins]</th>
<th>Model Behaviour</th>
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<tbody>
<tr>
<td>Glass</td>
<td>1.0 / 1.1</td>
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<td>CrNi steel 1.4435, polished</td>
<td>1.3 / 0.9</td>
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<td>1.0 / 1.2</td>
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<tr>
<td>PC</td>
<td>2.2 / 2.3</td>
<td>given</td>
</tr>
<tr>
<td>BI, commercially available</td>
<td>2.6 / 2.3</td>
<td>given</td>
</tr>
<tr>
<td>Tyvek</td>
<td>2.0 / 3.1</td>
<td>given</td>
</tr>
<tr>
<td>Laminated foil 2, outside</td>
<td>2.5 / 3.2</td>
<td>given</td>
</tr>
<tr>
<td>Butyl chaouchouc</td>
<td>2.9 / 3.1</td>
<td>given</td>
</tr>
<tr>
<td>Hypalon</td>
<td>3.0 / 4.1</td>
<td>given</td>
</tr>
<tr>
<td>HEPA-filter pad</td>
<td>3.6 / 3.6</td>
<td>given</td>
</tr>
<tr>
<td>PVC, soft</td>
<td>4.3 / 4.6</td>
<td>given</td>
</tr>
<tr>
<td>POM</td>
<td>4.6 / 4.4</td>
<td>given</td>
</tr>
<tr>
<td>Aluminium, anodized, commercially available</td>
<td>&gt; 3.1 / 7.9</td>
<td>not given</td>
</tr>
<tr>
<td>Aluminium, anodized, air sampler</td>
<td>&gt;8.3 / 10.1</td>
<td>given</td>
</tr>
<tr>
<td>Aluminium, anodized, filling line</td>
<td>&gt;17.1 / &gt; 33.7</td>
<td>not applicable</td>
</tr>
</tbody>
</table>
Selection of Bioindicators

**Box and Whisker Plot**

Significantly higher D-value for the various aluminium samples
Selection of Bioindicators Carrier Material

Unsuitable Carrier Material

- Negative influence on the Resistance of Bioindicators
- Negative influence on the Model Behavior of Bioindicators
- Materials are spongy or porous
- Test Organism penetrate into those Materials
- not or only hart to be reached by \( \text{H}_2\text{O}_2 \) Decontamination
Carrier Material  stainless steel, not polished

Volker Sigwarth, Skan AG, Switzerland  BI and Isolator Decontamination  February 2006
Carrier Material  stainless steel, not polished
Carrier Material: stainless steel, not polished
Carrier Material  stainless steel, not polished
Carrier Material: Aluminium; Air Sampler

Volker Sigwarth, Skan AG, Switzerland
BI and Isolator Decontamination
February 2006
Carrier Material: Aluminium; Air Sampler
Carrier Material  Aluminium; Air Sampler
Carrier Material  
Aluminium anodized
Carrier Material

Aluminium anodized

Volker Sigwarth, Skan AG, Switzerland

BI and Isolator Decontamination

February 2006
Carrier Material  Aluminium anodized
Carrier Material  Aluminium anodized
Carrier Material

Aluminium anodized
Selection of Bioindicators  

**Carrier Material**

**Unsuitable Carrier Material**

- Negative influence on the *Resistance* of Bioindicators
- Negative influence on the *Model Behavior* of Bioindicators
- Materials are *spongy* or *porous*
- Test Organism *penetrate into* those Materials
- *not or only hart* to be reached by H$_2$O$_2$ Decontamination

The *Penetration* of the Decontamination Effect of the H$_2$O$_2$ Process is

- *not ensured*
- *but also not expected*
Selection of Bioindicators

*Carrier Material*

- Polyoxymethylene, POM
- Polyvinylchlorid, PVC soft
- HEPA-filter pad
- Hypalon
- Butyl caoutchouc
- Reference BI
- Laminated foil 2
- Tyvek
- Polycarbonate, PC
- Laminated foil 1
- Polyvinylchlorid, PVC
- Polypropylene, PP
- Polyethylene, PE UHMW
- Polytetrafluorethylen PTFE
- Polyvinylchlorid, PVC hard
- Stainless steel 1.4301, polished
- Stainless steel 1.4435, not polished
- Stainless steel 1.4301, not polished
- Stainless steel 1.4435, polished
- Glass

![Graph showing the selection of bioindicators and carrier materials with D-values for different materials.](image)

**D-value [min]**

- Worst Case 4.33
- Best Case 1.45
- Reference 2.28
Selection of Bioindicators   Primary Packaging

Standards    EN/ISO 14161

„Primary Packaging should not effect the Inactivation and protect the inoculated Carrier against Destruction and Contamination“

$H_2O_2$ Decontamination

- semi permeable Membrane       Tyvek
- permeable for $H_2O_2$
- Barrier for Contamination
Primary Packaging

Test Organism: B. stearothermophilus
Initial Population: > 1.0 x 10^6
Carrier Material: Stainless Steel

<table>
<thead>
<tr>
<th>Group</th>
<th>Exposure Time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
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<tr>
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<td>+</td>
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<td></td>
</tr>
</tbody>
</table>

- Primary Packaging: Tyvek
- estimated D-value: 1.2 [min]
- Model Behavior: OK
**Primary Packaging**

- **Test Organism:** B. stearothermophilus
- **Initial Population:** > 1.0 x 10^6
- **Carrier Material:** Stainless Steel

<table>
<thead>
<tr>
<th>Group</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
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<td>14.0</td>
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<td>18.0</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- **Primary Packaging:** without primary Packaging
- **estimated D-value:** 0.8 [min]
- **Model Behavior:** OK
Selection of Biological Indicators

BI Composition has to reflect the Process Requirements

- Test organisms  
  *Bacteria Spores* generally suitable

- Initial Population  
  *Preparation* has to be suitable for Process

- Carrier Material  
  *Properties* has to be suitable for Process

- Primary Packaging  
  *Properties* has to be suitable for Process

- Bioindicators  
  *reflect* the Decontamination effect *realistic*

- Bioindicators  
  *show a conform* Model Behavior
Selection of Biological Indicators

• Bioindicators show **unrealistic** high Resistances
• Bioindicators show **Artifacts** in Model Behavior

**Interpretation of the Process**

• Bioindicators show **Process- Performance und Boundaries**
• Bioindicators are **to high Process Challenge**
• Bioindicators show **Process to be overrated**
• Bioindicators show **Kind and Extent of acceptable Bioburden**

**Definition:** **Process- Performance und Expectations**
# Biological Indicator for gaseous $H_2O_2$

**Testorganism:** B. stearothermophilus ATCC 12980, min $4.5 \times 10^5$

**Carrier / Package:** Glasfibre / Tyvek

**Specified D-value:** 1.5 [min]

### Run 01

<table>
<thead>
<tr>
<th>exposure time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
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<th>pos</th>
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</thead>
<tbody>
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<td>result</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>-</td>
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### Run 02

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<th>03</th>
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<th>06</th>
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<th>10</th>
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</thead>
<tbody>
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<td>-</td>
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<td>-</td>
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</tr>
</tbody>
</table>
# Biological Indicator for gaseous $H_2O_2$

**Testorganism:** B. stearothermophilus ATCC 7953, min $1.0 \times 10^6$

**Carrier / Package:** Glasfibre / Tyvek, PE

**Spezified D-value:** 3.1 [min]  

## Run 01

<table>
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<tr>
<th>exposure time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
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<th>06</th>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Positivity:** + growth  
**Negativity:** - no growth

## Run 02

<table>
<thead>
<tr>
<th>exposure time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
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<tbody>
<tr>
<td>result</td>
<td>+</td>
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<td>-</td>
</tr>
</tbody>
</table>
## Selection of Biological Indicators

**Steam**

**Test Organism:** B. stearothermophilus  
**Initial Population:** $> 1.0 \times 10^6$  
**Carrier Material:** Paper

### Results Table

<table>
<thead>
<tr>
<th>Group</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>pos</th>
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<tbody>
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<td>30.0</td>
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<td>+</td>
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</tr>
</tbody>
</table>

- estimated D-value: $> 17.0$ [min]  
- Model Behavior: not able to be evaluated
Selection of Biological Indicators

Test Organism: B. stearothermophilus
Initial Population: > 1.0 x 10^6
Carrier Material: CrNi- Stahl
Primary packaging: Tyvek

<table>
<thead>
<tr>
<th>Group</th>
<th>Exposure Time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
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<td>-</td>
</tr>
</tbody>
</table>

- estimated D-value: = 2.0 [min]
- Model Behavior: = OK

+ growth
- no growth
Selection of Biological Indicators

Alternative Sterilization Methods

• Resistance and Model Behavior describes Bioindicator
• Process- Performance and Expectations defines Bioindicator
• careful Selection of Composition Bioindicators

Artifacts in the Results of Bioindicators Process Boundaries
Biological Indicators and
H$_2$O$_2$ Decontamination of Isolator Systems

Volker Sigwarth
Skan AG, Switzerland

Alexandra Stärk
Novartis Pharma AG, Switzerland
Biological Indicators and
$H_2O_2$ Decontamination of Isolator Systems

Volker Sigwarth
Skan AG
Switzerland

Process Development
Biological Indicators and $H_2O_2$ Decontamination

Process Development

• Process Development of alternative Sterilization Method
Investigation on Germ Reducing Parameters of the $\text{H}_2\text{O}_2$ Decontamination Method

- **Process Development**

- Establish parameters of microbial reducing effects of $\text{H}_2\text{O}_2$ decontamination

- Target value is the *D-value* of a defined microbiological system

- Using the *Design of Experiment* method as statistical tool

- Statistical significant quantification of the influence of effects

- Correlation of *Process Parameter* versus *Kill Effect*
Investigation on Germ Reducing Parameters of the H$_2$O$_2$ Decontamination Method

• Comprehension of the decontamination process

• The nice and need to have parameters for the decontamination success

• Hints for Design, Qualification and Monitoring

• Method to describe, develop and quantify H$_2$O$_2$ decontamination cycles
Investigation on Germ Reducing Parameters of the H$_2$O$_2$ Decontamination Method

- Test - System and - Equipment
- Microbiological System
- Decontamination Process
- Selection of the experimental Factors
- Design of Experiment
- Result of Investigation and Interpretation
- Summary
**Test - Isolator**

- **Material:** stainless steel, glass
- **Volume:** 1,4 m³ (40 ft³)

---

Volker Sigwarth, Skan AG, Switzerland  
BI and Isolator Decontamination  
February 2006
# Sensors

<table>
<thead>
<tr>
<th>Process</th>
<th>$H_2O_2$ Gas-Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Electro-chemical Sensor</td>
</tr>
<tr>
<td>Humidity</td>
<td>UV-Spectrometer</td>
</tr>
<tr>
<td>Air Velocity</td>
<td>IMS-Spectrometer</td>
</tr>
<tr>
<td>Pressure</td>
<td>NIR-Spectrometer</td>
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<tr>
<td>Mass / Balance</td>
<td>Wet-chemistry Method</td>
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<td>Humidity</td>
<td>[m/s]</td>
</tr>
<tr>
<td>Pressure</td>
<td>[Pa]</td>
</tr>
<tr>
<td>Mass / Balance</td>
<td>[g]</td>
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</table>
Microbiological System

- Commercially available Biological Indicators
- Bacillus stearothermophilus ATCC 12980
- Stainless Steel Carriers
- Tyvek Pouches
Microbiological System

Model of Microbial Reduction

- Initial Population [log-scale]
- Inactivation Time [min]
- Survival Curve
- D-value [min]
- Survival - Kill Window [min]
**Minimized LSKM, Reactive Pattern**

<table>
<thead>
<tr>
<th>exposure</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
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</tbody>
</table>

estimated D-value = 2.0 [min]

+  growth
-  no growth
**Limited Spearman Karber Method (LSKM)**

<table>
<thead>
<tr>
<th>exposure</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
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</tr>
</tbody>
</table>

**D-value** = 2.06 [min]  

**95% CI D-value** = 2.06 ± 0.22 [min]  

+ growth  
- no growth
Decontamination Cycle

Decontamination Effect

1 2 3 4 Cycle Phase

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**Decontamination Cycle**

Phase 1: **Pre-conditioning**

to establish the initial conditions in the chamber
Phase 2: **Conditioning**

to establish the decontamination effect
Phase 3: **Decontamination**

to maintain stable decontamination effect
to ensure the total bacterial reduction over time
**Decontamination Cycle**

Phase 4: **Aeration**

to reach minimal residual $\text{H}_2\text{O}_2$ concentration
Selection of Factors

Vaporized Quantity of H$_2$O$_2$
Selection of Factors

Pre-conditioning
Initial conditions in the chamber

Temperature [°C]
Humidity [%rH]

Vaporized Quantity of H₂O₂

Cycle Phase

Volker Sigwarth, Skan AG, Switzerland
BI and Isolator Decontamination
February 2006
Selection of Factors

Conditioning
Vaporized initial quantity of pure $\text{H}_2\text{O}_2$ Quantity $[\text{g/m}^3]$
Selection of Factors

Decontamination
Rate of continuously vaporized pure $\text{H}_2\text{O}_2$ Redose [%/h]
Selection of Factors

Special
Concentration of H$_2$O$_2$ Solution $H_2O_2$ [%]

5 factors selected for the investigation

A: Quantity of pure H$_2$O$_2$ [g/m$^3$]
B: Rate of Redose [%A/h]
C: Temperature [°C]
D: Humidity [%rH]
E: Concentration of H$_2$O$_2$ [%]
## Range of Factors

<table>
<thead>
<tr>
<th>No:</th>
<th>Description</th>
<th>Unit</th>
<th>SP-</th>
<th>FF-</th>
<th>CP</th>
<th>FF+</th>
<th>SP+</th>
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</thead>
<tbody>
<tr>
<td>A:</td>
<td>Quantity of pure H$_2$O$_2$</td>
<td>g/m$^3$</td>
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<tr>
<td>B:</td>
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<td>% A/h</td>
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<td>40</td>
<td>70</td>
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<tr>
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<td>Humidity</td>
<td>% rH</td>
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<td>10</td>
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<td>20</td>
<td>24</td>
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<tr>
<td>E:</td>
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<td>%</td>
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<td>35</td>
<td>42.5</td>
<td>50</td>
<td>55</td>
</tr>
</tbody>
</table>
**Design of Experiment (DoE)**

- Fractional Factorial Plan (FF)
- Centre Point (CP)
- Star Point (SP)

Independent Estimation of the Parameters for:

- Main Effects
- Quadratic Effects
- Interactions

- + 4 independent determinations of Centre Point
## Range of Factors

<table>
<thead>
<tr>
<th>No:</th>
<th>Description</th>
<th>Unit</th>
<th>SP-</th>
<th>FF-</th>
<th>CP</th>
<th>FF+</th>
<th>SP+</th>
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<td>6.5</td>
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<tr>
<td>B:</td>
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<td>% A/h</td>
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<td>C:</td>
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<td>D:</td>
<td>Humidity</td>
<td>% rH</td>
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<td>10</td>
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<td>E:</td>
<td>Concentration of H₂O₂</td>
<td>%</td>
<td>30</td>
<td>35</td>
<td>42.5</td>
<td>50</td>
<td>55</td>
</tr>
</tbody>
</table>
Test Handling

- place BI`s in the isolator gastight wrapped
Test Handling

- establish required initial conditions
- start vaporizing H$_2$O$_2$ up to required quantity
**Test Handling**

- **Expose BI`s to the inactivation atmosphere**
- **Start redosing**

Diagram showing vaporized quantity of $\text{H}_2\text{O}_2$ over cycle phase.
Test Handling

- remove BI`s out of the chamber in constant time intervals
Results and Interpretation  

Centre Point

Independent Centre Point Determinations

- full LSKM 10 groups, 10 BI`s per group

<table>
<thead>
<tr>
<th>Run No.</th>
<th>D-value [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.75</td>
</tr>
<tr>
<td>2</td>
<td>1.80</td>
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<tr>
<td>3</td>
<td>1.74</td>
</tr>
<tr>
<td>4</td>
<td>1.71</td>
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</tbody>
</table>

- **Constant** parameters lead to a **reproducible** decontamination effect
Results and Interpretation Model

Standardized Pareto Chart for D-value

A: Quantity
AA
B: Redose
AB
E: H₂O₂
C: Temp.
D: Humidity
CD

Quantity of H₂O₂

Standardized effect
Results and Interpretation  Quantity (A)

Main Effects Plot for D-value

D-value

Quantity

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Results and Interpretation

Plot of D-value versus Quantity (A)

- Quantity [g/m³]
- D-value [min]
Results and Interpretation

quantity (A)

• Quantity of vaporized H₂O₂ is the Main Effect in decontamination process

• Nonlinear effect quadratic fit within range of model

• Asymptotic character observed with larger range than model
**Results and Interpretation**

- Starting with constant initial conditions boost the quantity of vaporized \( \text{H}_2\text{O}_2 \)
  leads to a *Steady State* in decontamination effect
Results and Interpretation  Redose (B)

- The **higher** the rates of Redose (B) the **better** the decontamination effect
Results and Interpretation Interaction (AB)

Interaction Plot for D-value

D-value

Redose 40.0

Redose 100.0

5.0  Quantity  8.0

Redose 40.0

Redose 100.0
Results and Interpretation Interaction (AB)

- Redose (B) loses its influence at higher values of factor Quantity (A)
- Only high rates of Redose (B) improve a low decontamination effect
Results and Interpretation Interaction (AB)

- **Stability** of decontamination effect over time depends on the **Rate of Redose**
Results and Interpretation

Temperature (C)
Humidity (D)

Main Effects Plot for D-value
**Results and Interpretation**  

**Temperature (C)**

- The *lower* the Temperature (C) the *better* the decontamination effect.

- Minor effect: change in D-value of only + 0.5 [min]  
  within a temperature range of 10 [°C]
Results and Interpretation

Temperature (°C)

- **Worst Case** positions for the decontamination effect are positions with *higher temperature*.
Results and Interpretation  

Humidity (D)

- The **higher** the Humidity (D) the **better** the decontamination effect

- Minor effect: change in D-value of only - 0.4 [min] within a humidity range of 10 [% rH]
Results and Interpretation  

Humidity (D)

- **Worst Case** positions for the decontamination effect are positions with **lower humidity**
Results and Interpretation  $H_2O_2 (E)$

Main Effects Plot for D-value

<table>
<thead>
<tr>
<th>D-value</th>
<th>1.27</th>
<th>1.87</th>
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<tbody>
<tr>
<td>35.0</td>
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<tr>
<td>$H_2O_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results and Interpretation  \( H_2O_2 (E) \)

- The lower the Concentration of H\(_2\)O\(_2\) Solution (E) the better the decontamination effect

- Minor effect: change in D-value of only + 0.6 [min] within a concentration range of 15 [%]
**Results and Interpretation**  \( \text{H}_2\text{O}_2 (E) \)

- Vaporization of the same quantity of pure \( \text{H}_2\text{O}_2 \) produces a **better** decontamination **effect**
  
  at **lower concentrations** of the \( \text{H}_2\text{O}_2 \) solution
Results and Interpretation

One main **Interrelationship** between all Factors?

A: Quantity  Steady State  higher saturation of gaseous phase
B: Redose  Stability  higher saturation of gaseous phase
C: Temp.  lower temperature  higher saturation of gaseous phase
D: Humidity  higher humidity  higher saturation of gaseous phase
E: $\text{H}_2\text{O}_2$  lower concentration  higher saturation of gaseous phase

- Decontamination effect depends on **Saturation of gaseous phase**
- **“Physical Pressure”** from gaseous phase to surface
Results and Interpretation  \( \text{H}_2\text{O}_2 \) Gas-Concentration

- No useful correlation to the microbial reduction
- Depends highly on the temperature
- Most sensors show the same shape of measurement curve, but highly differences in values
Results and Interpretation    \(\text{H}_2\text{O}_2\) Gas-Concentration
Results and Interpretation  \( \text{H}_2\text{O}_2 \) Gas-Concentration

- No useful correlation to the microbial reduction
- Depends highly on the temperature
- Most sensors show the same shape of measurement curve, but highly differences in values
  
  - **Calibration method !!!**

- Not yet a tool for describe microbial reduction
Results and Interpretation  $H_2O_2$ Gas-Concentration

Measurement of process concentration
• useful as indicative measurement

Measurement of residual $H_2O_2$ concentration
• important for the final release of the decontaminated area
**Description of Decontamination Effect**

The decontamination effect is described by:

- the results of the minimized LSKM
- the only useful sensor is the BI
- the minimized LSKM leads to quantifiable results

- the suitability of the BI has to be tested prior
- to “calibrate” the BI as
  
  *Sensor for the decontamination effect*

**Development and Quantification of H$_2$O$_2$ Decontamination Cycles**
Selection of Biological Indicators

Test Organism: B. stearothermophilus
Initial Population: > 1.0 x 10^6
Carrier Material: CrNi- Stahl
Primary packaging: Tyvek

<table>
<thead>
<tr>
<th>Group</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
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<td>Exposure Time [min]</td>
<td>6.0</td>
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<td>15.0</td>
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<td>-</td>
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<td></td>
</tr>
</tbody>
</table>

- estimated D-value: = 2.0 [min]
- Model Behavior: = OK

+ growth
- no growth
Description of Cycle

Physical

Vaporized Quantity of $\text{H}_2\text{O}_2$

Cycle Phase

1  2  3  4

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BI and Isolator Decontamination
February 2006
Description of Cycle

Physical

Reproducability of Decontamination Effect

Initial conditions in chamber

Temperature  ± 5 [°C]

Humidity ± 5 [%rH]
Description of Cycle

Achieved Decontamination Effect

Quantity of initial vaporized $\text{H}_2\text{O}_2$  mass control, balance $[g]$
Description of Cycle

**Physical**

![Graph showing vaporized quantity of H₂O₂ over cycle phases 1 to 4.]

Stability of Decontamination Effect

*Rate of Redose H₂O₂*  
mass control, balance  
[g/t]
Description of Cycle

**Physical**

![Graph showing vaporized quantity of H₂O₂ over cycle phase.](image)

- **Residual Concentration of H₂O₂**
- **Aeration curve**
- Low concentration sensor [ppm/t]
Description of Cycle  Decontamination effect

- Based on a well known and defined BI
- Using the minimized LSKM as a methodical tool
- Proof the decontamination effect over the whole cycle
- Define the required values for cycle parameters
Description of Cycle  Decontamination effect

Decontamination Effect

Cycle Phase

1 2 3 4
**Description of Cycle**  

**Decontamination effect**

Reproducability of Decontamination Effect

*Physically defined by:*

- Temperature: $\pm 5$ [°C]
- Humidity: $\pm 5$ [%rH]
Temperature and Humidity Mapping

%Hr

°C

Time

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BI and Isolator Decontamination
February 2006
## Description of Cycle

<table>
<thead>
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<th>Cycle Phase</th>
<th>Decontamination Effect</th>
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<tr>
<td>1</td>
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<tr>
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<td>Achieved D-value</td>
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<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

### Achieved Decontamination Effect

Achieved D-value, defined initial Quantity of $H_2O_2$ [g]

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BI and Isolator Decontamination  
February 2006
Achieved Decontamination effect

Achieved decontamination effect

Quantity 5 [g/m³]

<table>
<thead>
<tr>
<th>exposure time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
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</tbody>
</table>

+ growth
- no growth
Achieved Decontamination effect

Achieved decontamination effect

Quantity 7.5 [g/m$^3$]

<table>
<thead>
<tr>
<th>exposure time [min]</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
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</tr>
<tr>
<td>result</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>neg</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>3</td>
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<td></td>
</tr>
</tbody>
</table>

+  growth
-  no growth
Achieved Decontamination effect

Plot of D-value versus Quantity
### Description of Cycle and Decontamination Effect

#### Decontamination Effect

<table>
<thead>
<tr>
<th>Cycle Phase</th>
<th>Decontamination Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

#### Stability of Decontamination Effect

- **Stability of D-values** defined
- **Rate of Redose [g/t]**

---

Volker Sigwarth, Skan AG, Switzerland

BI and Isolator Decontamination

February 2006
# Stability of Decontamination effect

Rate of Redose 25 [%A/h]

### LSKM 1, exposition 5 [min] after conditioning

<table>
<thead>
<tr>
<th>exposure</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>exposure time [min]</td>
<td>7.5</td>
<td>10.0</td>
<td>12.5</td>
<td>15.0</td>
<td>17.5</td>
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<td></td>
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<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>result</td>
<td>1</td>
<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>neg</td>
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<td>2</td>
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</tr>
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</table>

### LSKM 2, exposition 30 [min] after conditioning

<table>
<thead>
<tr>
<th>exposure</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>pos</th>
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</thead>
<tbody>
<tr>
<td>exposure time [min]</td>
<td>7.5</td>
<td>10.0</td>
<td>12.5</td>
<td>15.0</td>
<td>17.5</td>
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<tr>
<td>result</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>neg</td>
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<td></td>
<td>2</td>
<td>+</td>
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</tr>
</tbody>
</table>
**Stability of Decontamination effect**

Rate of Redose 100 [%A/h]

**LSKM 1, exposition 5 [min] after conditioning**

<table>
<thead>
<tr>
<th>exposure</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
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</table>

**LSKM 2, exposition 30 [min] after conditioning**

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<th>03</th>
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</tr>
</tbody>
</table>
**Description of Cycle**  
**Worst Case Positions**

Definition of Worst Case Positions, (BI- Positions)

- Air Flow and Distribution  
- Temperature, Humidity Distribution  
- Chemo Indicator Mapping  
- deeply consider the Process

**Extreme Point of Application**  
**no Assumptions**
Description of Cycle  Decontamination effect

Duration of Decontamination Phase

Worst Case Study defined D-value Worst Case

Duration of Decontamination [t]
Description of Cycle  Worst Case Positions

Assessment of Worst Case Positions

• 3x BI’s per defined WC Position  fractional results possible

• Deco time 10 x D-value  best place  shortest cycle to total kill

• Use resulting BI pattern to estimate kill  - - - 10log, - - + 6log, + + + ??

• Calculate D-value worst case  Deco time / achieved reduction

• longer Deco time if required  10 x D-value worst case
Description of Cycle  Decontamination effect

<table>
<thead>
<tr>
<th>Cycle Phase</th>
<th>Decontamination Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><strong>D-value</strong></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Required log Reduction

- observed *D*-values
- **defined**
- achieved log Reduction

Volker Sigwarth, Skan AG, Switzerland

BI and Isolator Decontamination

February 2006
**H₂O₂ Decontamination Process**

- Reproducable and stable process *if well developed*
- Development contents two systems  
  1. Decontamination System  
  2. Microbiological System
- Recognize the suitability of the microbiological system before hand
- Develop the decontamination cycle using:  
  the “calibrated BI *and*  
  the whole physical range *no assumption*
- Transparent and systematic process development and comprehension
- Strong evidence for the following step  
  Performance Qualification
Development and Quantification of $H_2O_2$ Decontamination Cycles

- Validation Article
  PDA Journal

  “Development and Quantification of $H_2O_2$ Decontamination Cycles”

  Volker Sigwarth, Dr. Claude Moirandat, Skan AG

  PDA Journal, Vol. 54, July / August 2000
Reference Studies for $H_2O_2$ Decontamination

- Suitability study for all commercially available BI`s
- D-value studies for a wide range of spores and vegetative germs
- Customized determination of virus decontamination
- Special studies for devices and equipment

- Effect of Carrier Materials on the Resistances of BI`s
- Co-operation with Novartis, Stein
Material Study

- Materials used in isolator as construction materials as disposables in routine work
- Inoculate with min 1.0x10^6 spores of B. stearothermophilus
- Determination of D-value and Reactive Pattern
- Compare the results with commercially available BI
- Transfer results to different Production Isolator Systems

- Conclusion “HOW to handle the Material Question”
## Materials Study Results

<table>
<thead>
<tr>
<th>Carrier Material of BI</th>
<th>D-value Estimations [mins]</th>
<th>Model Behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>1.0 / 1.1</td>
<td>given</td>
</tr>
<tr>
<td>CrNi steel 1.4435, polished</td>
<td>1.3 / 0.9</td>
<td>given</td>
</tr>
<tr>
<td>CrNi steel 1.4301, unpolished</td>
<td>1.0 / 1.2</td>
<td>given</td>
</tr>
<tr>
<td>CrNi steel 1.4435, unpolished</td>
<td>1.0 / 1.4</td>
<td>given</td>
</tr>
<tr>
<td>CrNi steel 1.4301, polished</td>
<td>1.3 / 1.4</td>
<td>given</td>
</tr>
<tr>
<td>PVC, hard</td>
<td>1.0 / 1.8</td>
<td>given</td>
</tr>
<tr>
<td>PTFE</td>
<td>1.3 / 1.6</td>
<td>given</td>
</tr>
<tr>
<td>PE, UHMW</td>
<td>1.6 / 1.6</td>
<td>given</td>
</tr>
<tr>
<td>PP</td>
<td>1.3 / 2.0</td>
<td>given</td>
</tr>
<tr>
<td>PVC</td>
<td>2.0 / 1.6</td>
<td>given</td>
</tr>
<tr>
<td>Laminated foil 1, outside</td>
<td>1.6 / 2.5</td>
<td>given</td>
</tr>
<tr>
<td>PC</td>
<td>2.2 / 2.3</td>
<td>given</td>
</tr>
<tr>
<td>BI, commercially available</td>
<td>2.6 / 2.3</td>
<td>given</td>
</tr>
<tr>
<td>Tyvek</td>
<td>2.0 / 3.1</td>
<td>given</td>
</tr>
<tr>
<td>Laminated foil 2, outside</td>
<td>2.5 / 3.2</td>
<td>given</td>
</tr>
<tr>
<td>Butyl chaouchouc</td>
<td>2.9 / 3.1</td>
<td>given</td>
</tr>
<tr>
<td>Hypalon</td>
<td>3.0 / 4.1</td>
<td>given</td>
</tr>
<tr>
<td>HEPA-filter pad</td>
<td>3.6 / 3.6</td>
<td>given</td>
</tr>
<tr>
<td>PVC, soft</td>
<td>4.3 / 4.6</td>
<td>given</td>
</tr>
<tr>
<td>POM</td>
<td>4.6 / 4.4</td>
<td>given</td>
</tr>
<tr>
<td>Aluminium, anodized, commercially available</td>
<td>&gt; 3.1 / 7.9</td>
<td>not given</td>
</tr>
<tr>
<td>Aluminium, anodized, air sampler</td>
<td>&gt; 8.3 / 10.1</td>
<td>given</td>
</tr>
<tr>
<td>Aluminium, anodized, filling line</td>
<td>&gt; 17.1 / &gt; 33.7</td>
<td>not applicable</td>
</tr>
</tbody>
</table>
Significantly higher D-value for the various aluminium samples
Materials Study Interpretation Aluminium

- Extremely porous surface structure
- Suspension was absorbed into surface
- H$_2$O$_2$ decontamination is not able to penetrate into a surface

→ No or only a bad inactivation effect

→ Not suitable for H$_2$O$_2$ decontamination
Carrier Material

Aluminium anodized

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BI and Isolator Decontamination

February 2006
Carrier Material

Aluminium anodized

Volker Sigwarth, Skan AG, Switzerland  BI and Isolator Decontamination  February 2006
Materials Study Results

<table>
<thead>
<tr>
<th>Material</th>
<th>D-value [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyoxymethylen, POM</td>
<td>4.33</td>
</tr>
<tr>
<td>Polyvinylchlorid, PVC soft</td>
<td></td>
</tr>
<tr>
<td>HEPA-filter pad</td>
<td></td>
</tr>
<tr>
<td>Hypalon</td>
<td></td>
</tr>
<tr>
<td>Butyl caoutchuc</td>
<td></td>
</tr>
<tr>
<td>Reference BI</td>
<td>2.28</td>
</tr>
<tr>
<td>Laminated foil 2</td>
<td></td>
</tr>
<tr>
<td>Tyvek</td>
<td></td>
</tr>
<tr>
<td>Polycarbonate, PC</td>
<td></td>
</tr>
<tr>
<td>Laminated foil 1</td>
<td></td>
</tr>
<tr>
<td>Polyvinylchlorid, PVC</td>
<td></td>
</tr>
<tr>
<td>Polypropylen, PP</td>
<td></td>
</tr>
<tr>
<td>Polyethylen, PE UHMW</td>
<td></td>
</tr>
<tr>
<td>Polytetrafluorehylen PTFE</td>
<td></td>
</tr>
<tr>
<td>Polyvinylchlorid, PVC hard</td>
<td></td>
</tr>
<tr>
<td>Stainless steel 1.4301, polished</td>
<td>1.45</td>
</tr>
<tr>
<td>Stainless steel 1.4301, not polished</td>
<td></td>
</tr>
<tr>
<td>Stainless steel 1.4301, not polished</td>
<td></td>
</tr>
<tr>
<td>Stainless steel 1.4301, polished</td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Worst Case: 4.33
Best Case: 1.45
Reference: 2.28
Materials Study

- 1 x Reference Isolator 1,4 m³
- 2 x Sterility Test Isolator 2,2 m³
- 5 x Filling Isolator 9,0 m³
- 2 x Material Pass Through 15,0 m³

Transferability

- IQ / OQ finished
- Cycle development based on commercial BI’s finished
- Initial condition and cycle parameter defined
### Materials Study

- **1 x Reference Isolator**: 1.45 m³
- **2 x Sterility Test Isolator**: 2.2 m³
- **5 x Filling Isolator**: 9.0 m³
- **2 x Material Pass Through**: 15.0 m³

### Transferability

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. stearothermophilus on Glass</strong></td>
<td>1.45 ± 0.07</td>
<td>1.41 ± 0.04</td>
<td>0.96 ± 0.05</td>
<td>1.18 ± 0.20</td>
</tr>
<tr>
<td><strong>Biological Indicator Reference</strong></td>
<td>2.28 ± 0.06</td>
<td>2.22 ± 0.06</td>
<td>1.62 ± 0.08</td>
<td>1.72 ± 0.07</td>
</tr>
<tr>
<td><strong>B. stearothermophilus on POM</strong></td>
<td>4.33 ± 0.20</td>
<td>4.00 ± 0.30</td>
<td>3.38 ± 0.16</td>
<td>3.41 ± 0.23</td>
</tr>
</tbody>
</table>
Materials Study

Implementation

- Initial Population BI
- Bio load worst case material; POM
- D-value Reference BI
- D-value Worst case
- Qualified Cycle Time

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BI and Isolator Decontamination
February 2006
Materials Study

- Reproducible *different resistances* on *different carrier materials*
- Some Materials *seems* to be *not suitable* for $\text{H}_2\text{O}_2$ decontamination

  *borders of the process*

- Resistances were *transferable* to *different systems*
- Cycle development and qualification based on *commercial BI`s*
- *Knowledge* of all inactivation factors
Effect of Carrier Materials on the Resistance of 
*B. stearothermophilus* to gaseous $H_2O_2$

- Research Article  
  PDA Journal

“Effect of Carrier Materials on the Resistance of Spores of Bacillus 
Stearothermophilus to gaseous Hydrogen Peroxide”

Volker Sigwarth, Skan AG  
Alexandra Stärk, Novartis Pharma AG

PDA Journal, Vol. 57, No.1 January / February 2003

- As Reference Study for Isolator Validation
**Effect of Carrier Materials on the Resistance of B. stearothermophilus to gaseous H$_2$O$_2$**

- **Implementation in PIC/S Recommendation**

  “The carrier type e.g. plastic, paper, metal or other, of the biological indicator organism should be relevant to the materials being gassed or shown to be irrelevant”

  “If studies have been carried to show that lethality on carrier type a is similar to materials c, d, e, etc. with a similar sporicidal process, this would mean that *in house studies need not be carried out.*”

  “The data would need to be from a reputable source”
Materials Study  

FDA; Comment

• FDA Comment, R. Friedman, ISPE Washington Conference 2003

Reproducibly “different resistances of B. stearothermophilus to a H2O2 decontamination on different carrier materials”.

“Because of their surface structure and properties, certain materials seems to be not suitable for the H2O2 decontamination ...”

But... resistances were found to be essentially “transferable", so development work may greatly reduce the need to address extensively during decontamination cycle validation.

Development of decontamination cycle parameters should incorporate knowledge of material-effects. Commercial BI used as control for this study.

Authors conclude that no single factor on its own is normally responsible for material effect
Development of alternative Sterilization Methods

• Book Chapter  “Process Development of alternative Sterilization Methods”

Title:  “Contamination Control in Parenteral Processing”
Published by:  Marcel Dekker Inc., USA
Edited by:  Kevin Williams, Eli Lilly & Co., USA
Author:  Volker Sigwarth, Skan AG, Switzerland
published:  Middle of 2004
Decontamination of Isolator Systems

Risks in working with Biological Indicators

• “total kill approach” one surviving BI stops production
• Bioindicators are hand made reliable resistance of single BI
• use enough Bioindicators You will find a survivor

How to handle surviving Bioindicators during qualification work?
Decontamination of Isolator Systems

Risks in working with Biological Indicators

• “total kill approach”

• Where come the requirement “total kill” from?

• Steam Sterilization *versus* Isolator Decontamination

Regulatory Requirements and Possibilities
Decontamination of Isolator Systems

Regulatory Requirements; FDA Aseptic Guideline

“Normally a four- to six-log reduction can be justified depending on the application. The specific spore titer used and the selection of BI placement site should be justified. For example, demonstration of a four-log reduction should be sufficient for controlled, very low bioburden material introduced into a transfer isolator including wrapped sterile supplies that are briefly exposed to the surrounding environment”
**Microbiological System**

**Model of Microbial Reduction**

- Initial Population [log-scale]
- Inactivation Time [min]
- Survival Curve
- D-value [min]
- Survival - Kill Window [min]
Microbiological System

Probability Distribution, positive / negative

P(m) = e^{-m}

Probability of negative

63%

Probability of positive

37%
Decontamination of Isolator Systems

Regulatory Requirements; USP 28, <1208>

The sterilization methods used to treat isolators, test articles, and sterility testing supplies are capable of reproducibly yielding a six-log kill against an appropriate, highly resistant biological indicator (BI; see Biological Indicators for Sterilization (1035)), as verified by the fraction-negative or total-kill analysis methods. Total-kill analysis studies are suitable for BIs with a population of $10^6$ spores per unit, while fraction-negative studies are suitable for BIs with a population of $10^8$ or greater. A sufficient number of BIs are used to prove statistical reproducibility and adequate distribution of the sterilizing agent. Particular attention is given to areas that pose problems relative to the concentration of the agent. A larger number of BIs are used in isolators that are heavily loaded with equipment and materials. Also, when it is not possible to use one or more calibrated sensors to directly measure the concentration of the sterilizing agent, the placement of additional BIs is considered. The ability of the process to reproducibly deliver a six-log kill is confirmed in three consecutive validation studies.
Decontamination of Isolator Systems

Possibilities in working with Biological Indicators

• “fractional kill approach”

• relation between positive and negative Bioindicator results

• proof the required log reduction

Successfully applied for Filling and Sterility Test Isolators
Decontamination of Isolator Systems

Problems in using "fractional kill approach"

- Basics of microbial inactivation has to be deeply understood
- Argumentations during audits and with authorities often much harder
- Steam Sterilization \textit{versus} Isolator Decontamination
- Root cause of positive result? BI, Cycle Parameter, Isolator System

Will be the future approach for isolator cycle qualification
Decontamination of Isolator Systems

Possibilities in working with Biological Indicators

• Initial control of Bioindicators
• D-value based Cycle Development quantifiable for each phase
• one unexpected positive BI result at one position
• followed by one more run with multiple BI samples at this position

Successfully applied “Back Up Tool” for Cycle Qualification
Biological Indicators and $H_2O_2$ Decontamination of Isolator Systems

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Process Development