Biological Indicators and
H_2O_2 Decontamination of Isolator Systems

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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

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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

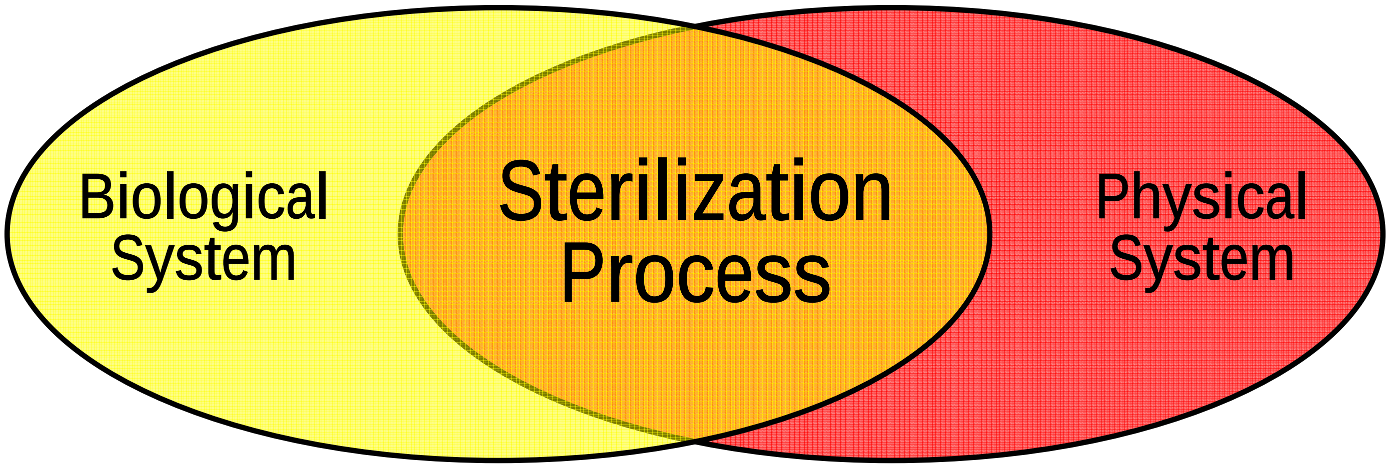
Overview of current Regulation and Standards
Requirements for Validation

• 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

- H_2O_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, H_2O_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*

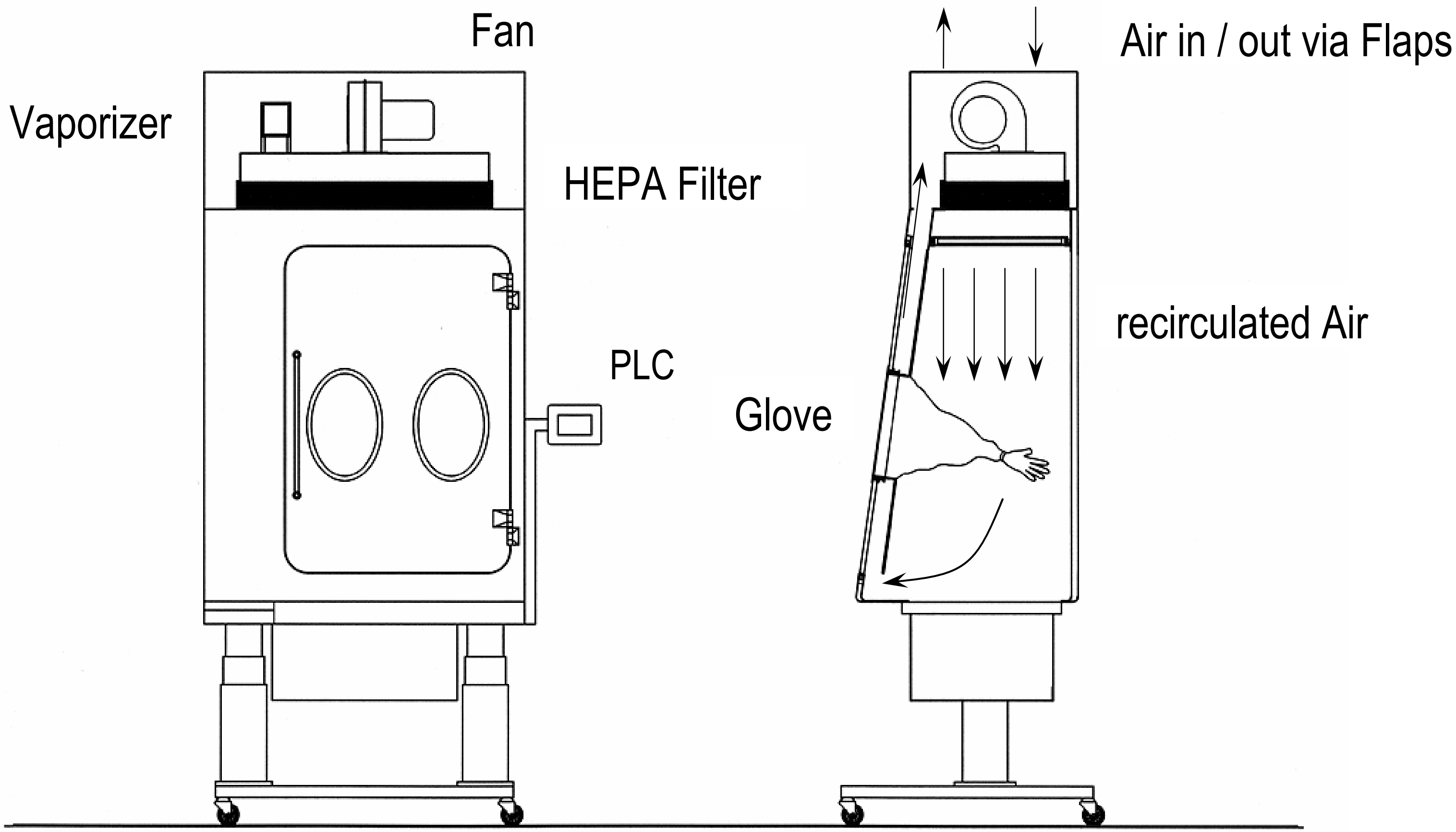
Alternative Sterilization Methods

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



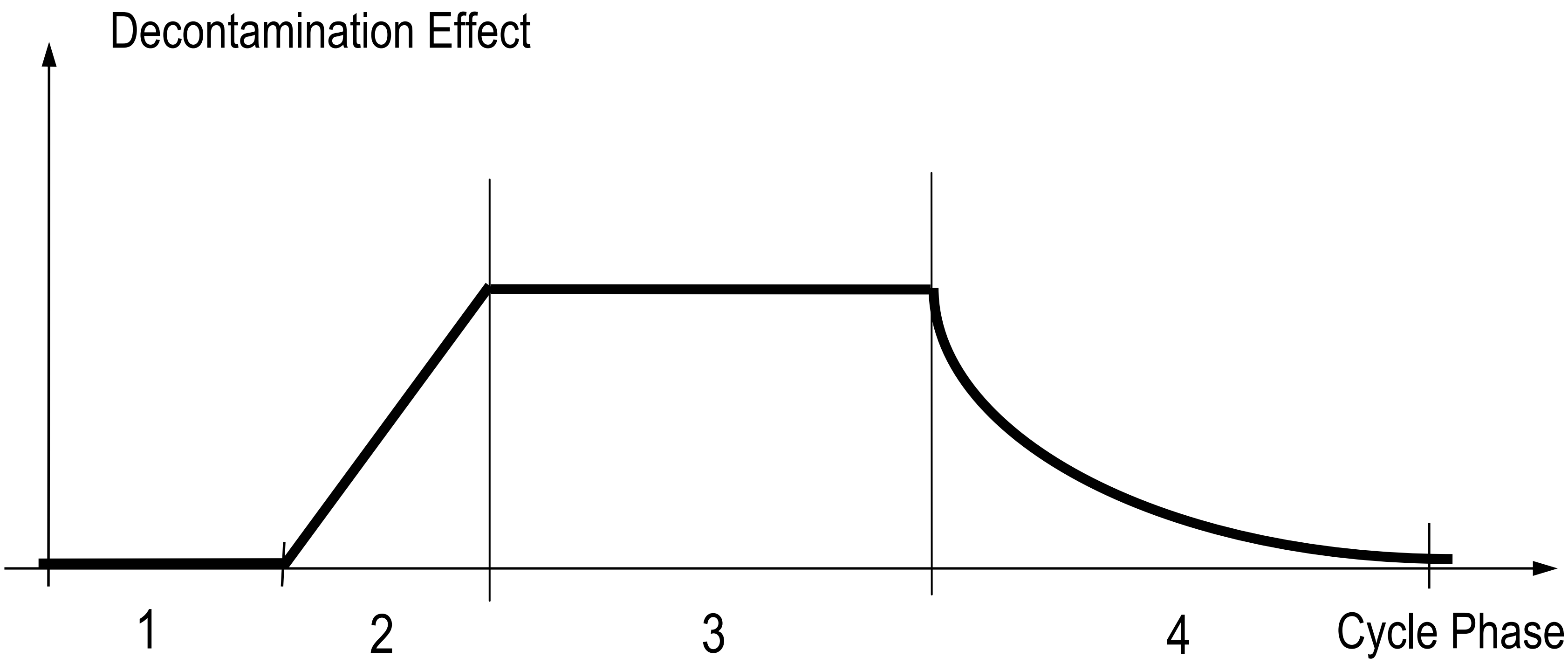
Material: stainless steel, glass

Volume: 1,4 m^3 (40 ft^3)

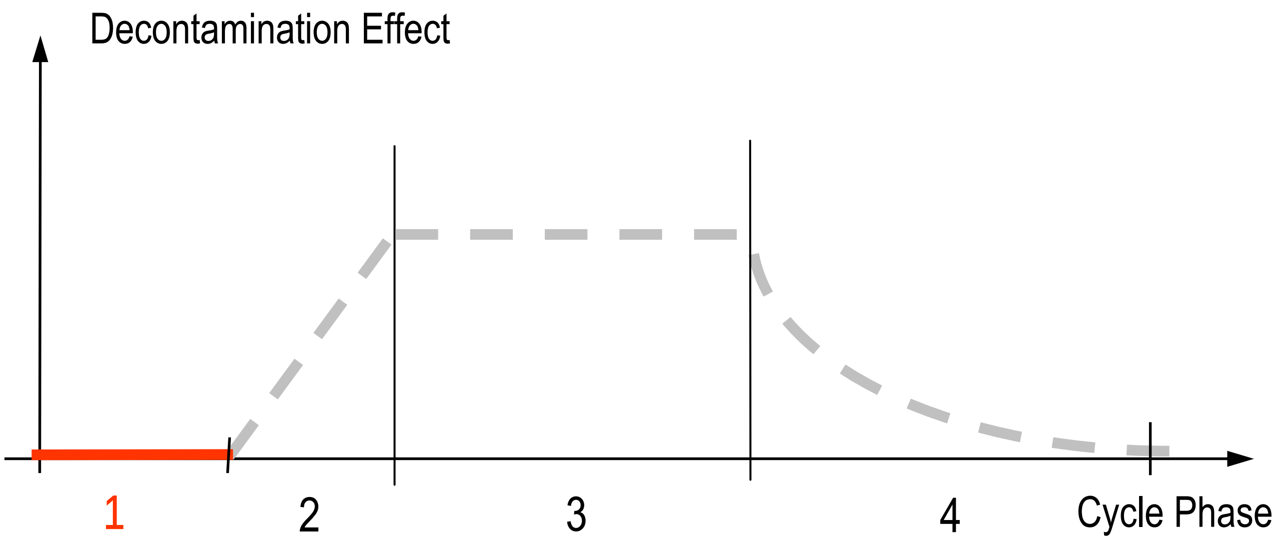
Process Control

Process		*H_2O_2 Gas-Concentration*
• Temperature	[°C]	• Electro-chemical Sensor
• Humidity	[% rH]	• UV-Spectrometer
• Air Velocity	[m/s]	• IMS-Spectrometer
• Pressure	[Pa]	• NIR-Spectrometer
• Mass / Balance	[g]	• Wet-chemistry Method

Decontamination Cycle



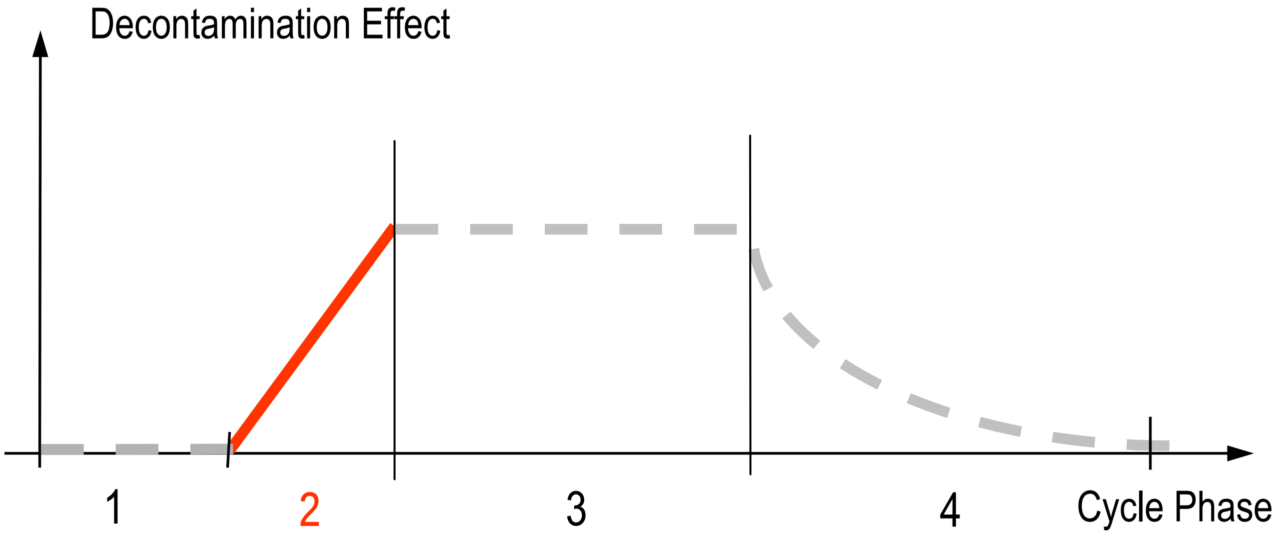
Decontamination Cycle



Phase 1: **Pre-conditioning**

to establish the initial conditions in the chamber

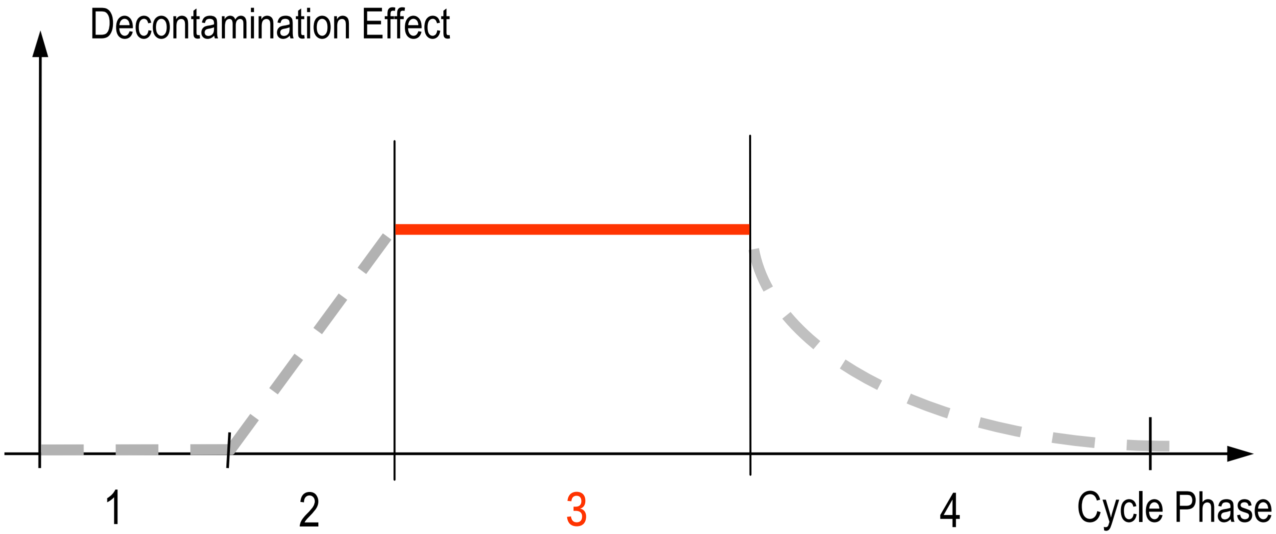
Decontamination Cycle



Phase 2: Conditioning

to establish the decontamination effect

Decontamination Cycle

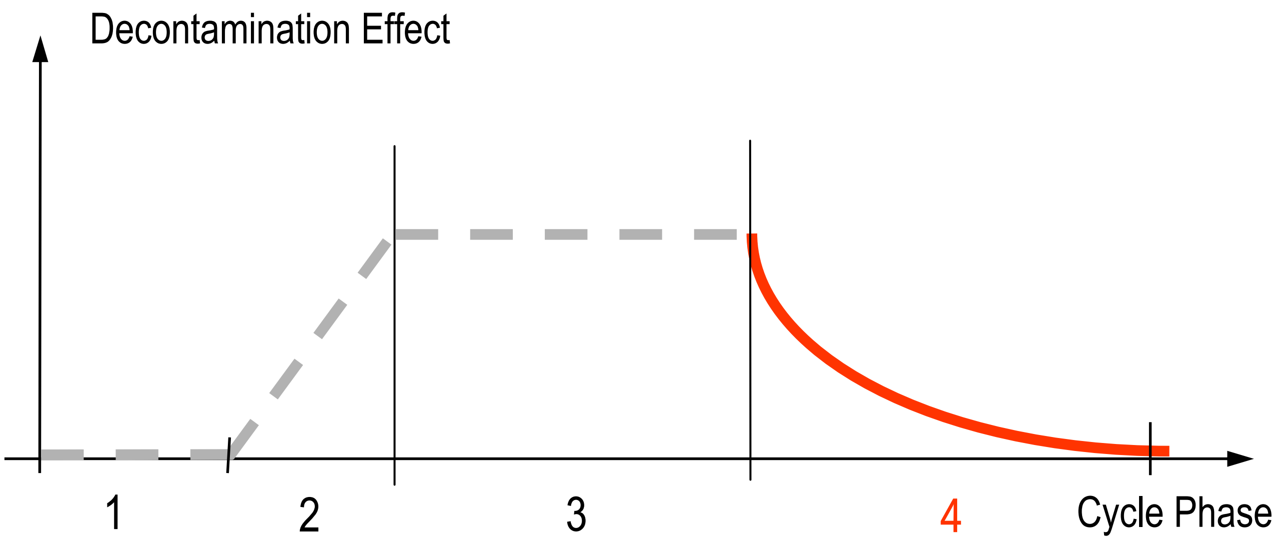


Phase 3: **Decontamination**

to maintain stable decontamination effect

to ensure the total bacterial reduction over time

Decontamination Cycle



Phase 4: **Aeration**

to reach minimal residual H_2O_2 concentration

Alternative Sterilization Methods

- No useful Correlation of:

  Physical Parameters → *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:

Physical Parameters → *Inactivation Effect*

- The use of alternative Inactivation Methods requires often:
- *Description of the Inactivation Effect directly*
- *Measure of the Inactivation Effect*
- *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

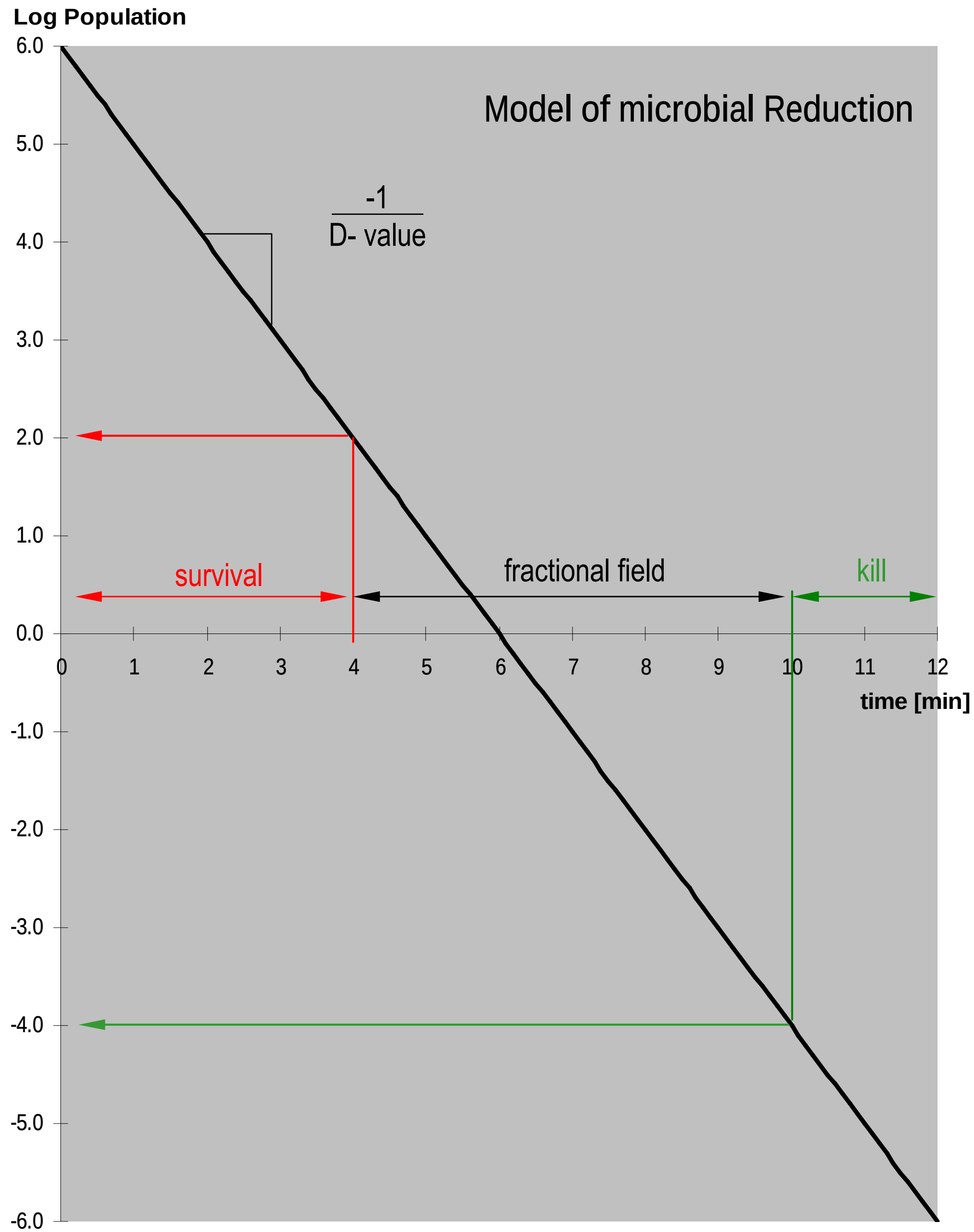
Resistance Description of Biological Indicator

- Initial Population of the Test Organisms [CFU/Carrier]
- D-value [min]
- Survival - Kill Window [min]
  - Survival time [min] = D-value x ([log] Population – 2)
  - Kill time [min] = D-value x ([log] Population + 4)

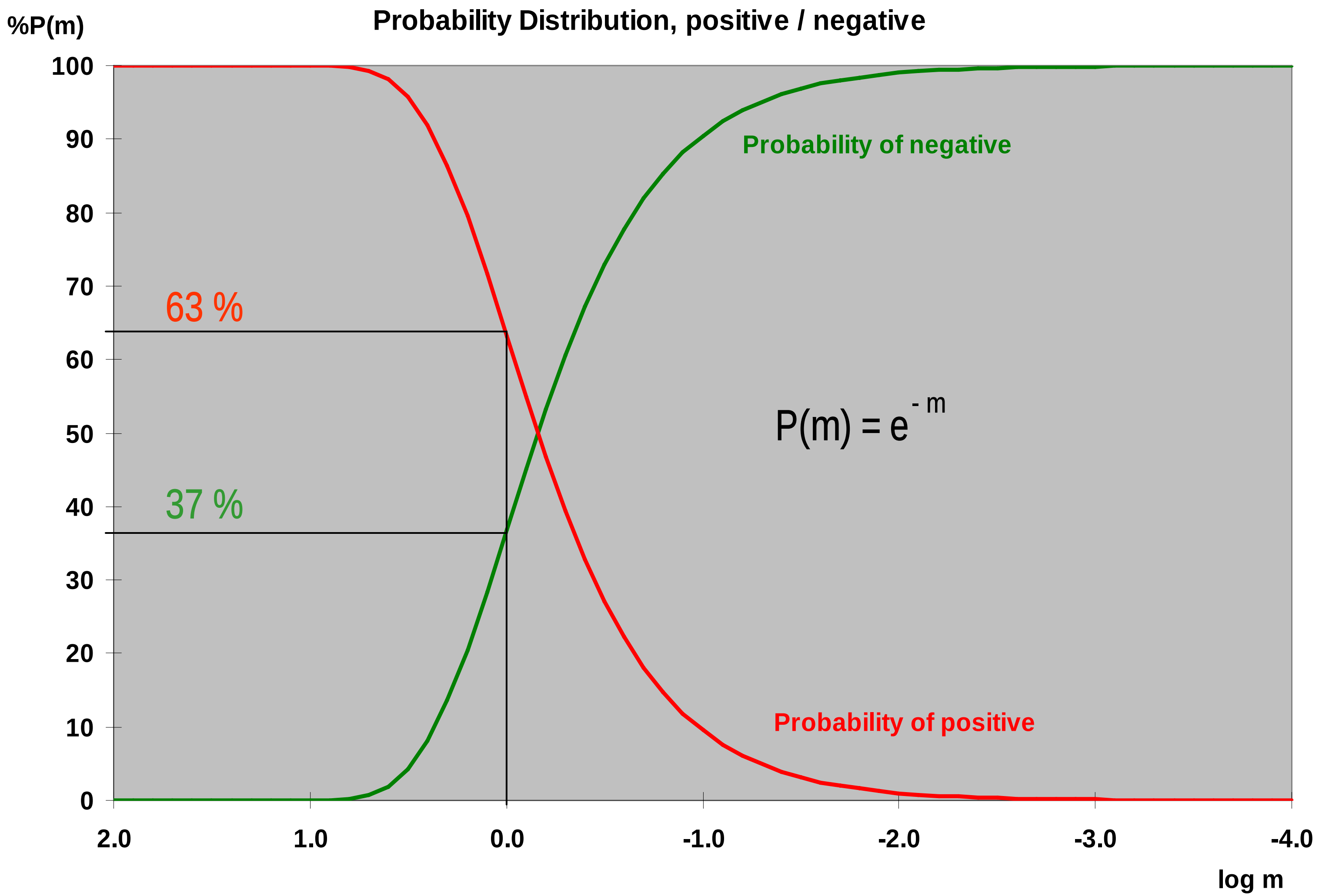
Microbiological System

Model of Microbial Reduction

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



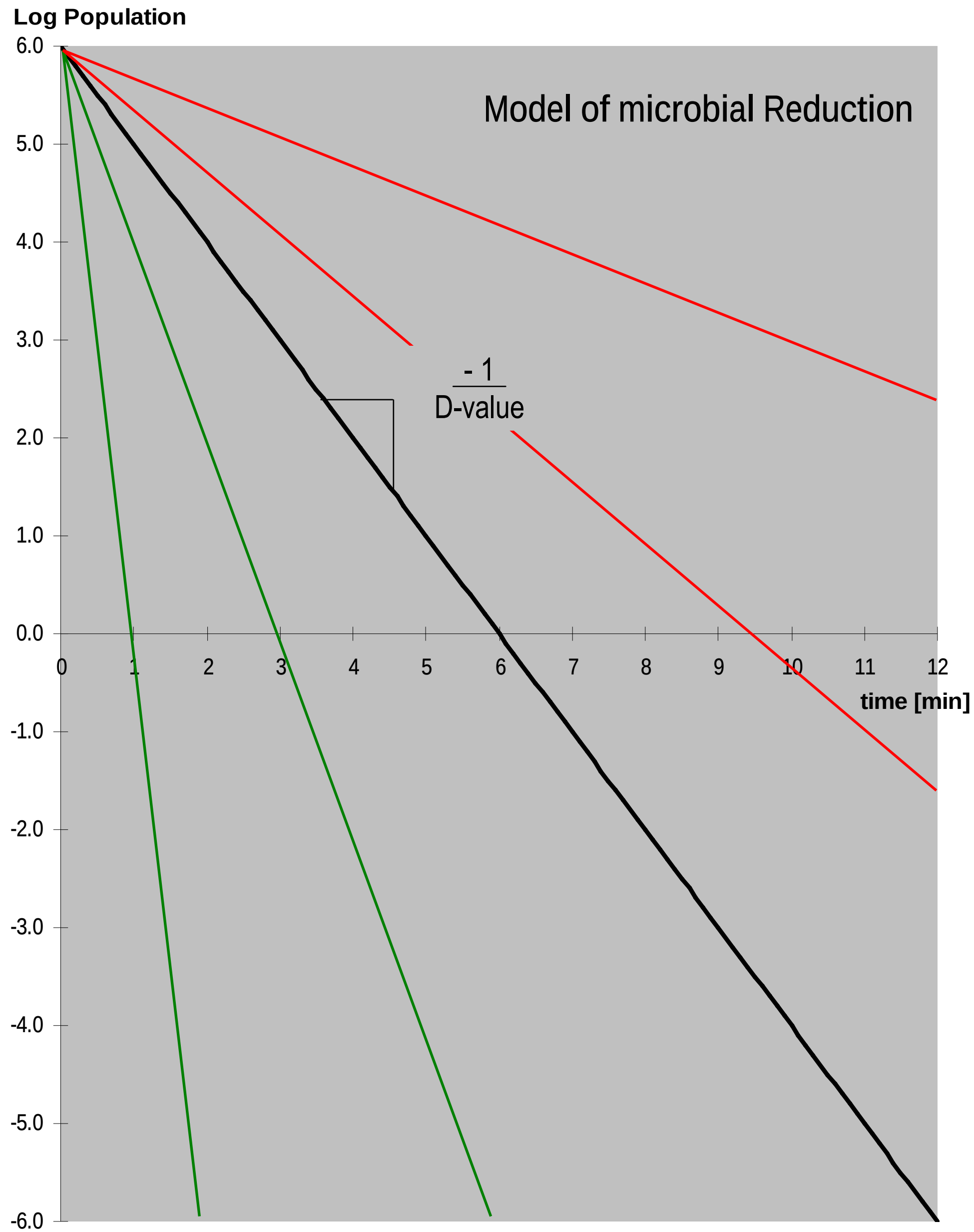
Microbiological System



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

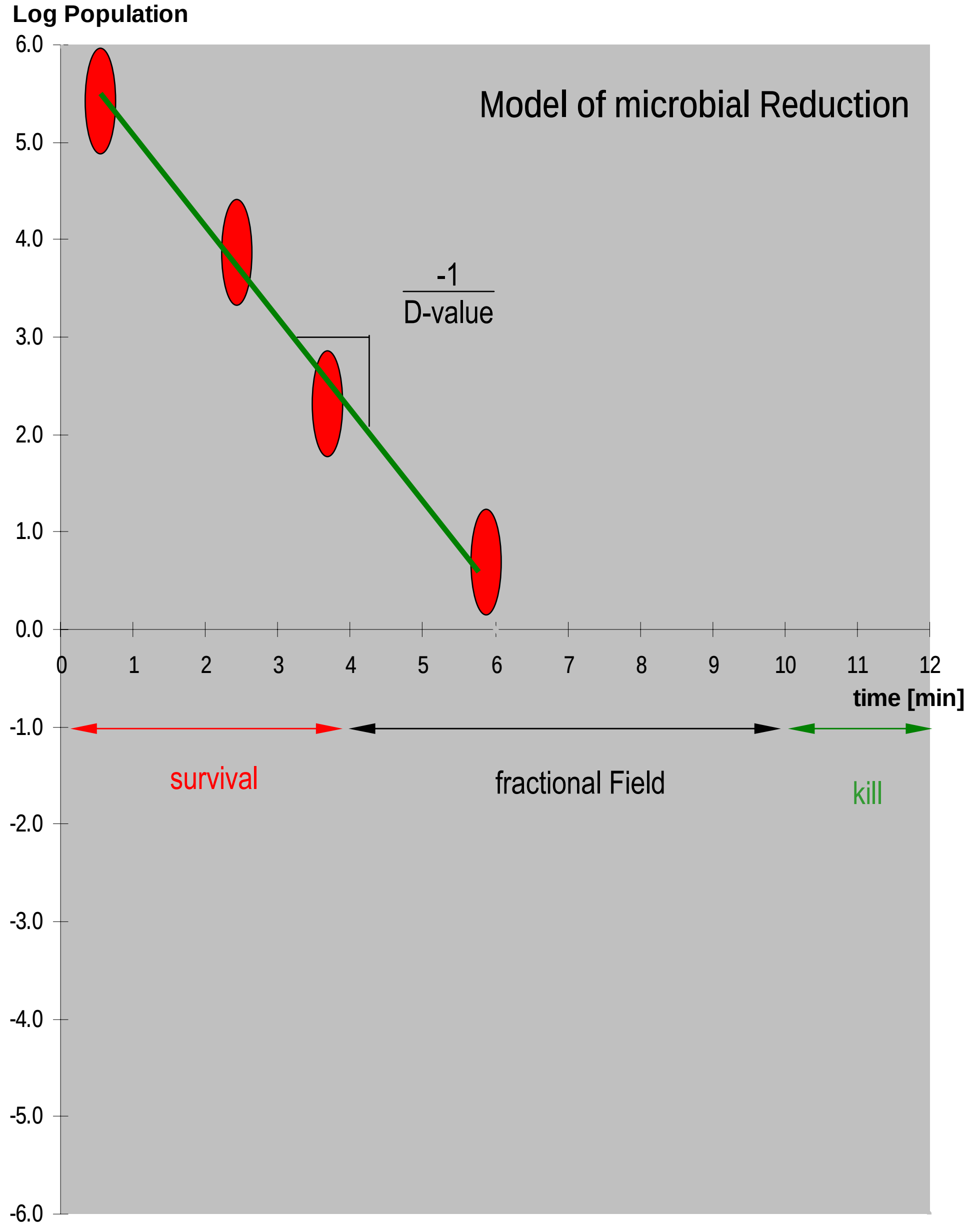
Description of the Inactivation Effect

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

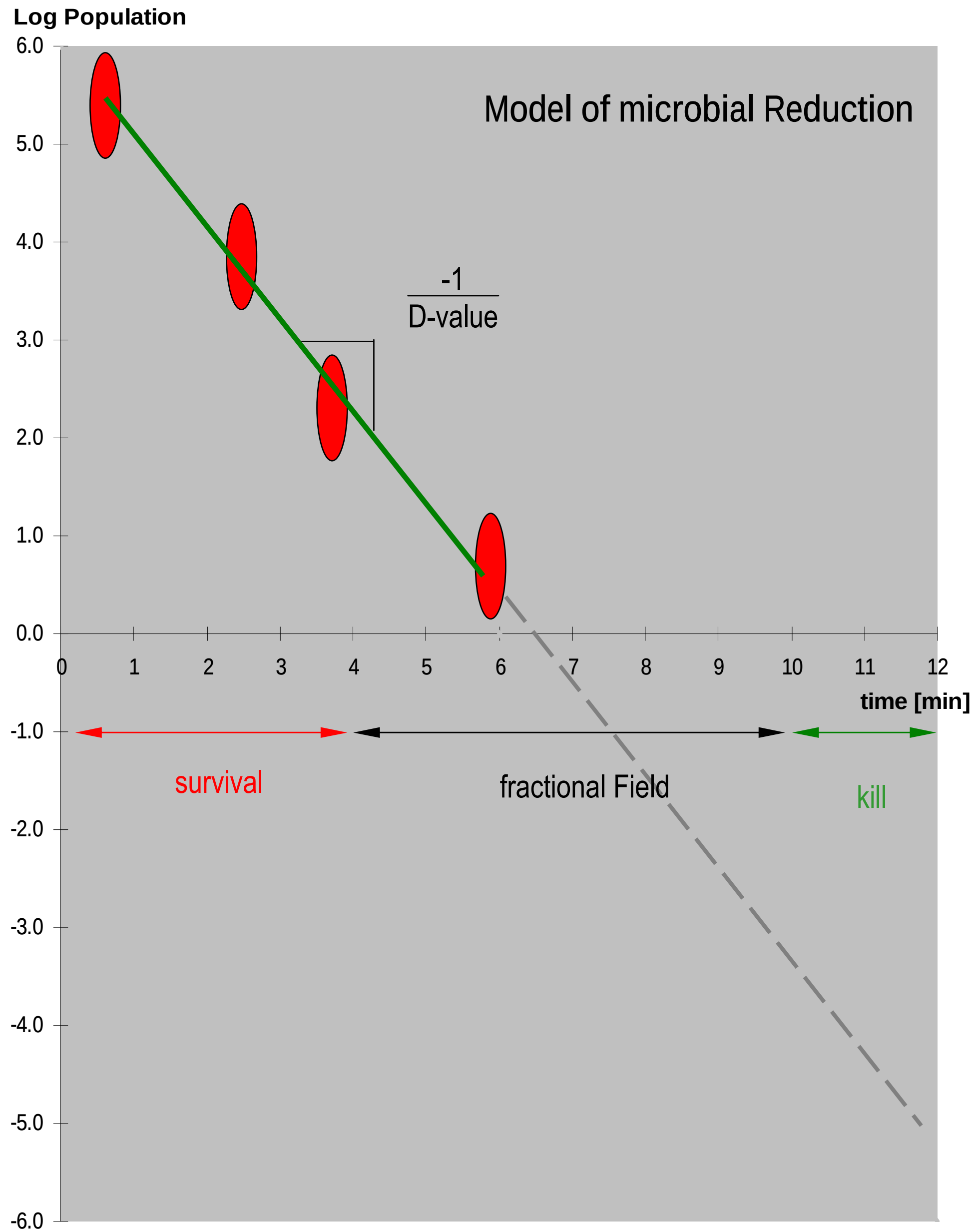
SC Method

- Survival Window
- Beginning of fractional Field

- high Lab Effort
- good if *Total Kill* is not required
- Disinfection Testing
- *"Last Chance Method"*

No Information about:

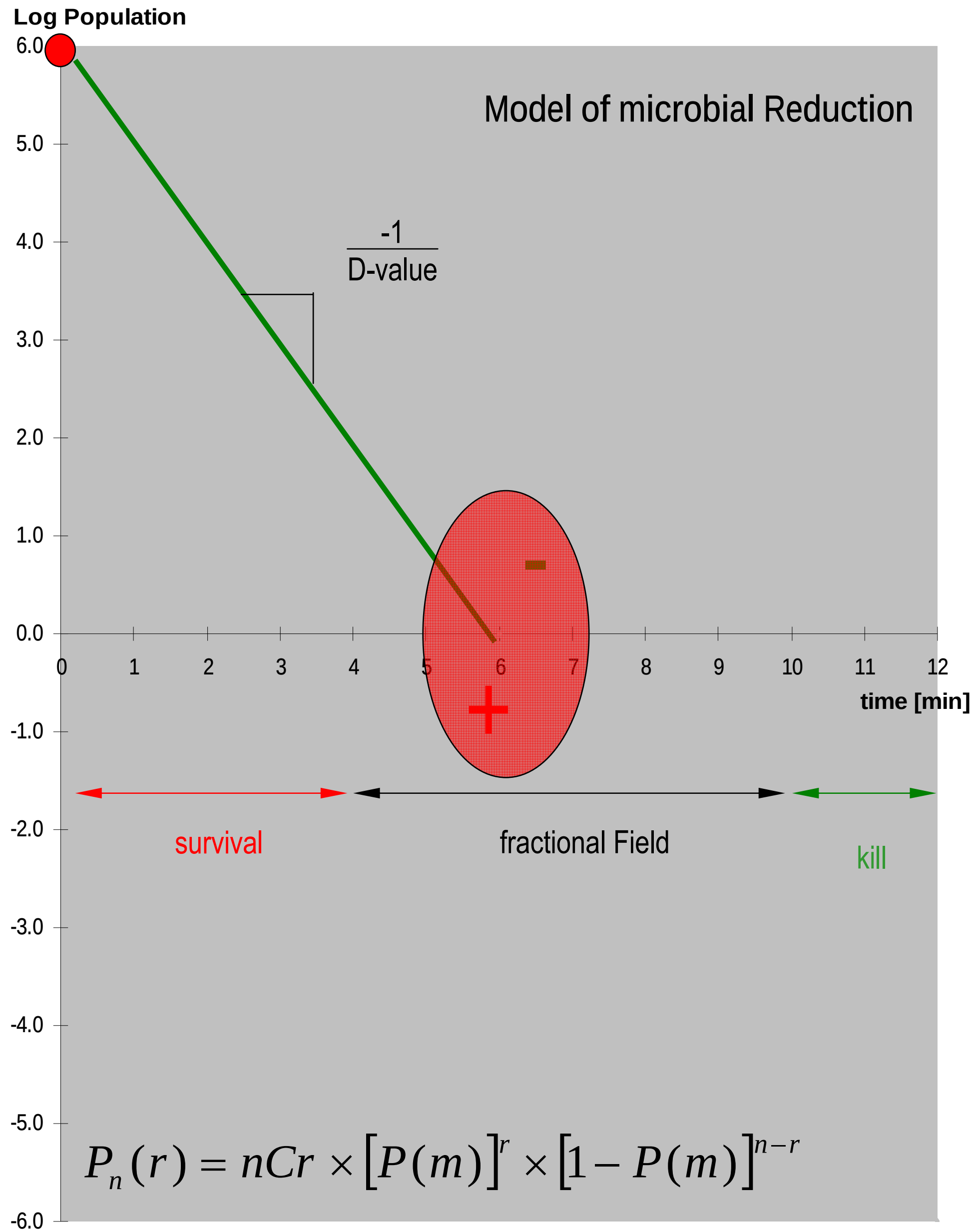
- *Kill Window*
- *Model Behaviour*



D-value Determination

SMCM

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



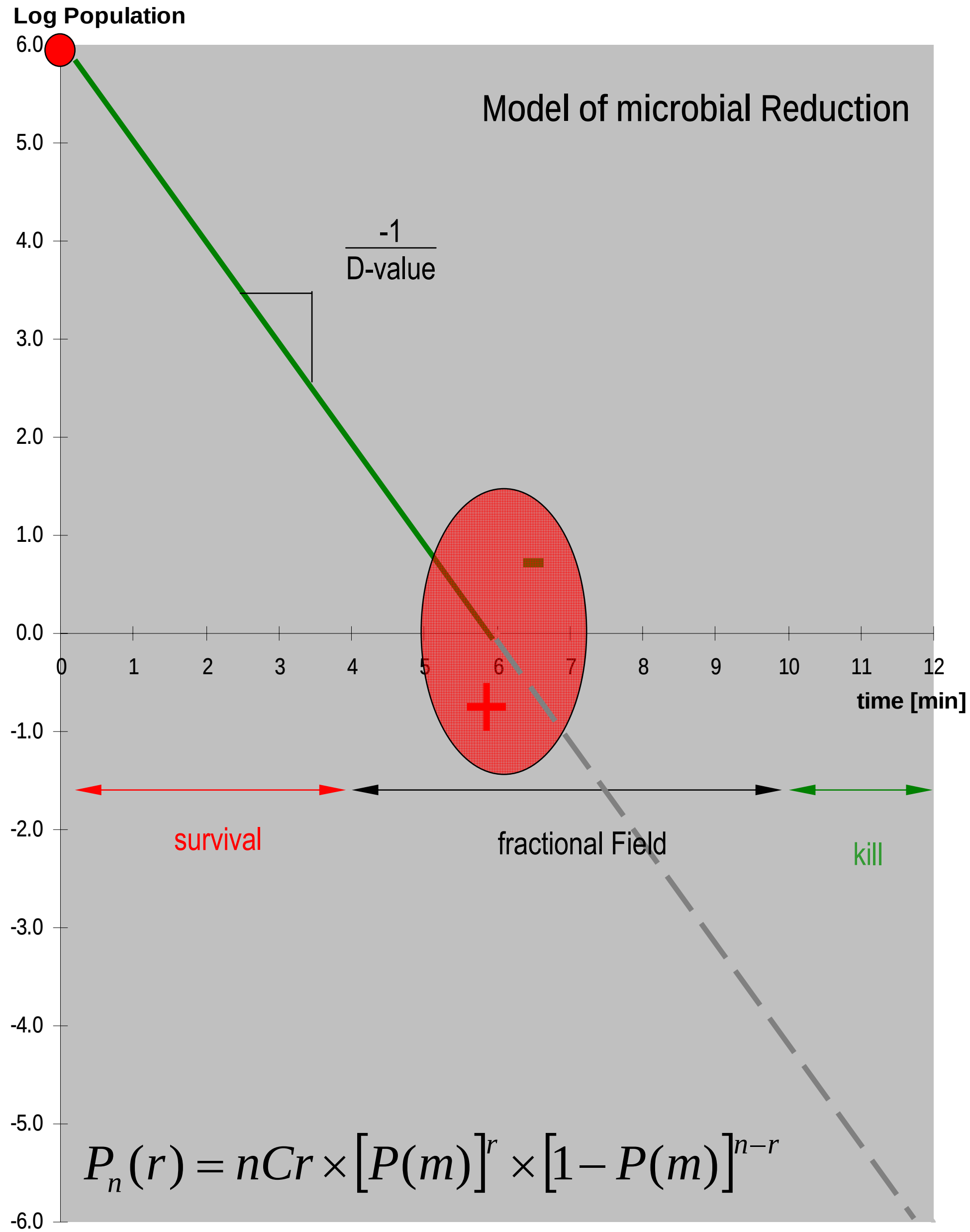
D-value Determination

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*



Stumbo Murphy Cochran Method *SMCM*

Initial Population N_0: 1.0x 10^6

exposure	01										
exposure time [min]	13										
result	-	-	-	-	+	+	+	+	+	+	neg
	-	-	-	-	+	+	+	+	+	+	-
	-	-	-	+	+	+	+	+	+	+	pos
	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	+	+	+	+	+	+	+	

+ growth

- no growth

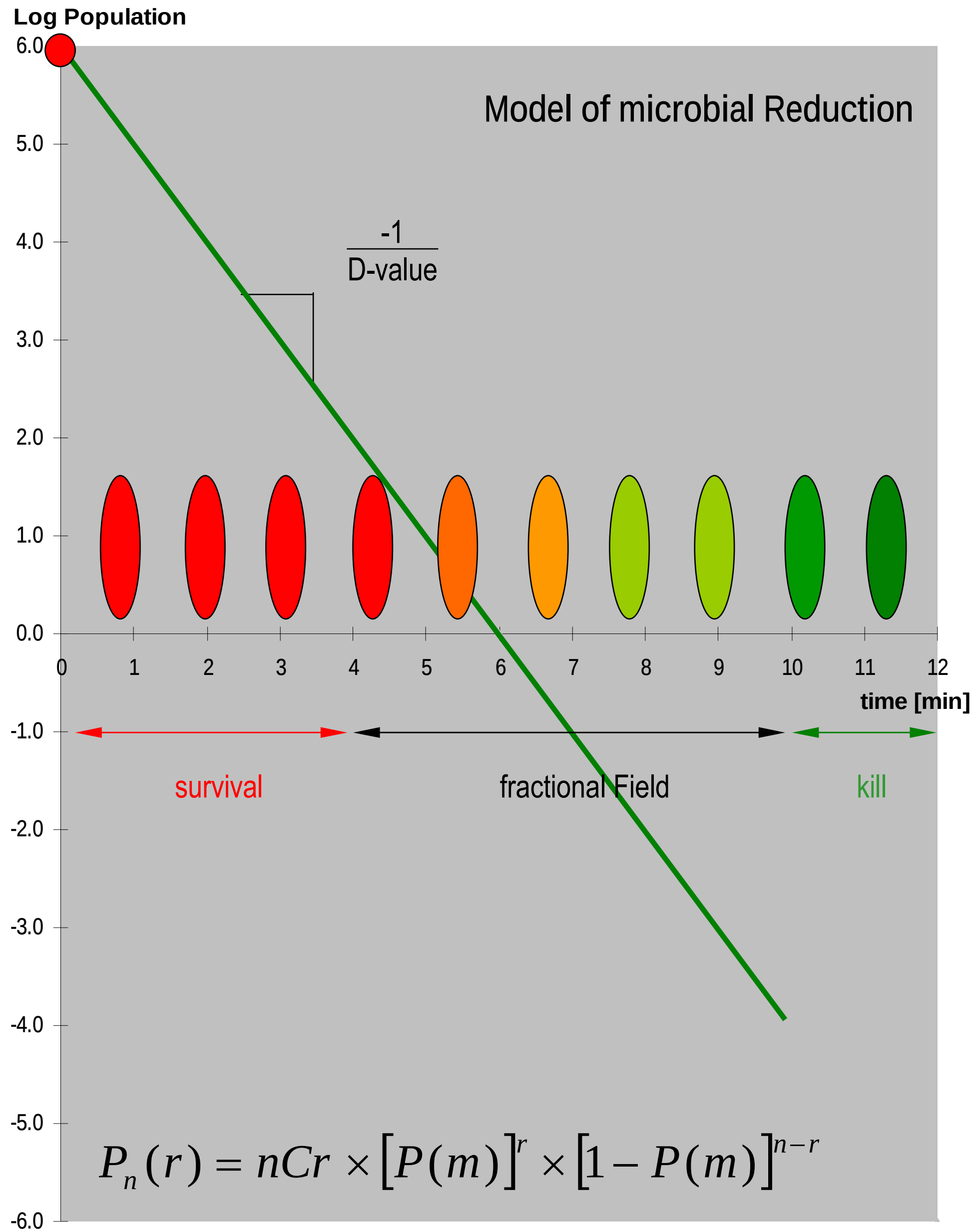
D-value = 2.03 [min]

95% CI D-value = 2.03 ± 0.05 [min]

D-value Determination

LSKM

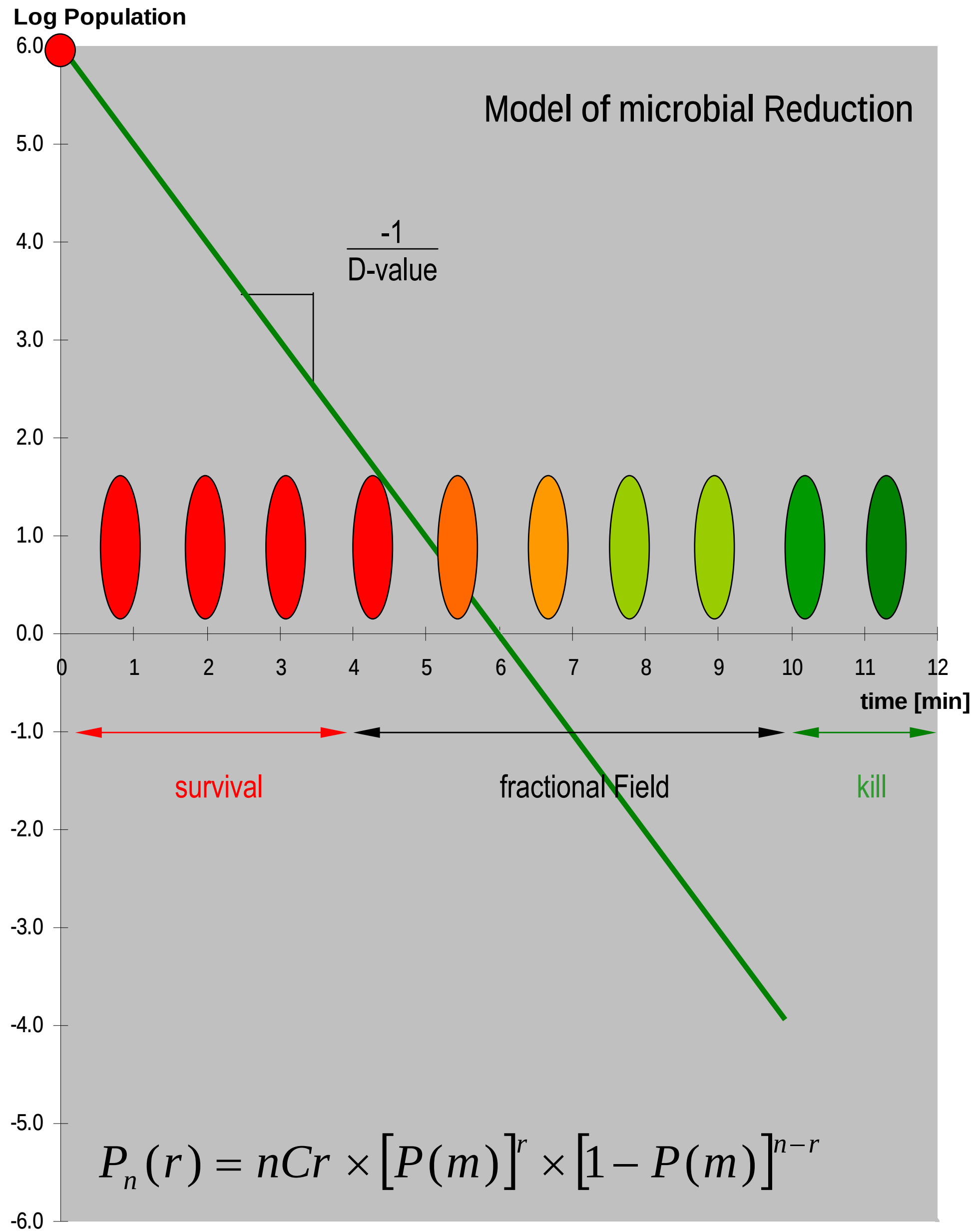
- several Groups of Bioindicators
- different Exposure Times
- observed Model Behavior
- initial Population
- D-value Calculation



D-value Determination

LSKM

- Kill Window is covered
- Model Behavior is completely shown



Limited Spearman Karber Method   LSKM

Initial Population N_0:1.0 x 10^6

exposure		01	02	03	04	05	06	07	08	09	10	
exposure time [min]		6.0	8.5	11.0	13.5	16.0	18.5	21.0	23.5	26.0	28.5	
result	1	+	+	+	+	+	+	-	-	-	-	neg
	2	+	+	+	+	+	+	-	-	-	-	-
	3	+	+	+	+	-	-	-	-	-	-	-
	4	+	+	+	+	-	-	-	-	-	-	pos
	5	+	+	+	-	-	-	-	-	-	-	+
	6	+	+	+	-	-	-	-	-	-	-	+
	7	+	+	+	-	-	-	-	-	-	-	
	8	+	+	+	-	-	-	-	-	-	-	
	9	+	+	-	-	-	-	-	-	-	-	
	10	+	-	-	-	-	-	-	-	-	-	

D-value = 2.06 [min]

95% CI D-value = 2.06 ± 0.22 [min]

\+ growth

\- no growth

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 x 10^6

exposure	01	02	03	04	05	06	07	08	09	10	
exposure time [min]	6.0	8.5	11.0	13.5	16.0	18.5	21.0	23.5	26.0	28.5	
result 1	+	+	+	+	+	+	-	-	-	-	neg
2	+	+	+	+	+	+	-	-	-	-	-
3	+	+	+	+	-	-	-	-	-	-	-
4	+	+	+	+	-	-	-	-	-	-	pos
5	+	+	+	-	-	-	-	-	-	-	+
6	+	+	+	-	-	-	-	-	-	-	+
7	+	+	+	-	-	-	-	-	-	-	
8	+	+	+	-	-	-	-	-	-	-	
9	+	+	-	-	-	-	-	-	-	-	
10	+	-	-	-	-	-	-	-	-	-	

D-value = 2.06 [min]

95% CI D-value = 2.06 ± 0.22 [min]

\+ growth

\- no growth

Minimized LSKM, Reactive Pattern

Initial Population N_0:1.0 x 10^6

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time[min]	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
Result 1	+	+	+	+	-	-	-	-	-	-	neg
2	+	+	+	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	

\+ growth
\- no growth

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

Test Organism	D-value [min]	Carrier Material
Staphylococcus aureus	< 0.3	Glass
Pseudomonas aeroginosa	< 0.3	Glass
Micrococcus luteus	0.3 – 0.6	Glass
Acinetobaccter lwoffii	0.6	Glass
Penicillium species	0.3 – 0.6	Glass
Aspergillus species	0.3	Glass

Method Deviation ± 10%

Selection of Bioindicators *Test Organism*

Bacteria Spores

Test Organism	D-value [min]	Carrier Material
Bacillus sphaericus	1.2	Glass
Bacillus subtilis var. niger	1.3	Glass
Bacillus stearothermophilus ATCC 12980	1.3	Glass
Bacillus stearothermophilus ATCC 7953	1.4	Glass

Method Deviation ± 10%

Selection of Bioindicators Test Organism

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

Selection of Bioindicators Test Organism

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_2O_2

Testorganism: B. stearothermophilus ATCC 12980, min $1.0x10^6$
Carrier / Package: Stainless steel / Tyvek
Spezified D-value: 0.9 to 1.8 [min]

+ growth
- no growth

Lot 01

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
result	1	+	+	+	+	-	-	-	-	-	-	neg
	2	+	+	+	-	-	-	-	-	-	-	-
	3	+	+	-	-	-	-	-	-	-	-	

Lot 02

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
result	1	+	+	-	-	-	-	-	-	-	-	neg
	2	+	-	-	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-	-	

Biological Indicator for gaseous H_2O_2

Testorganism: B. stearothermophilus ATCC 7953, min $1.0x10^6$
Carrier / Package: Stainless steel / Tyvek
Spezified D-value: 1.0 to 1.7 [min]

\+ growth
\- no growth

Lot 01

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
result	1	+	+	+	+	-	+	-	-	-	-	neg
	2	+	+	+	+	-	-	-	-	-	-	-
	3	+	+	+	-	-	-	-	-	-	-	

Lot 02

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
result	1	+	-	-	-	-	-	-	-	-	-	neg
	2	+	-	-	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-	-	

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 ?*

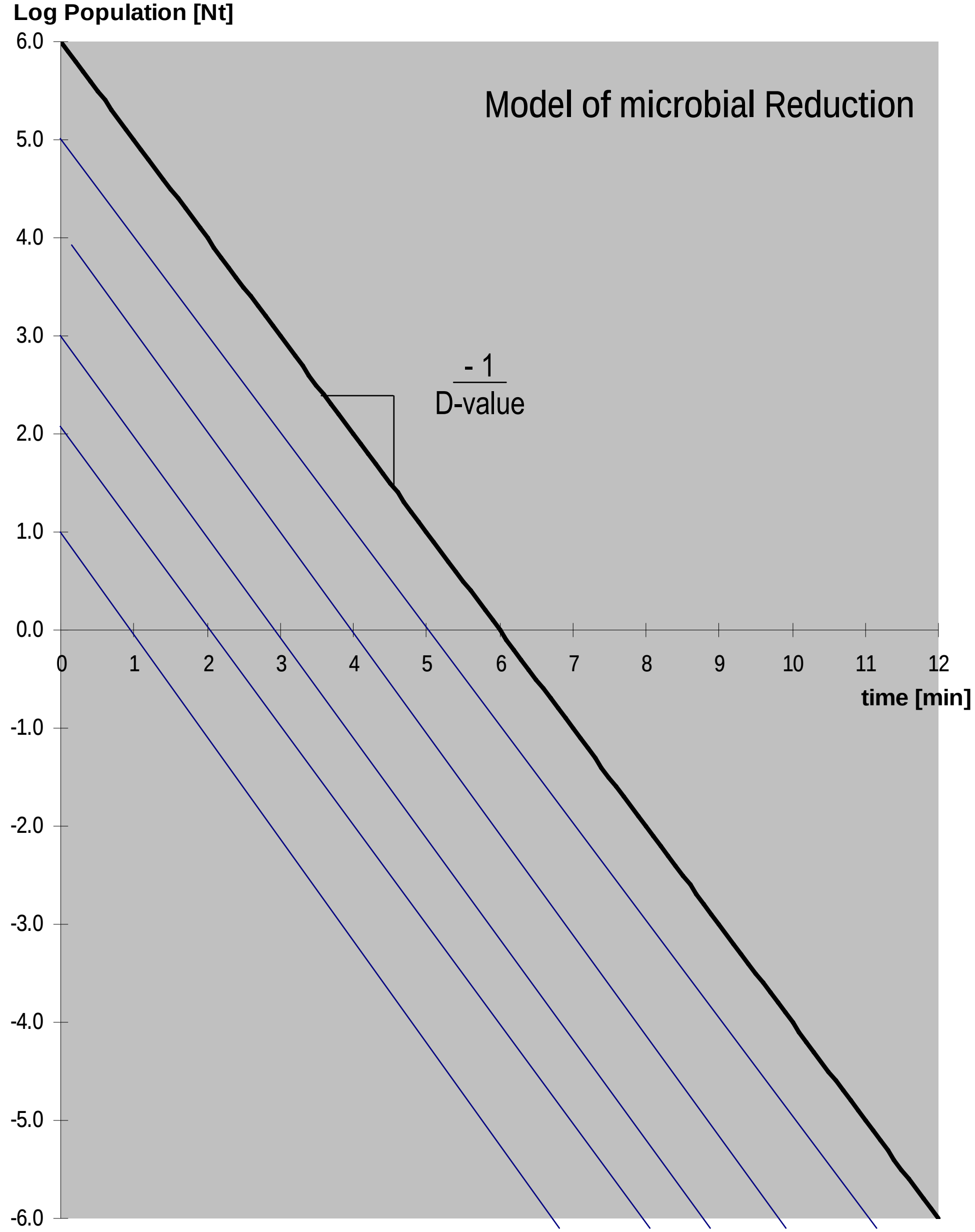
- D-value *independent* of the initial Population
- D-value *only depends* Resistance of the individual Microbe

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

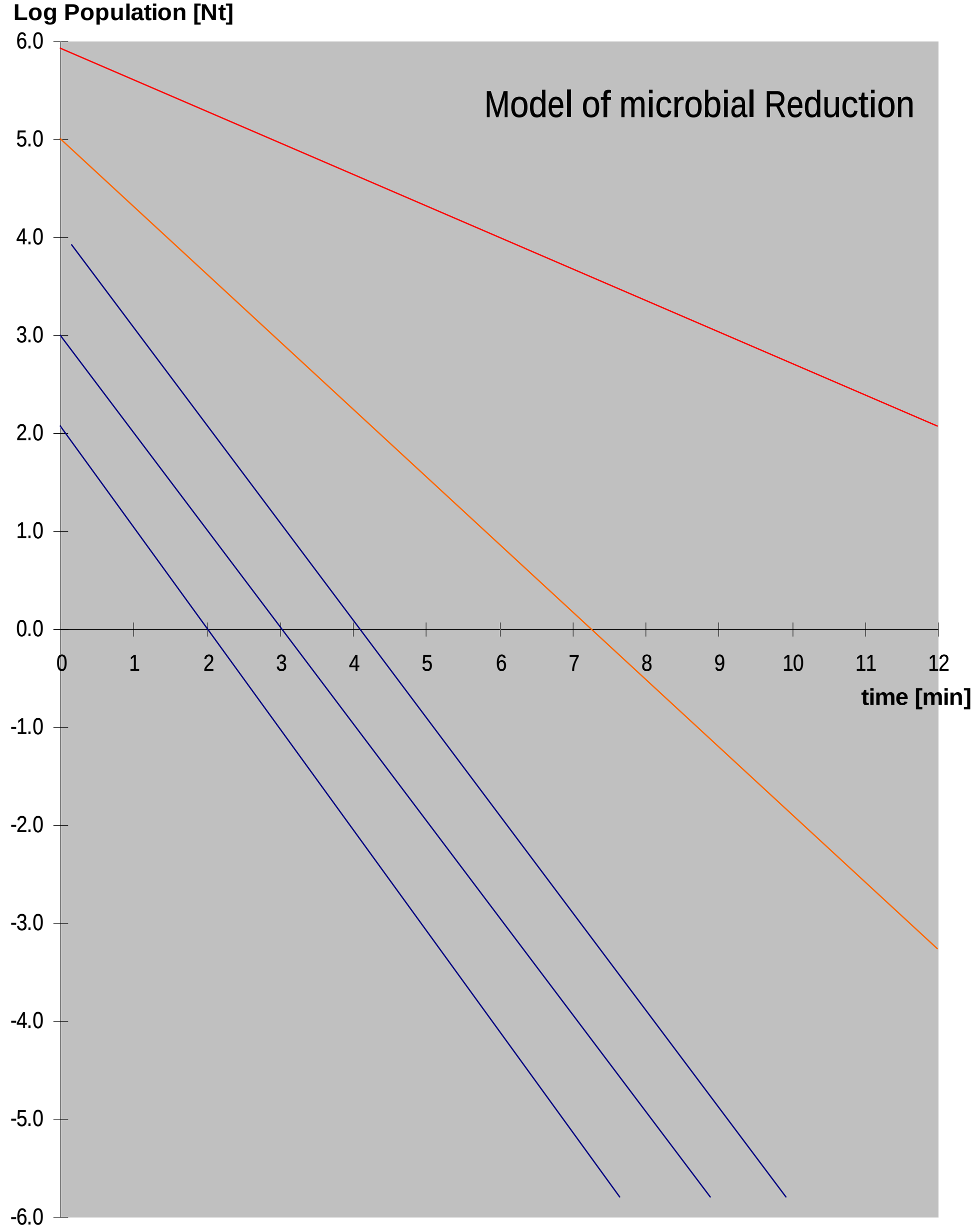
Test Organism	Population, N_0 [log Steps]	D-value [min]
Micrococcus luteus	$\geq 1.0 \times 10^3$	0.3
Micrococcus luteus	$\geq 1.0 \times 10^4$	0.3
Micrococcus luteus	$\geq 1.0 \times 10^5$	0.6
Micrococcus luteus	$\geq 1.0 \times 10^6$	> 3.6

Method Deviation ± 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

\+ growth
\- no growth

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	+
Result 1	+	+	+	-	-	-	-	-	-	-	neg
2	+	+	-	-	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	-	

- Initial Population: 1×10^5
- estimated D-value: 1.3 [min]
- Model behavior: OK

Population N_0 Model Behavior

Test Organism: B. stearothermophilus
carrier Material: Glass

+ growth
- no growth

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure time [min]	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	+
Result 1	+	+	+	-	-	-	+	-	+	-	neg
2	+	+	+	-	-	-	+	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	

- Initial Population: 1×10^6
- 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

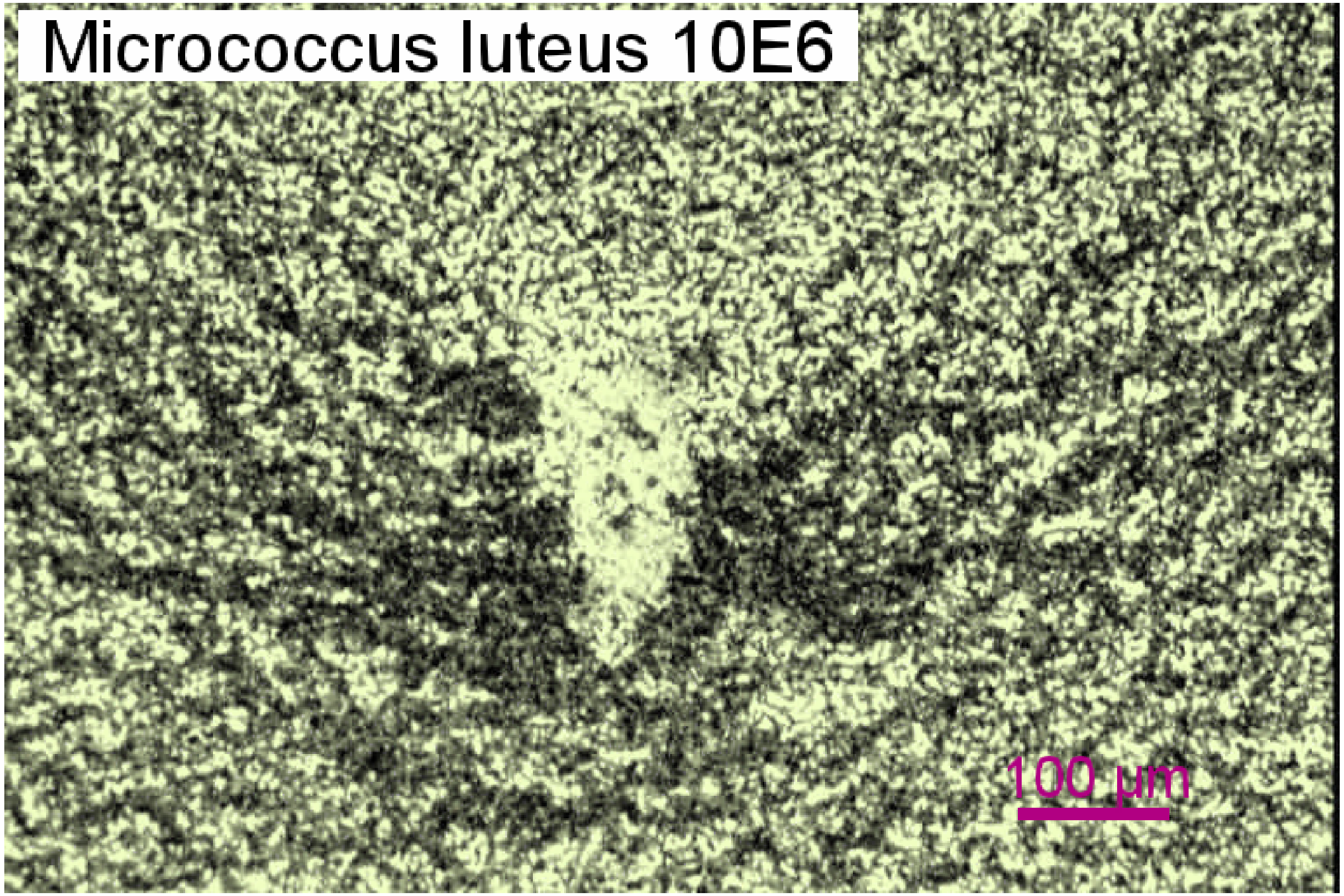
H_2O_2 Decontamination Process seems to be *not confidence*

Selection of Bioindicators *Population, N_0*

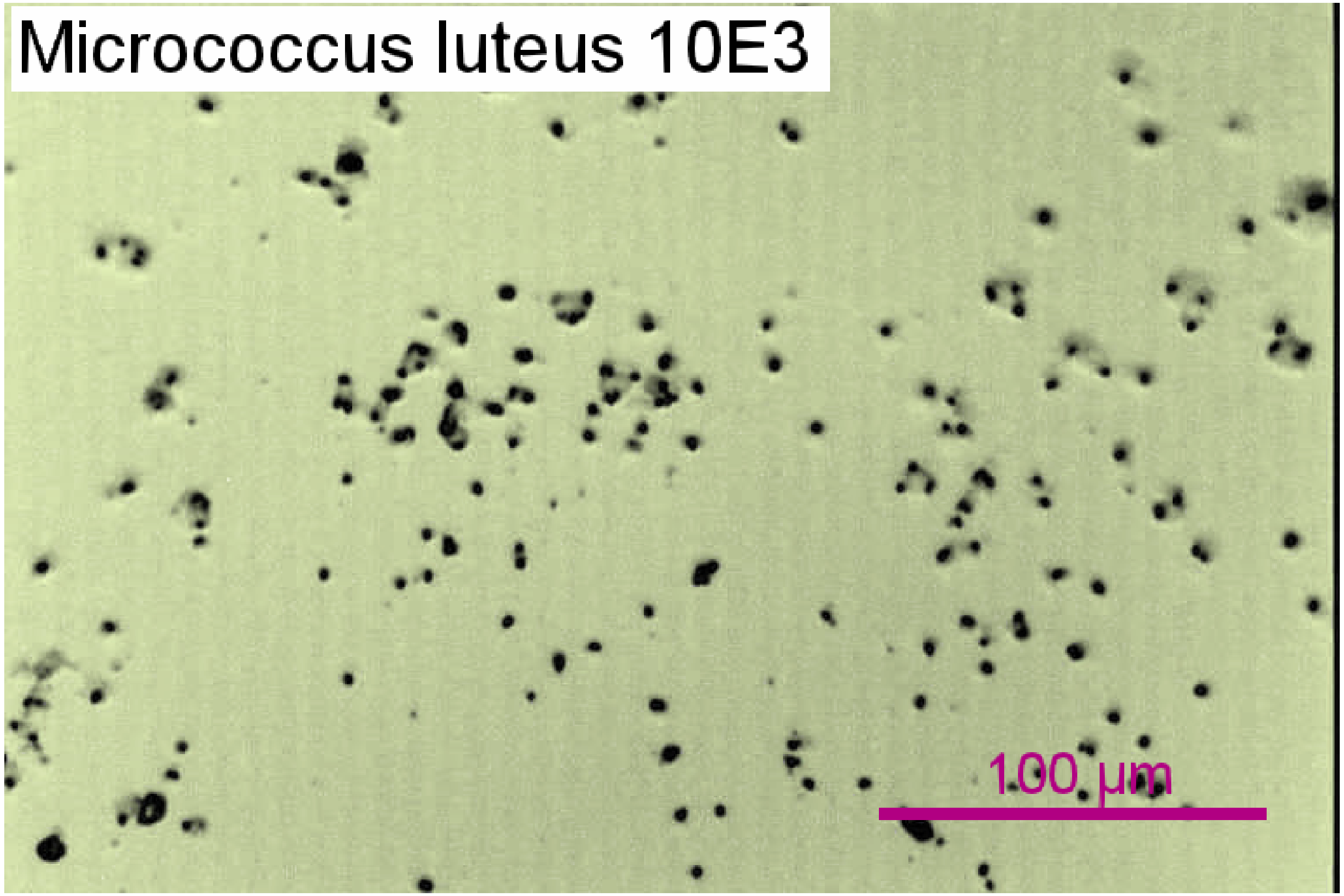
Dependency of Initial *Population* and *Resistance*

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

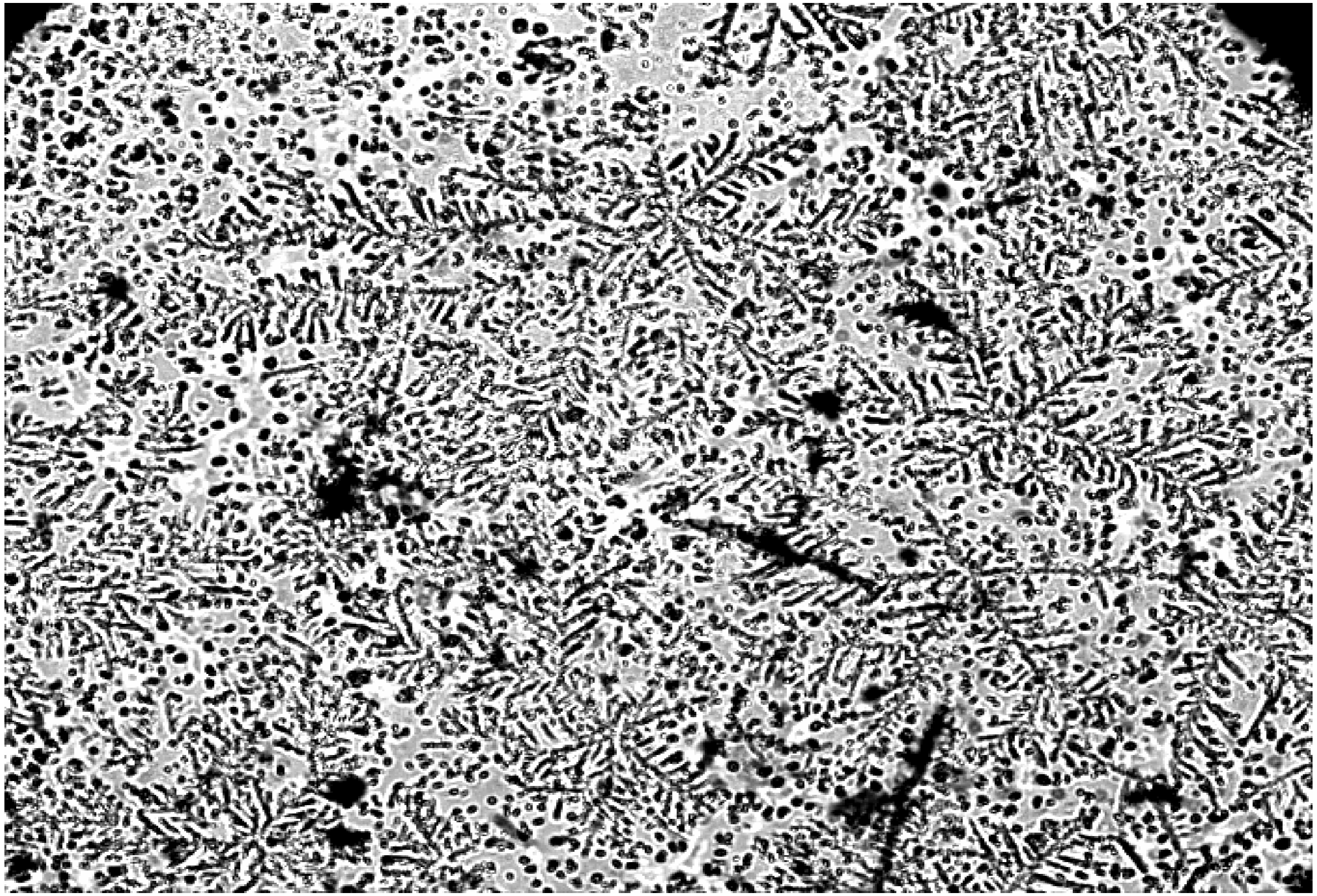
Selection of Bioindicators *Population, N_0*



Selection of Bioindicators *Population, N_0*



Selection of Bioindicators *Population, N_0*



Selection of Bioindicators *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 H_2O_2
- *inconsistent* Results of Bioindicators

The *Penetration* of the Decontamination Effect of the H_2O_2 Process is

- *not ensured*
- *but also not expected*

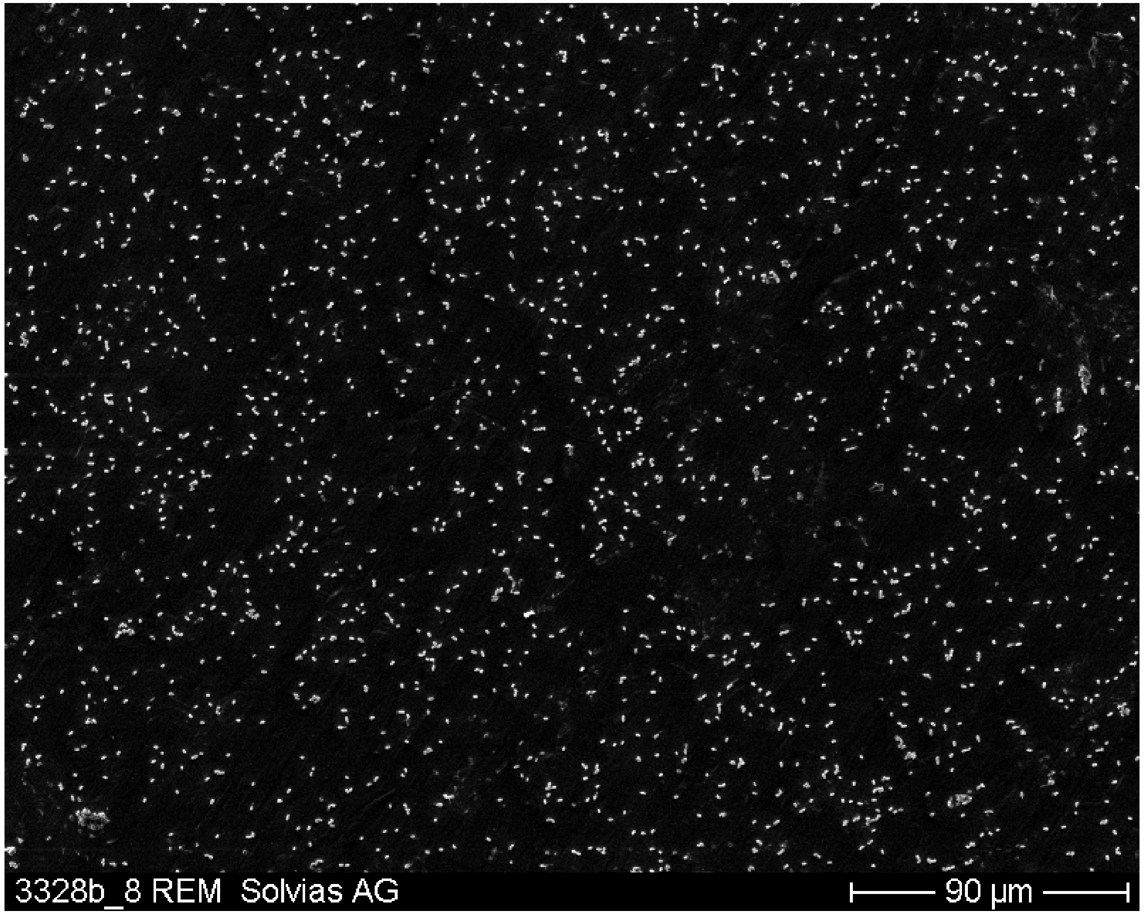
Selection of Bioindicators Population, N_0

Dependency of Initial Population and Resistance

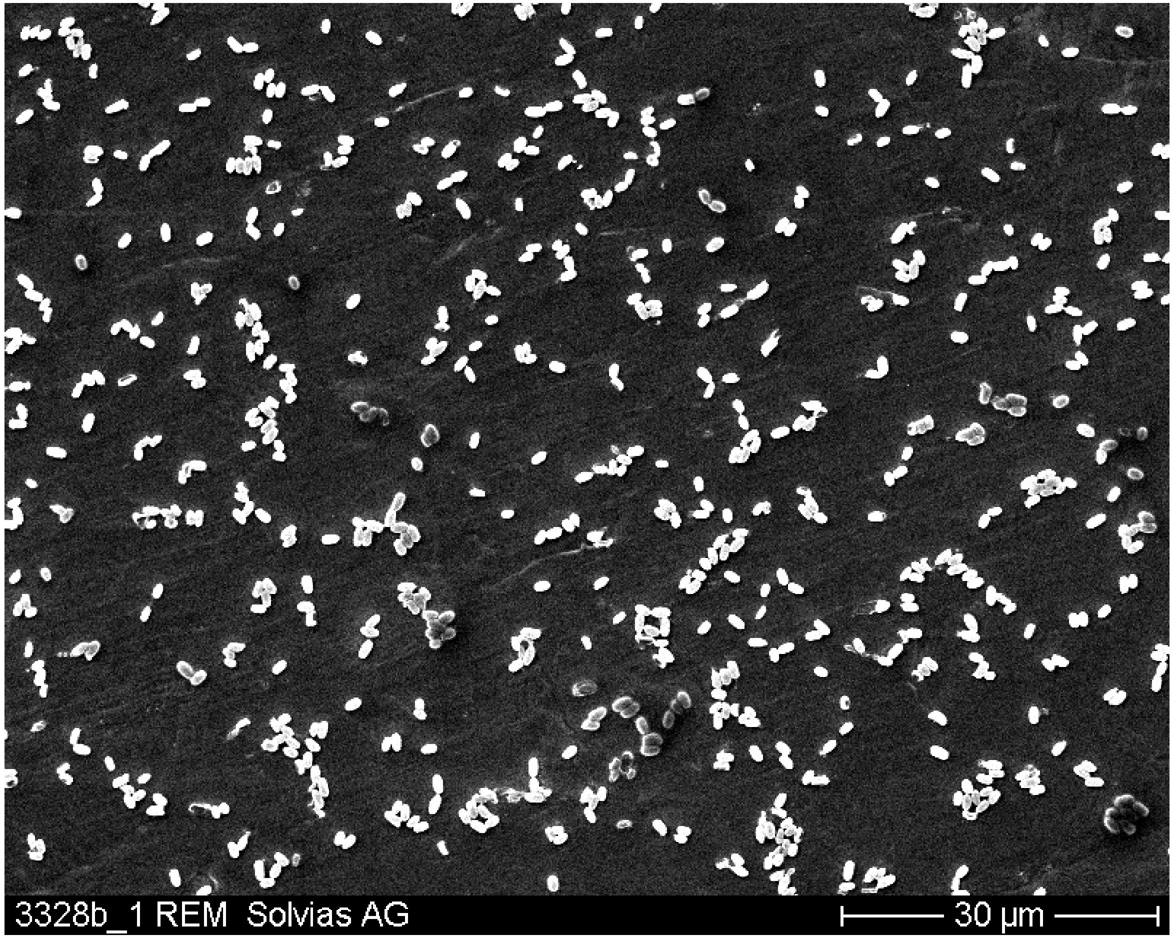
Test Organism	Population, N_0 [log Steps]	D-value [min]
Bacillus stearothermophilus	$\geq 1.0 \times 10^2$	1.6
Bacillus stearothermophilus	$\geq 1.0 \times 10^4$	1.3
Bacillus stearothermophilus	$\geq 1.0 \times 10^6$	1.4

Method Deviation ± 10%

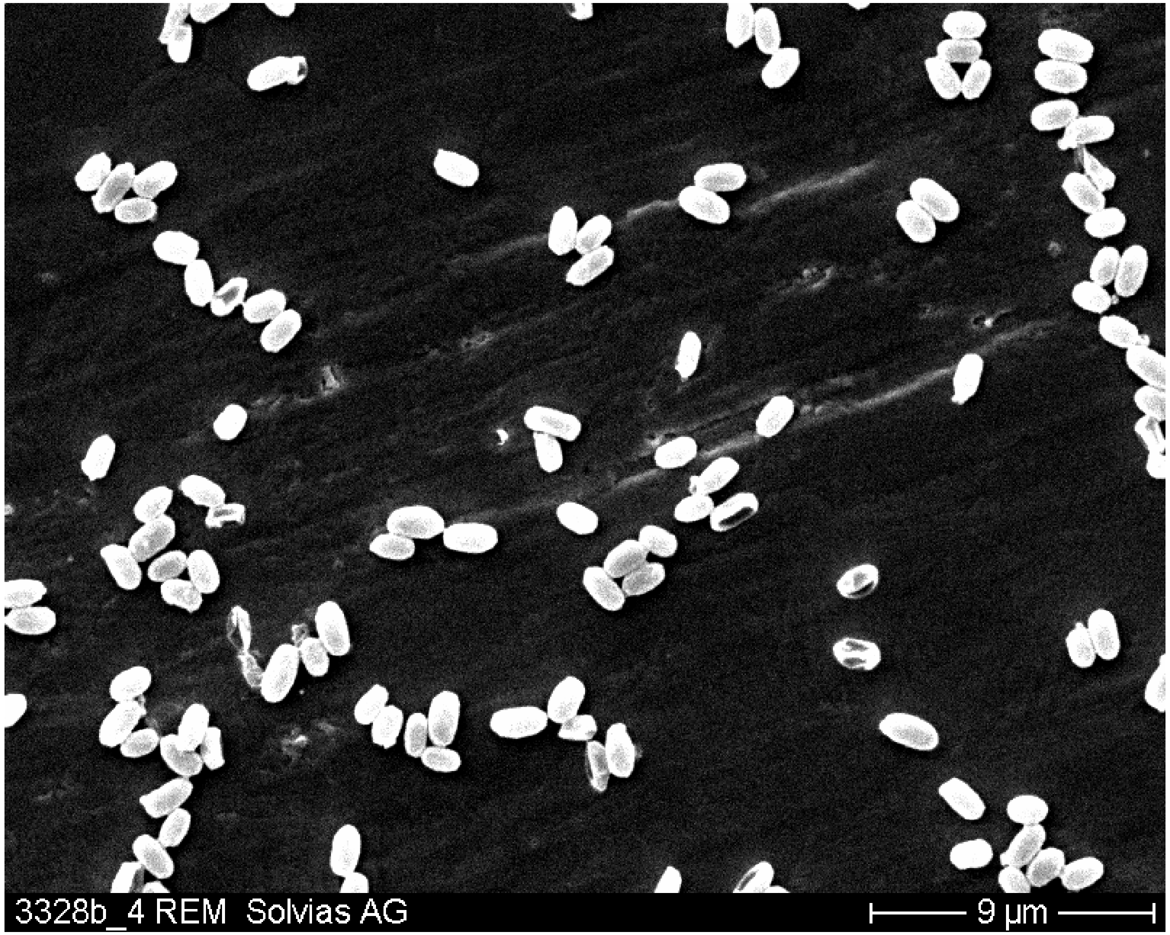
Selection of Bioindicators *Population, N_0*



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. Plastic Materials	Gloves, Sleeves, div. Product Enclosures
• dif. Metals	e.g. Aluminum

Biological Indicator

- Should *reflect* the Process
- Should behave according the *Model of microbial Reduction*

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

Selection of Bioindicators Carrier Material

Materials	Use
CrNi steel, different qualities: 1.4301 (304) unpolished 1.4301 (304) polished (Ra < 0.8 µm) 1.4435 (316L) unpolished 1.4435 (316L) polished (Ra < 0.8 µm)	Main chamber material Parts of filling line Steritest pump
Glass	Window and door material Media bottles, product units
Polycarbonate, PC	Window material
Hypalon	Glove material
Polyvinylchloride, PVC, soft	Material of glove gauntlets
Polyvinylchloride, PVC, hard	Glove ports
Polyvinylchloride, PVC	Package of steritest units
Polyoxymethylene, POM	Conveyer system of filling line, shelfs of steritest isolator
Polypropylene, PP	RTP-ports
Polyethylene, PE-UHMW	Conveyer system of filling line, RTP-ports
Polytetrafluorethylene PTFE / (Teflon)	Tubings, parts of filling line
Aluminium anodized, different qualities	Material sample commercially available Material sample filling line Material sample air sampler
Butyl caoutchouc	Stoppers of media bottles and product units
Laminated foil 1; from inside to outside: Polyethylene, Aluminium, Polyester	Package of media plates for cleanroom monitoring
Laminated foil 2; from inside to outside: Polyethylene, Polypropylene	Package of media plates for cleanroom monitoring
Tyvek	Package of Steritest units
HEPA-filter pad	HEPA-filter

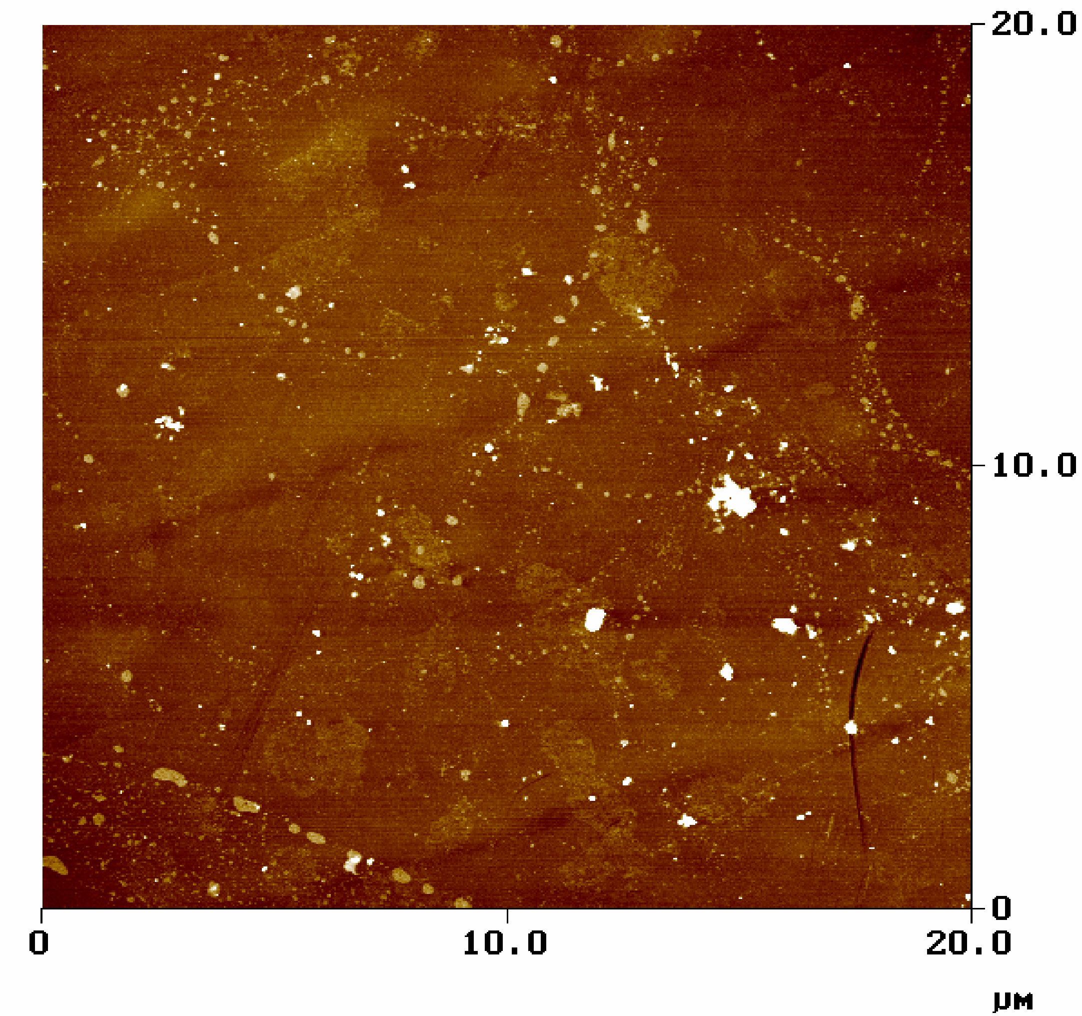
Carrier Material; Glass

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

+ growth
- no growth

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	+
Result 1	+	+	+	+	-	-	-	-	-	-	neg
2	+	+	+	-	-	-	-	-	-	-	-
3	+	+	+	-	-	-	-	-	-	-	

- estimated D-value: 1.2 [min]
- Model Behavior: OK

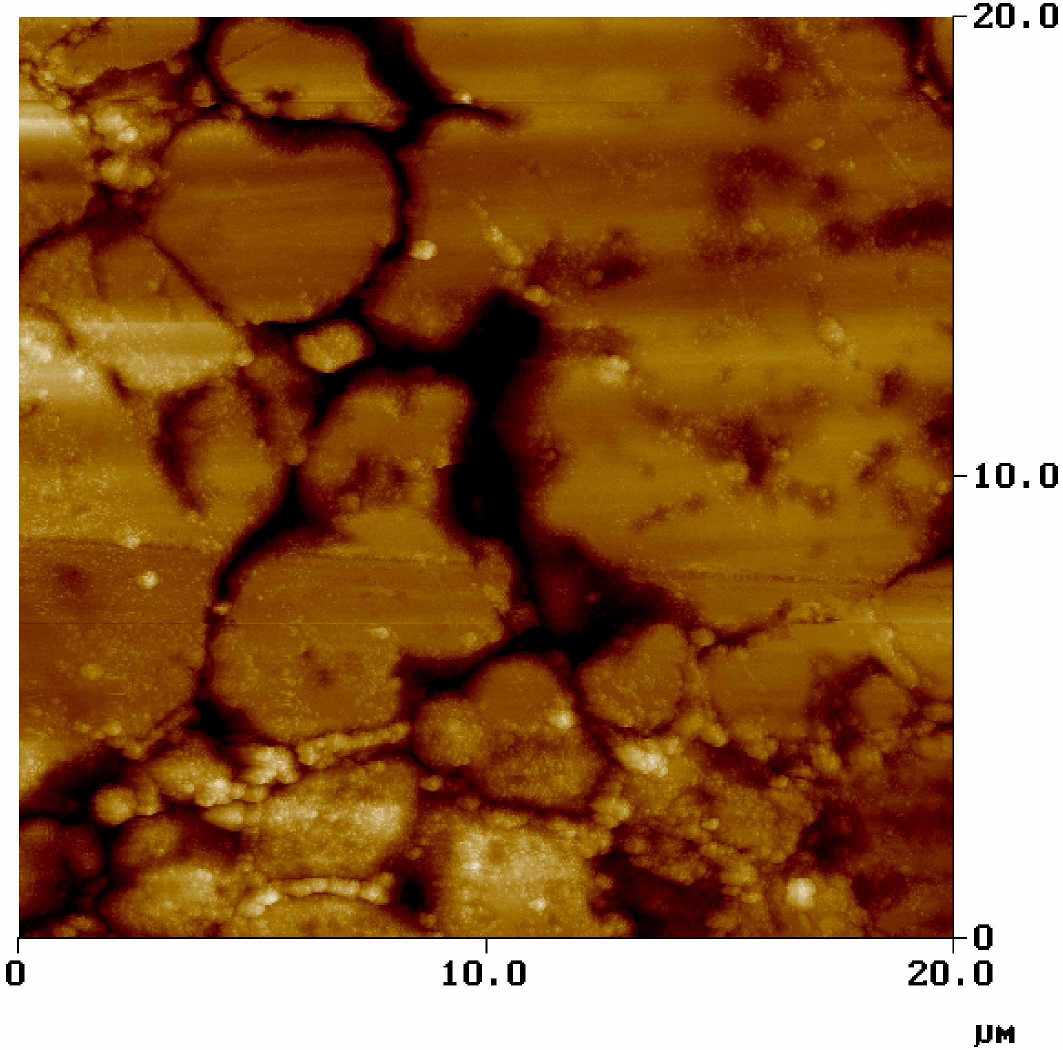
Carrier Material; stainless steel 1.4435, not polished

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

\+ growth

\- no growth

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	+
Result 1	+	+	+	+	+	-	-	-	-	-	neg
2	+	+	+	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	

- estimated D-value: 1.3 [min]
- Model Behavior: OK

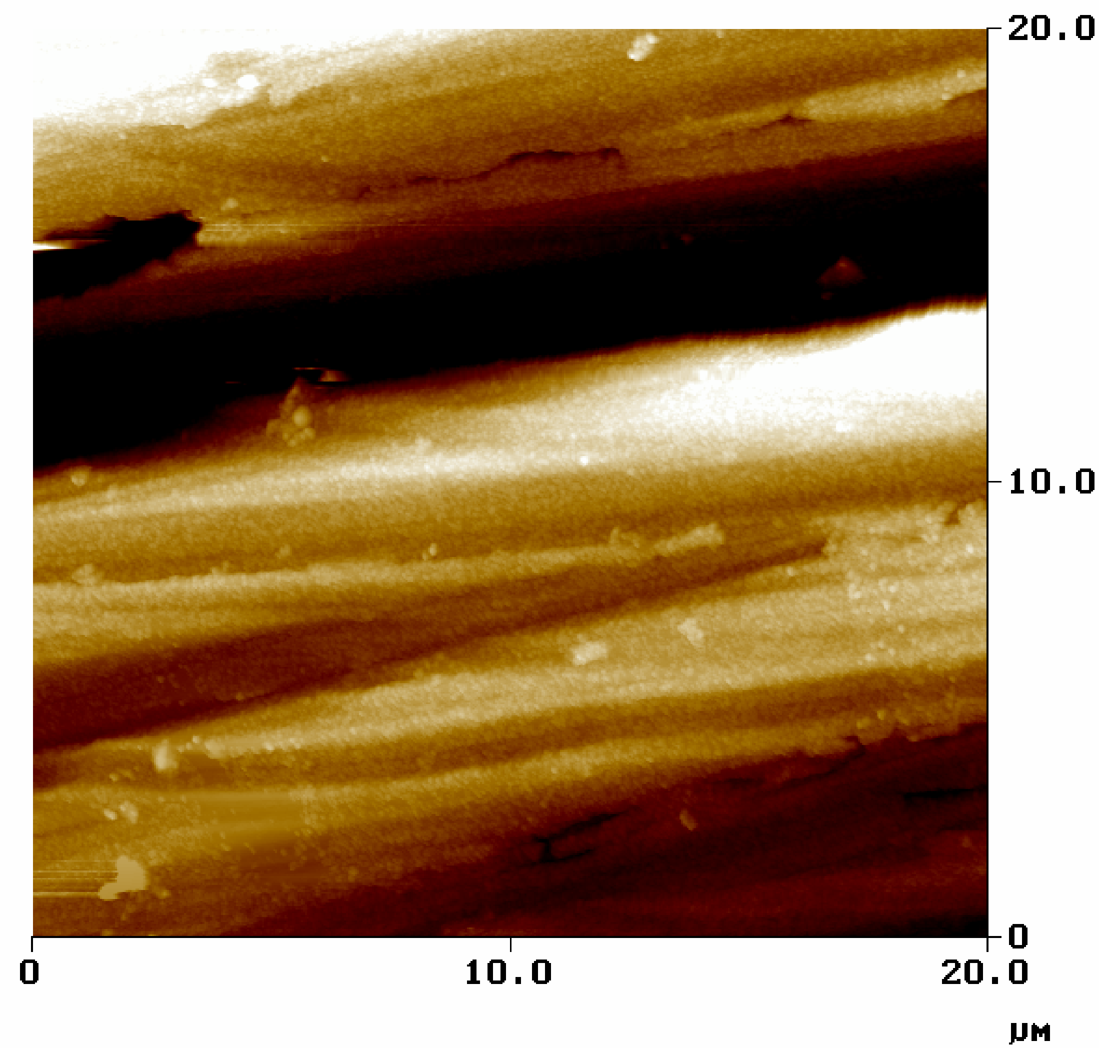
Carrier Material; stainless steel 1.4435, polished

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 x 10^6
Carrier Material: Stainless Steel, polished

+ growth
- no growth

Group		01	02	03	04	05	06	07	08	09	10	pos
Expousure [min]		2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	+
Result	1	+	+	+	-	-	-	-	-	-	-	neg
	2	+	+	-	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-	-	

- estimated D-value: 0.9 [min]
- Model Behavior: OK

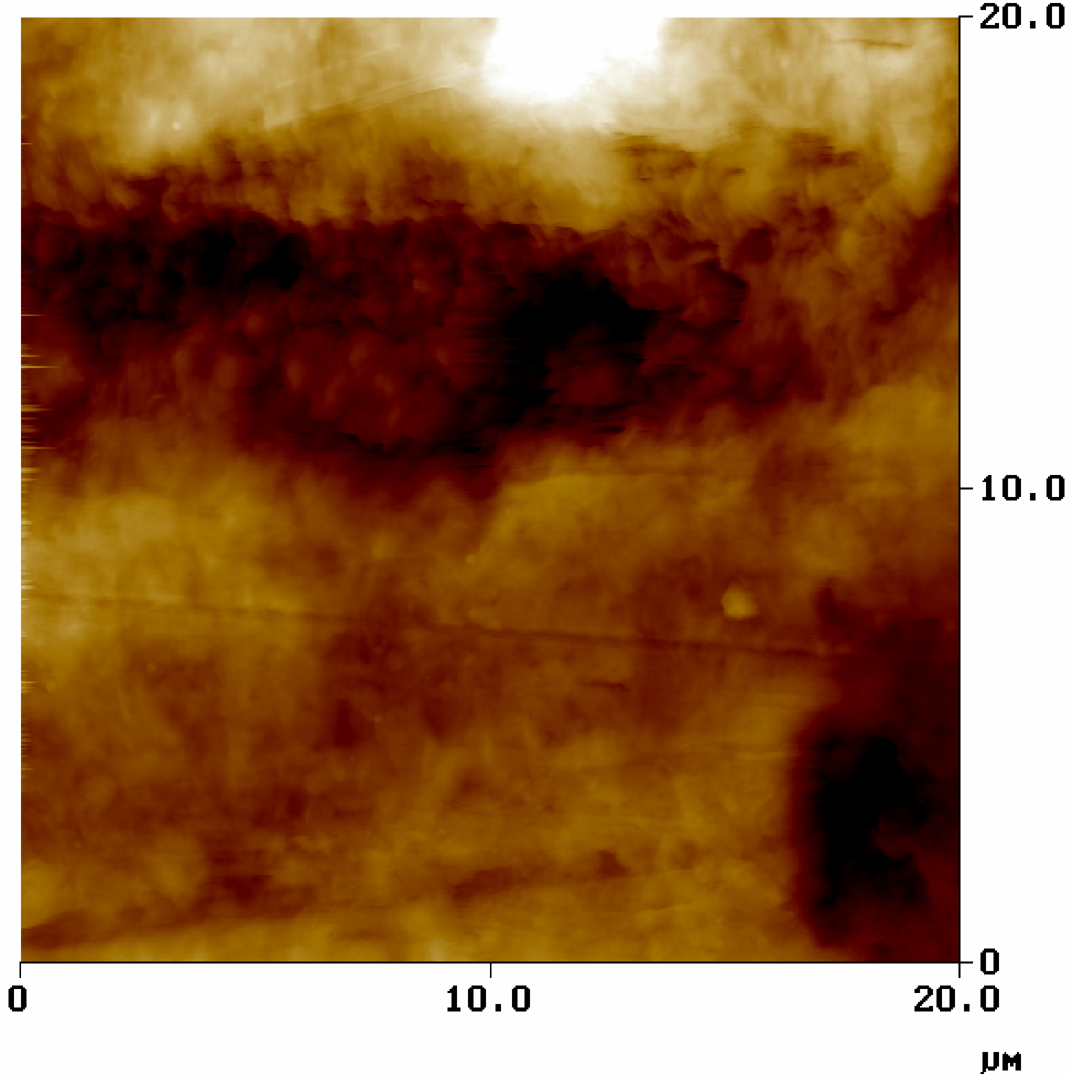
Carrier Material; PTFE, Teflon

Roughness

- P-P: 1266 nm
- RMS: 180 nm
- R_a: 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

Group		01	02	03	04	05	06	07	08	09	10	pos
Expousure [min]		2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	+
Result	1	+	+	+	+	+	-	-	-	-	-	neg
	2	+	+	+	+	-	-	-	-	-	-	-
	3	+	+	+	-	-	-	-	-	-	-	

- estimated D-value: 1.6 [min]
- Model Behavior: OK

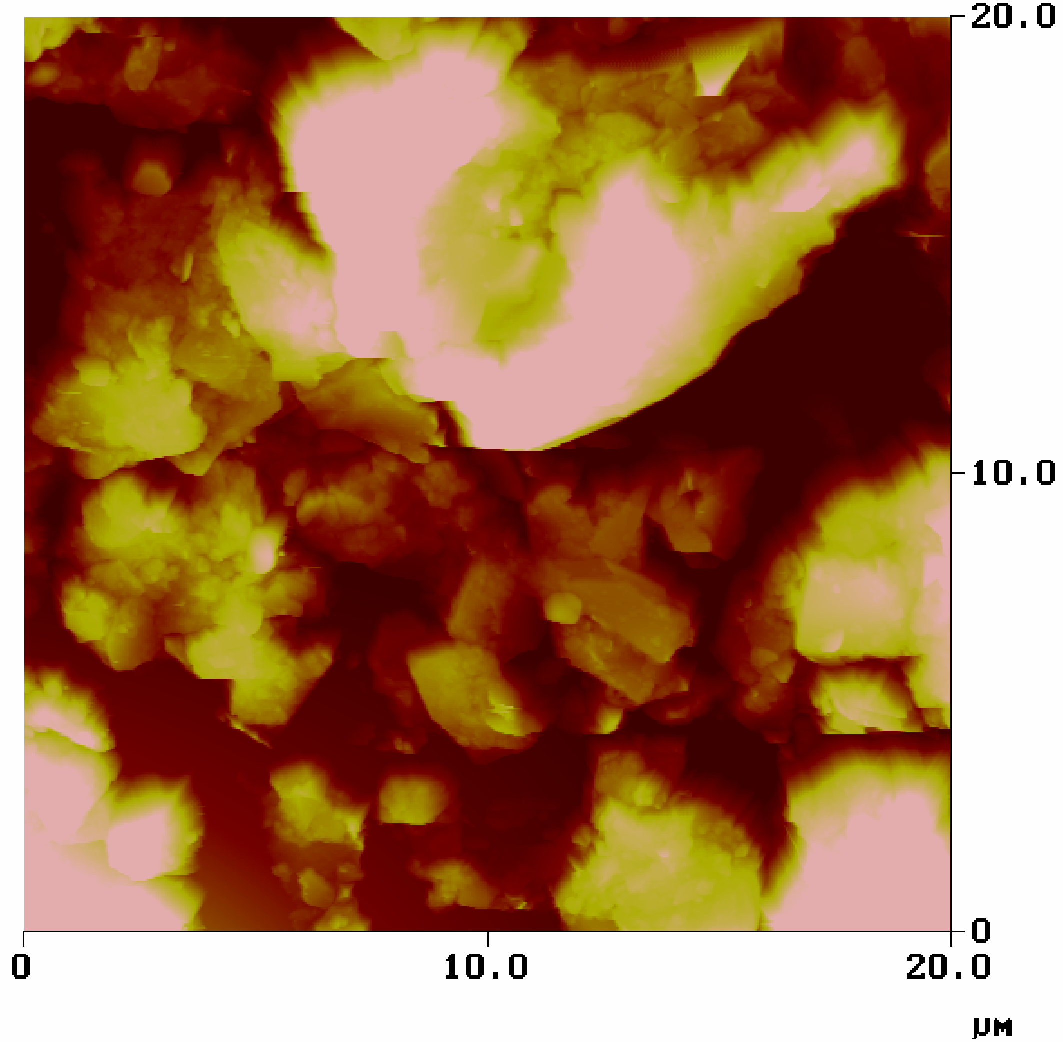
Carrier Material, Hypalon

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 x 10^6
Carrier Material: Hypalon

\+ growth
\- no growth

Group		01	02	03	04	05	06	07	08	09	10	pos
Expousure [min]		10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0	+
Result	1	+	+	-	-	-	-	-	-	-	-	neg
	2	+	-	-	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-	-	

- estimated D-value: 3.0 [min]
- Model Behavior: OK

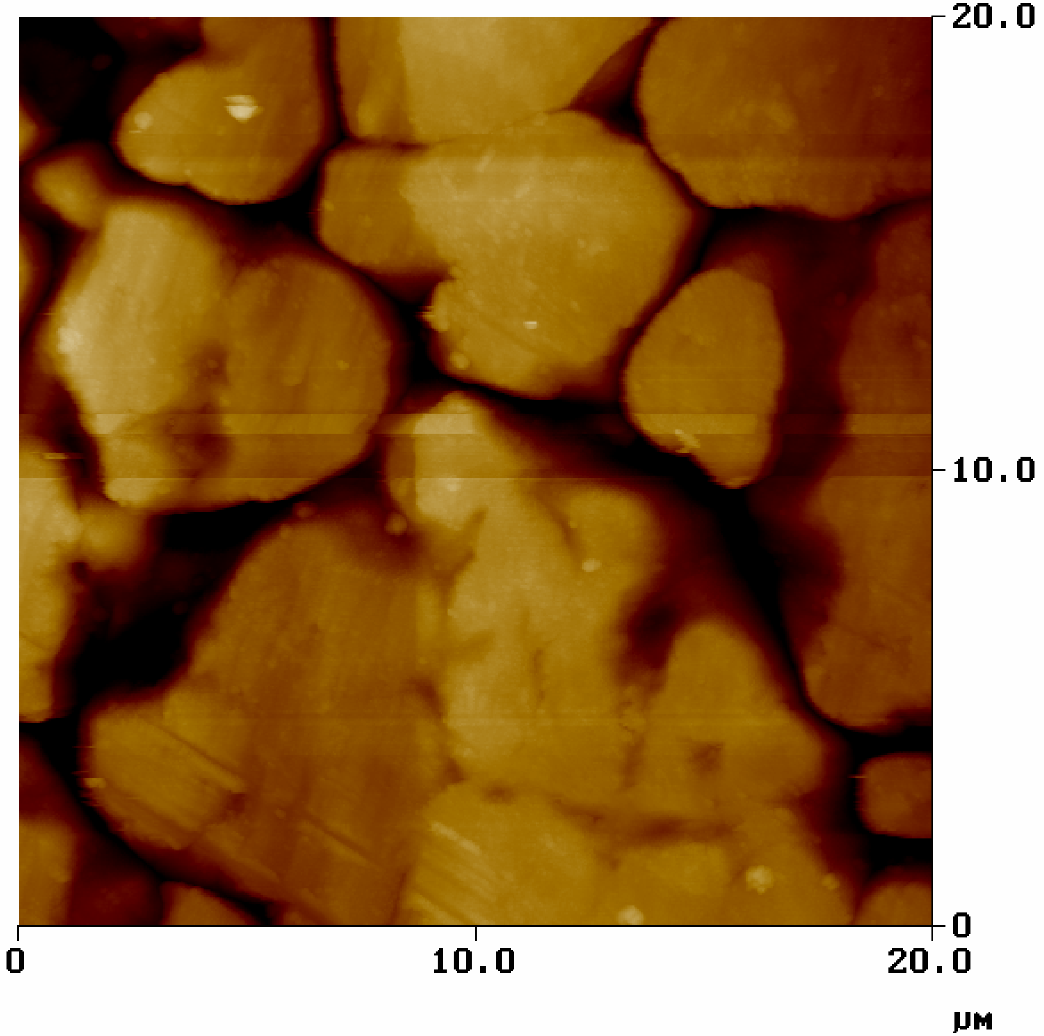
Carrier Material, Aluminium; commercial Sample

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 10^6
Carrier Material: Aluminum, anodized

\+ growth
\- no growth

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]	10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0	+
Result 1	+	+	+	+	+	+	+	-	-	+	neg
2	+	-	-	+	+	-	+	-	-	-	-
3	+	-	-	+	+	-	-	-	-	-	

- estimated D-value: 8.4 [min]
- Model Behavior: not OK

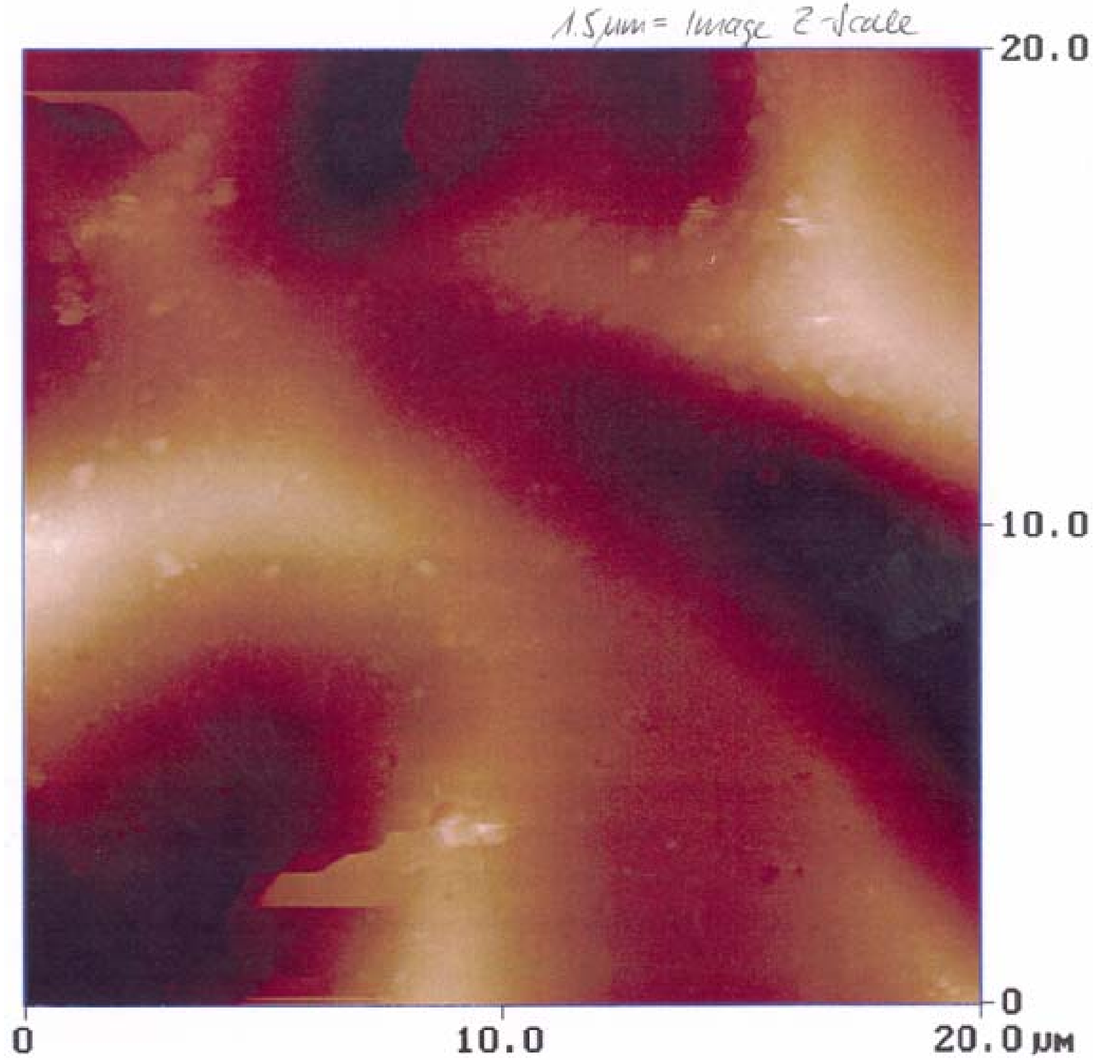
Carrier Material, Aluminium; Air Sampler

Roughness

- P-P: 1436 nm
- RMS: 290 nm
- R_a: 236 nm

Wettability

- high



Carrier Material, Model Behavior

Test Organism: B. stearothermophilus
Initial Population: > 1.0×10^6
Carrier Material: Aluminum, Air Sampler

\+ growth
\- no growth

Group	01	02	03	04	05	06	07	08	09	10	pos
Expousure [min]	10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0	+
Result 1	+	+	+	+	+	+	+	-	-	-	neg
2	+	+	+	+	+	-	-	-	-	-	-
3	+	+	+	+	+	-	-	-	-	-	

- estimated D-value: 7.9 [min]
- Model Behavior: OK

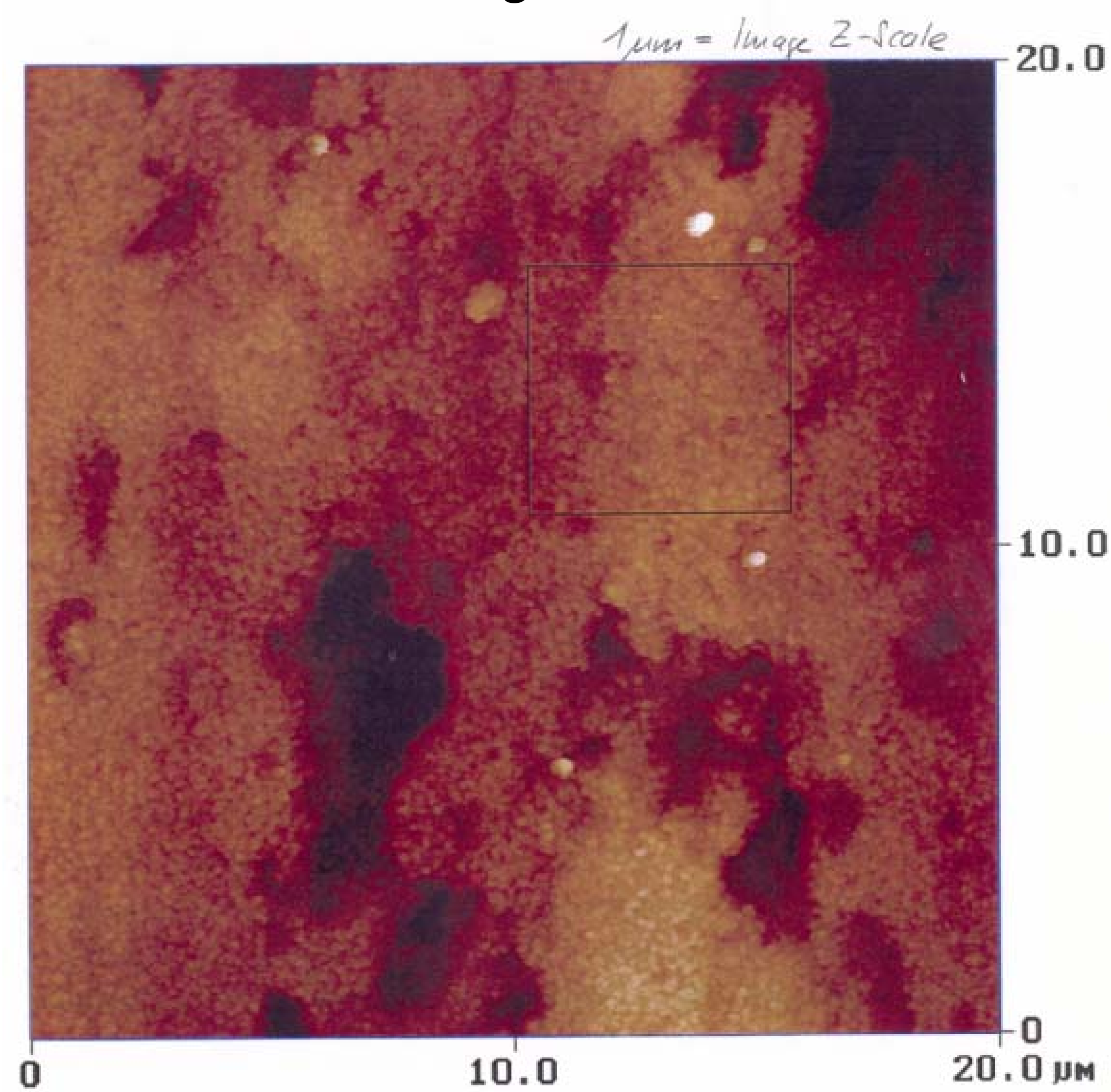
Carrier Material, Aluminium; Filling Line

Roughness

- P-P: 1478 nm
- RMS: 132 nm
- R_a: 94 nm

Wettability

- high



Carrier Material, Model Behavior

Test Organism: B. stearothermophilus

Initial Population: > 1.0 x 10^3

Carrier Material: Aluminum, anodized

\+ growth

\- no growth

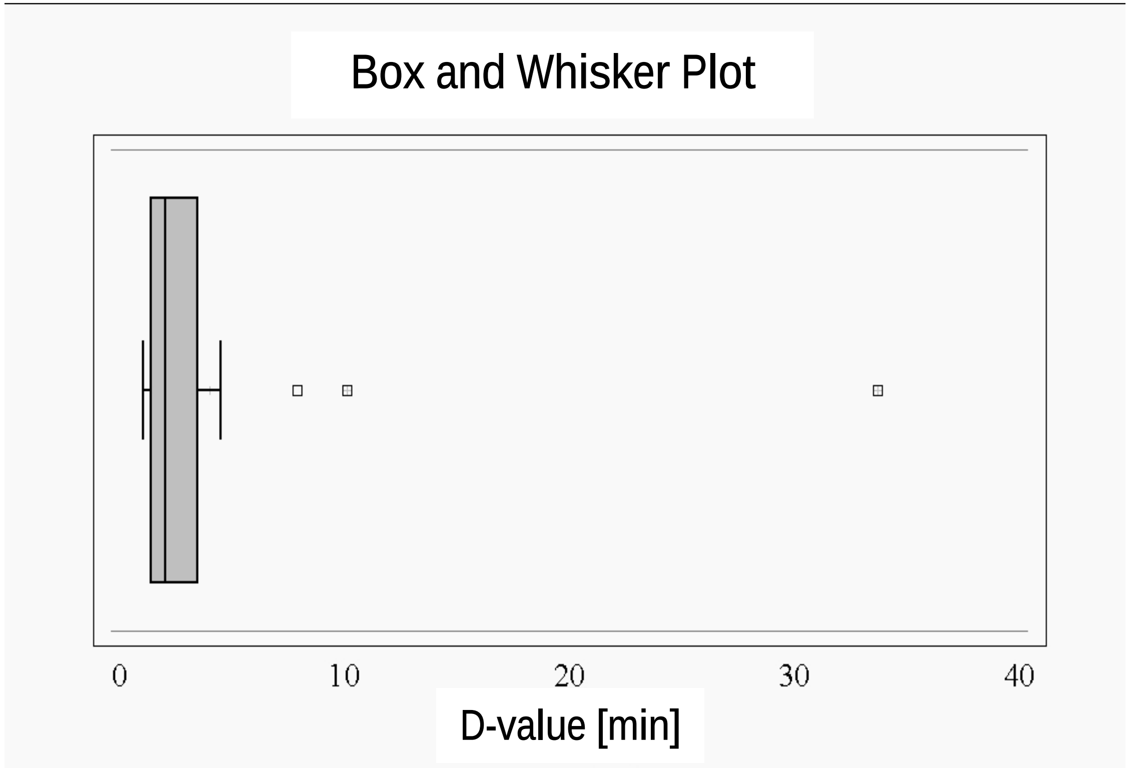
Group	01	02	03	04	05	06	07	08	09	10	pos
Expousure [min]	10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0	+
Result 1	+	+	+	+	+	+	+	+	+	+	neg
2	+	+	+	+	+	+	+	+	+	+	-
3	+	+	+	+	+	+	+	+	+	+	

- estimated D-value: > 33.7 [min]
- Model Behavior: not able to be evaluated

Selection of Bioindicators Carrier Material

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

Selection of Bioindicators *Carrier Material*



Significantly higher D-value for the various *aluminium* samples

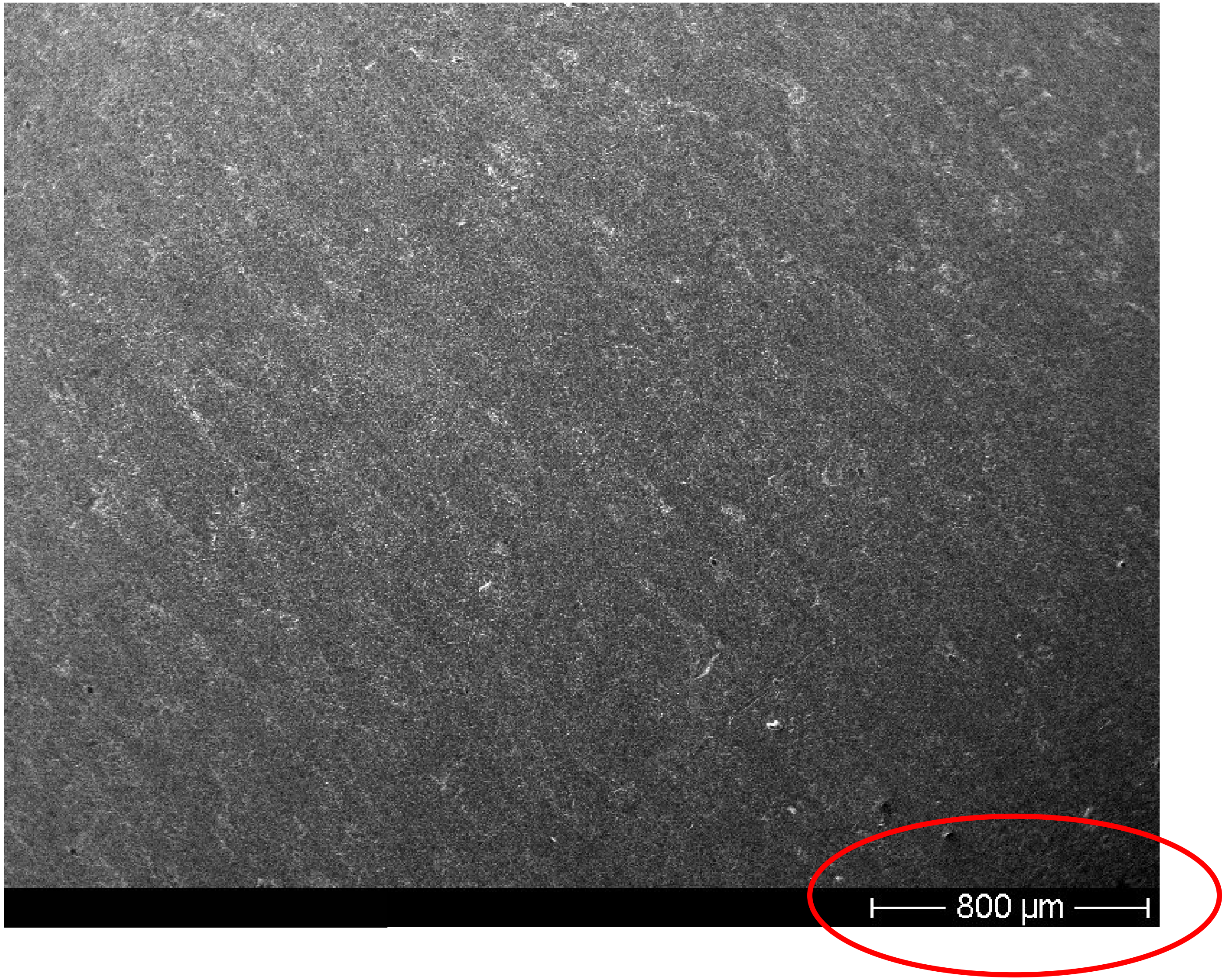
Selection of Bioindicators *Carrier Material*

Unsuitable Carrier Material

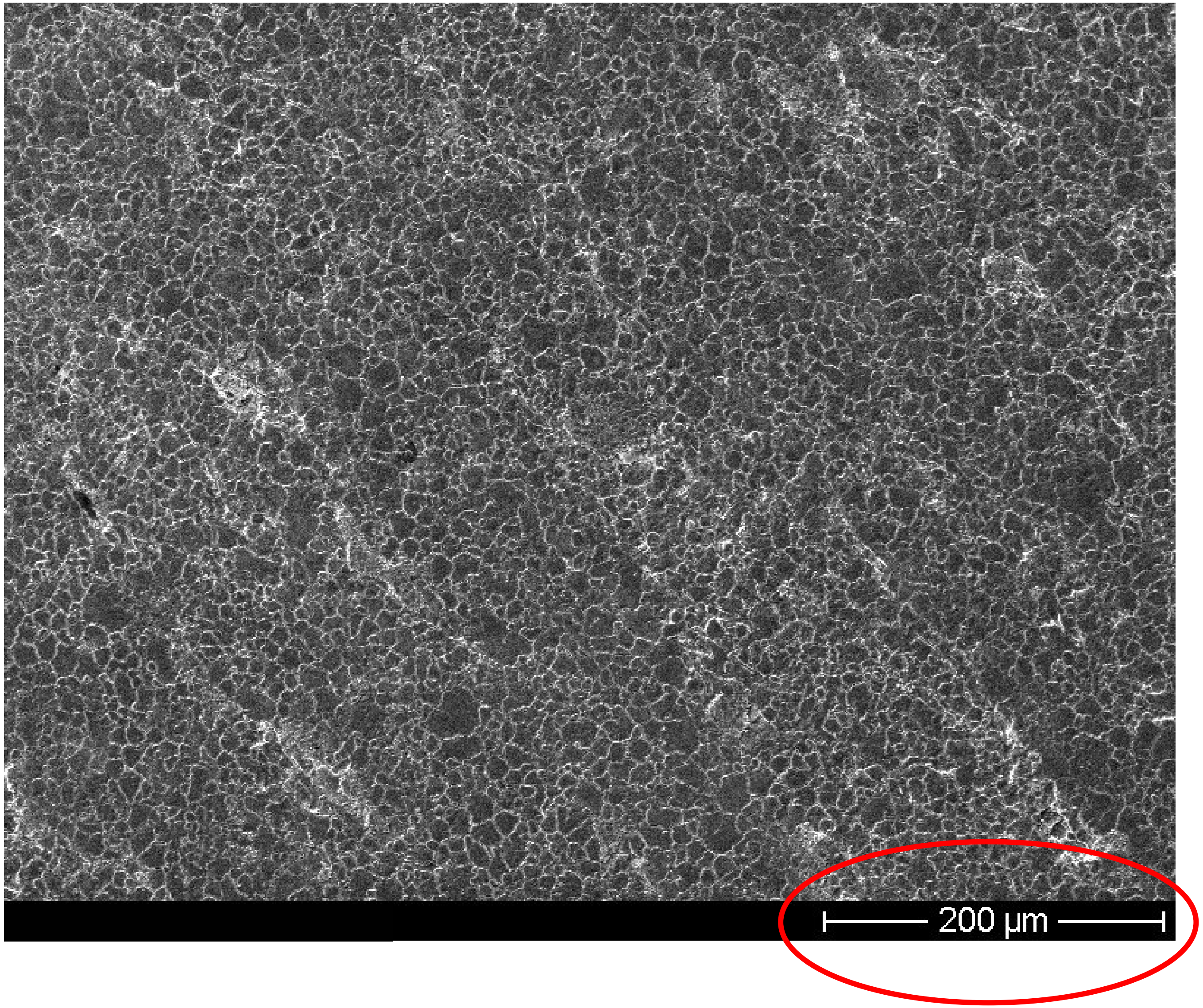
- Negative influence on the *Resistance* of Bioindicators
- Negative influence on the *Model Behavior* of Bioindicators

- Materials are *spongy* or *porous*
- Test Organism *penetrate into* those Materials
- *not or only hart* to be reached by H_2O_2 Decontamination

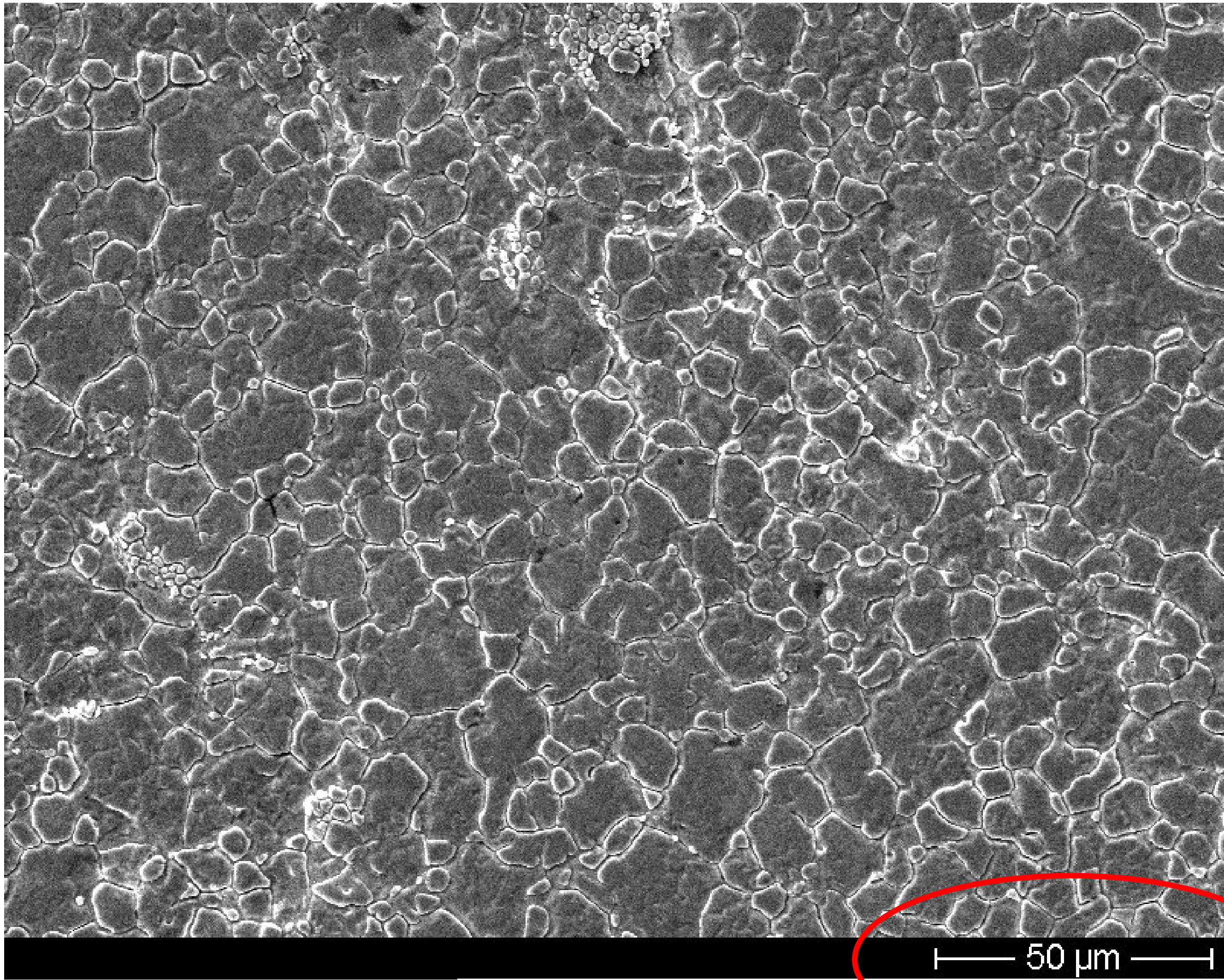
Carrier Material *stainless steel, not polished*



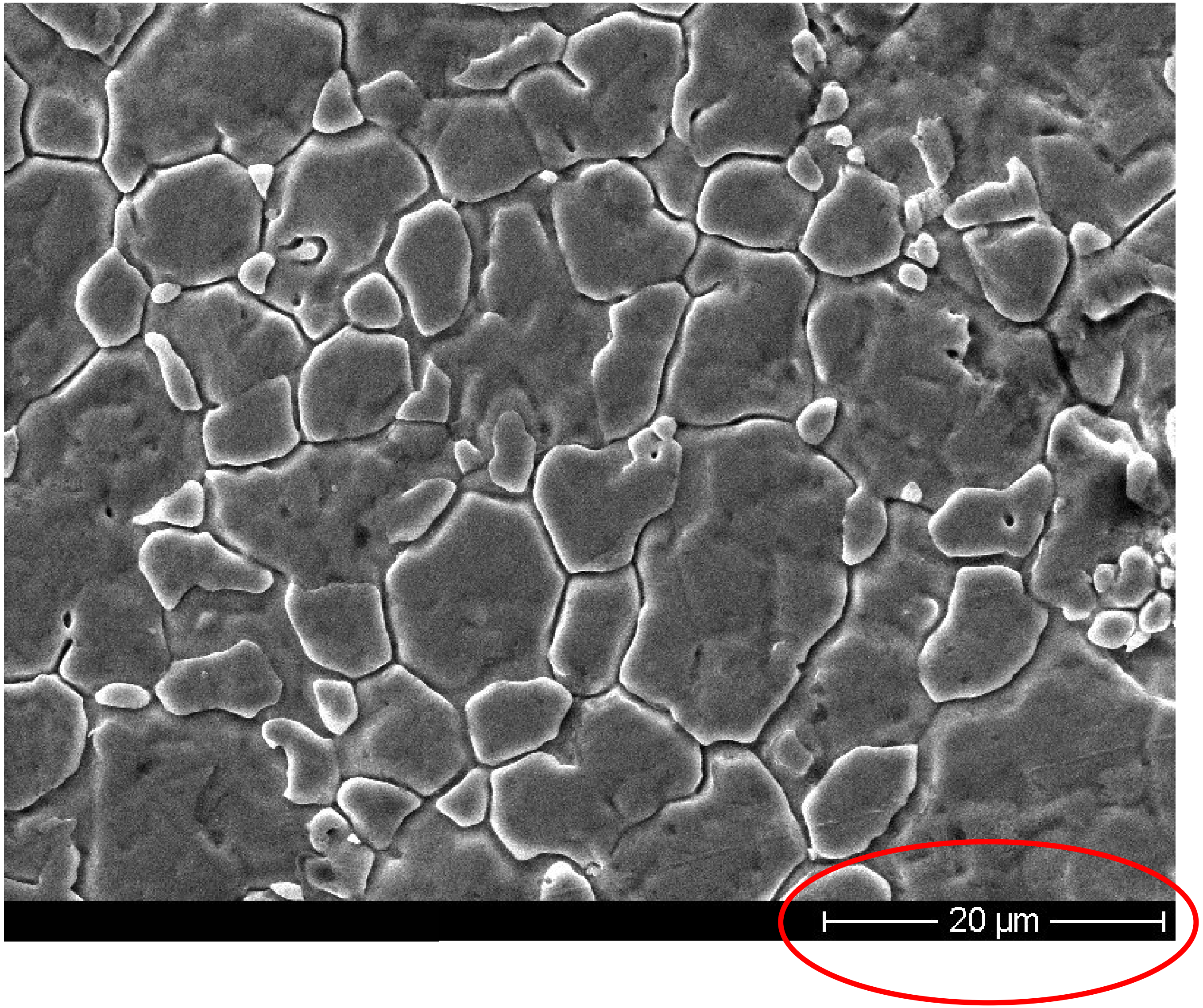
Carrier Material *stainless steel, not polished*



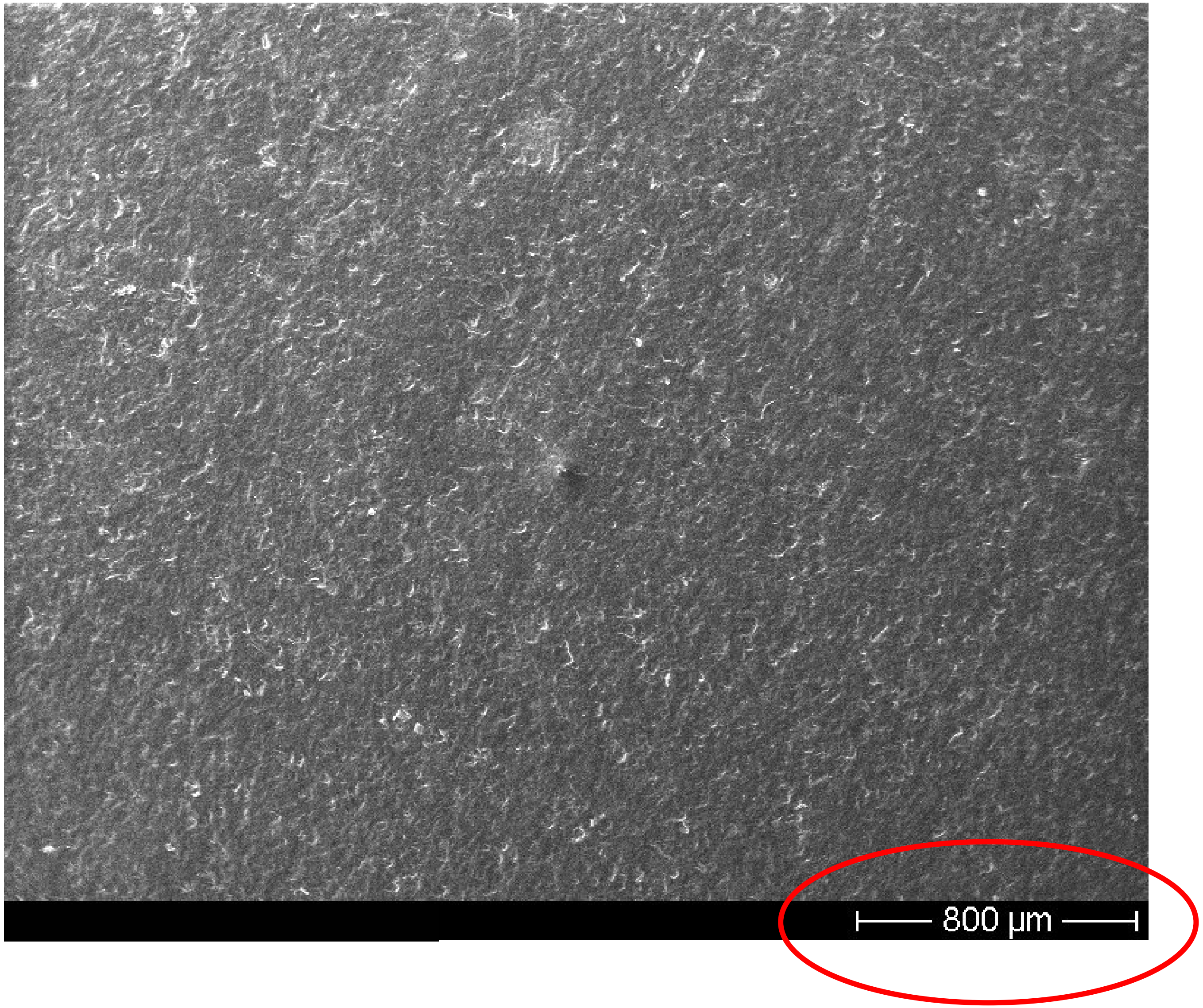
Carrier Material *stainless steel, not polished*



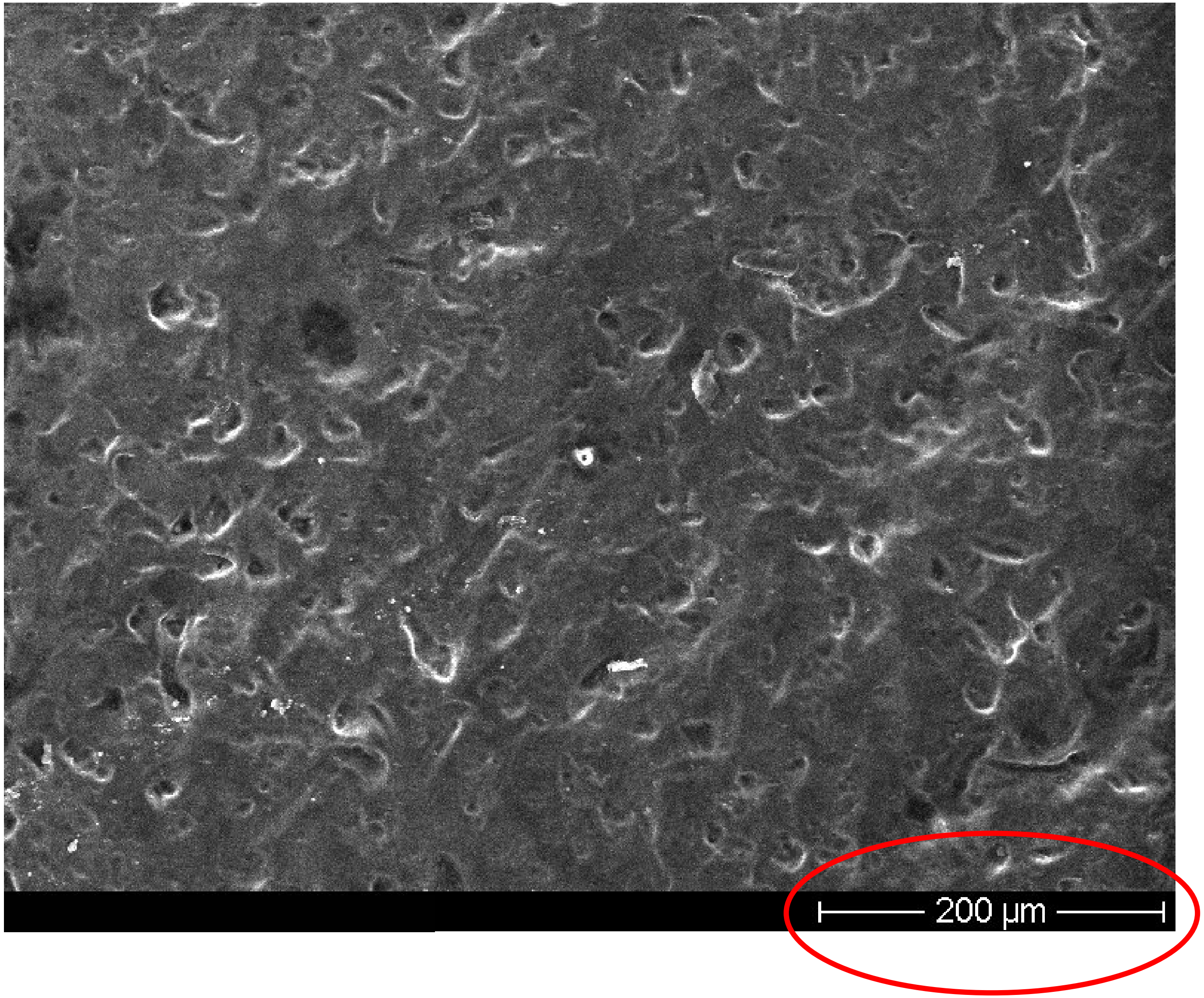
Carrier Material *stainless steel, not polished*



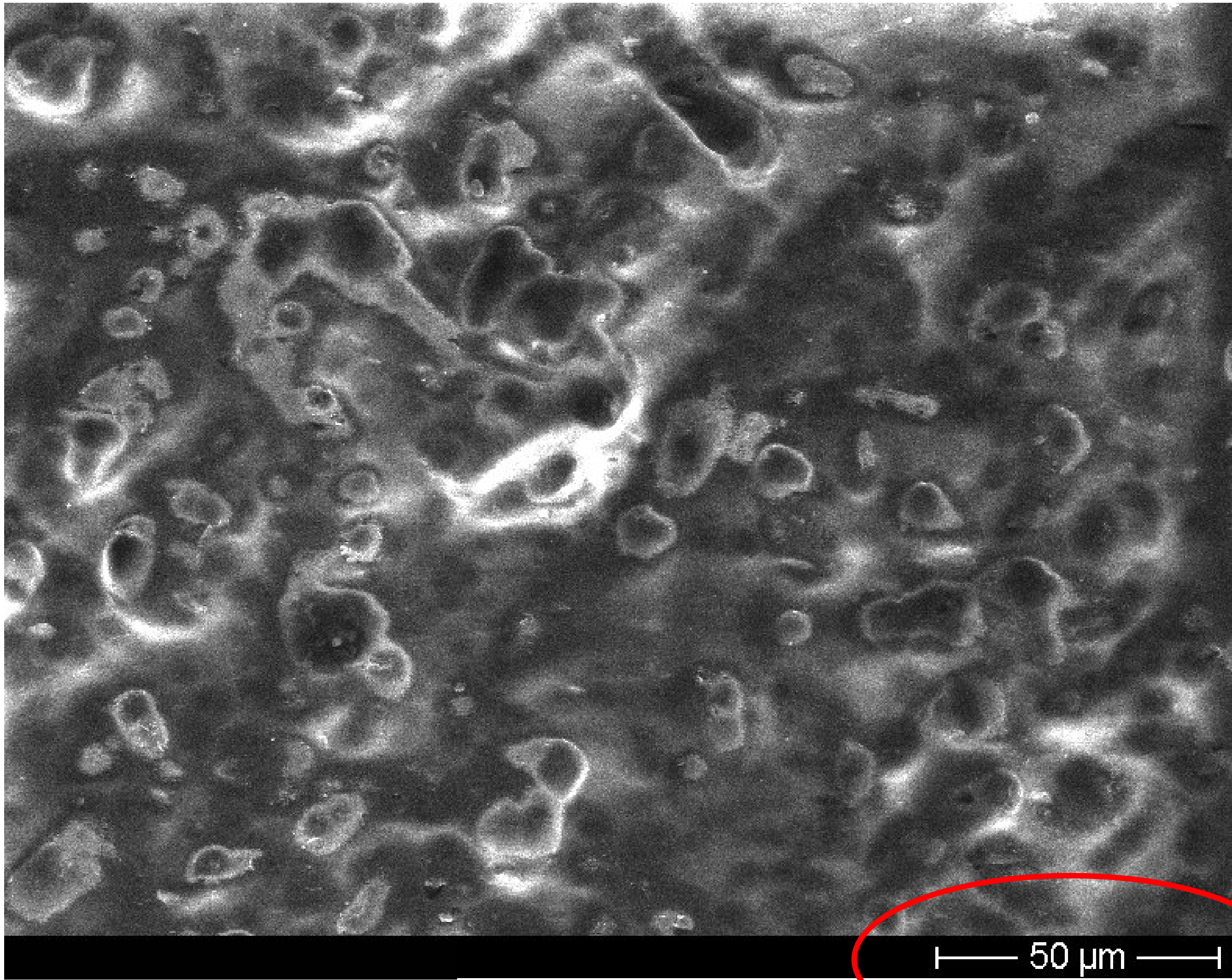
Carrier Material *Aluminium; Air Sampler*



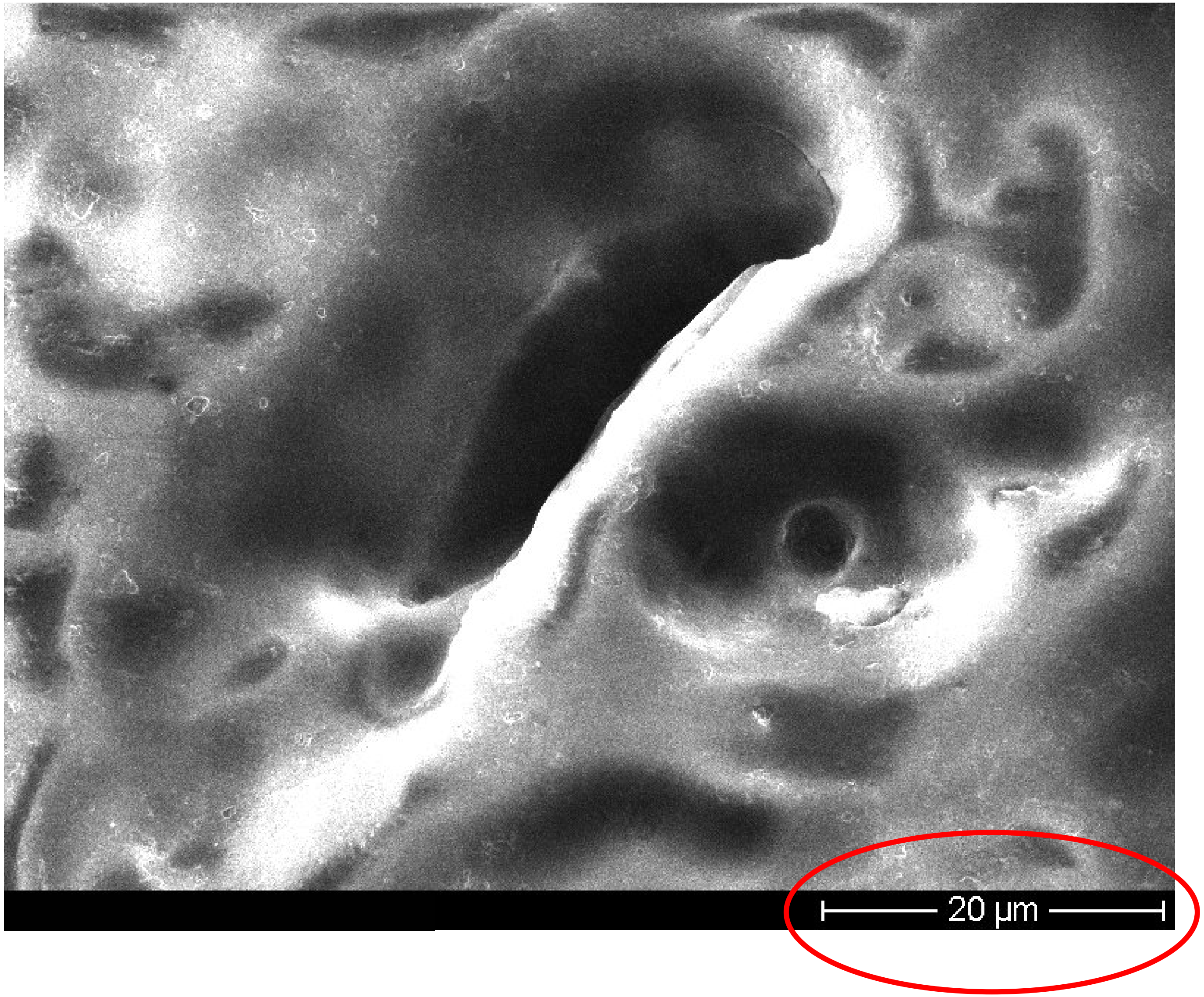
Carrier Material *Aluminium; Air Sampler*



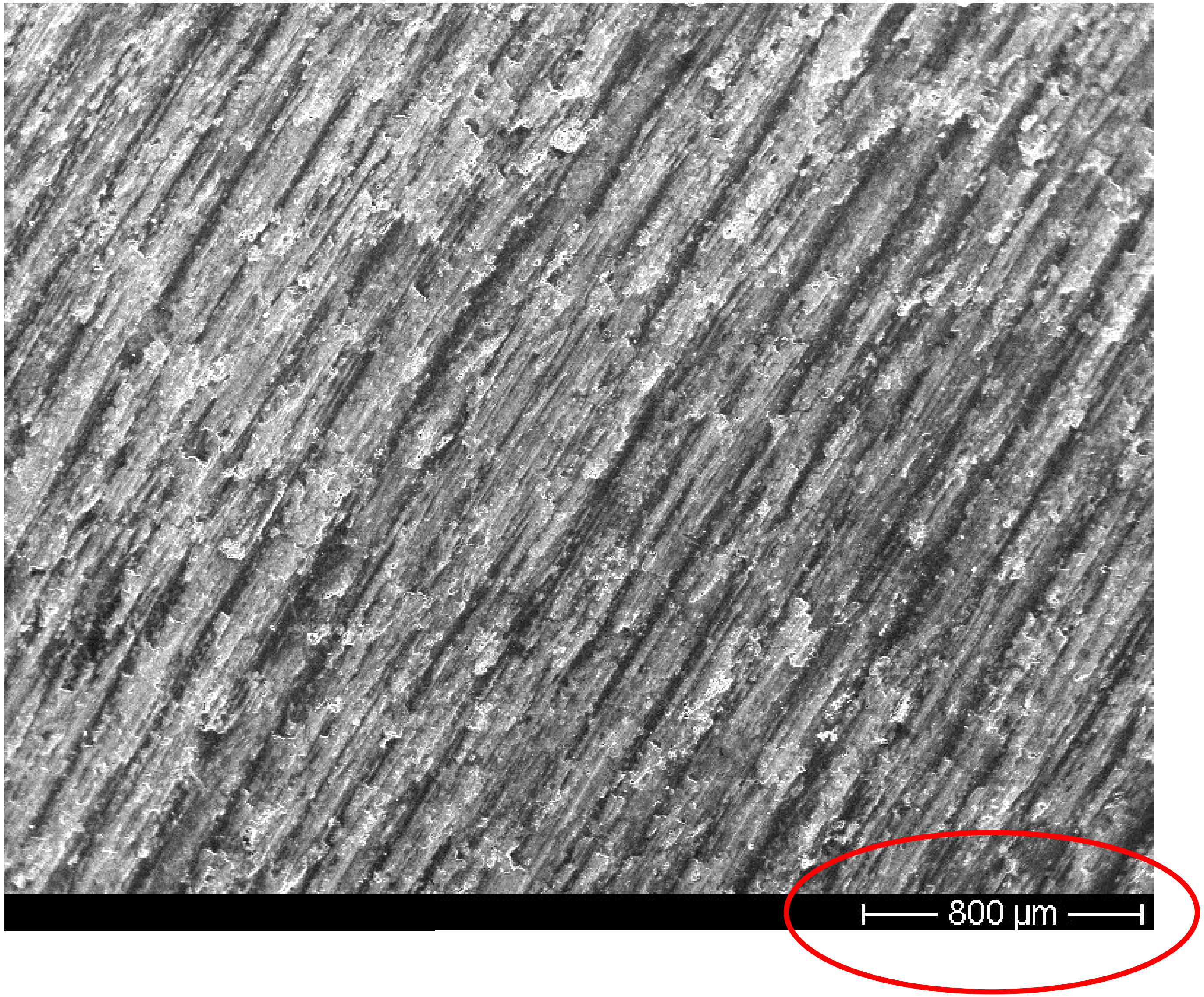
Carrier Material *Aluminium; Air Sampler*



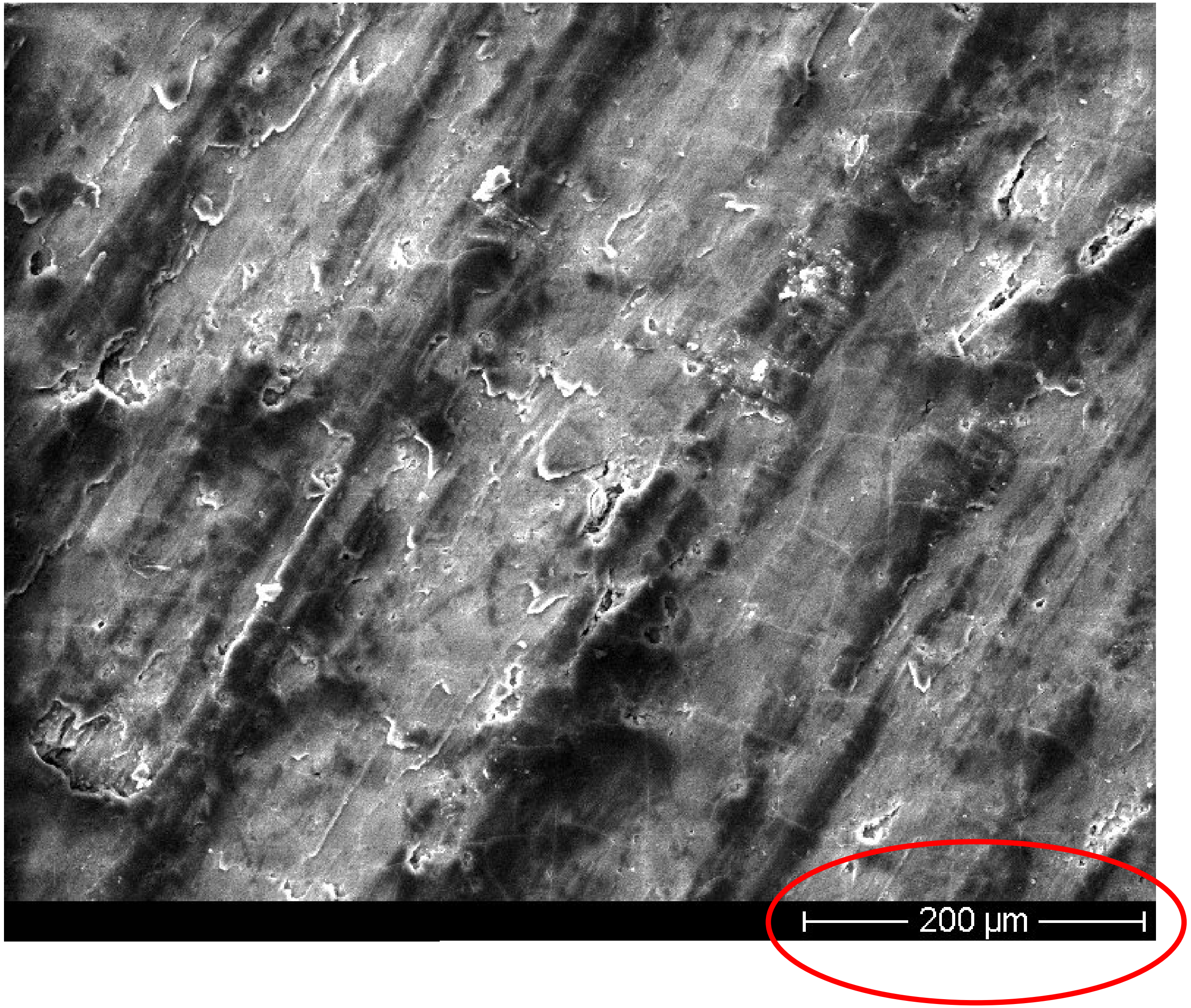
Carrier Material *Aluminium; Air Sampler*



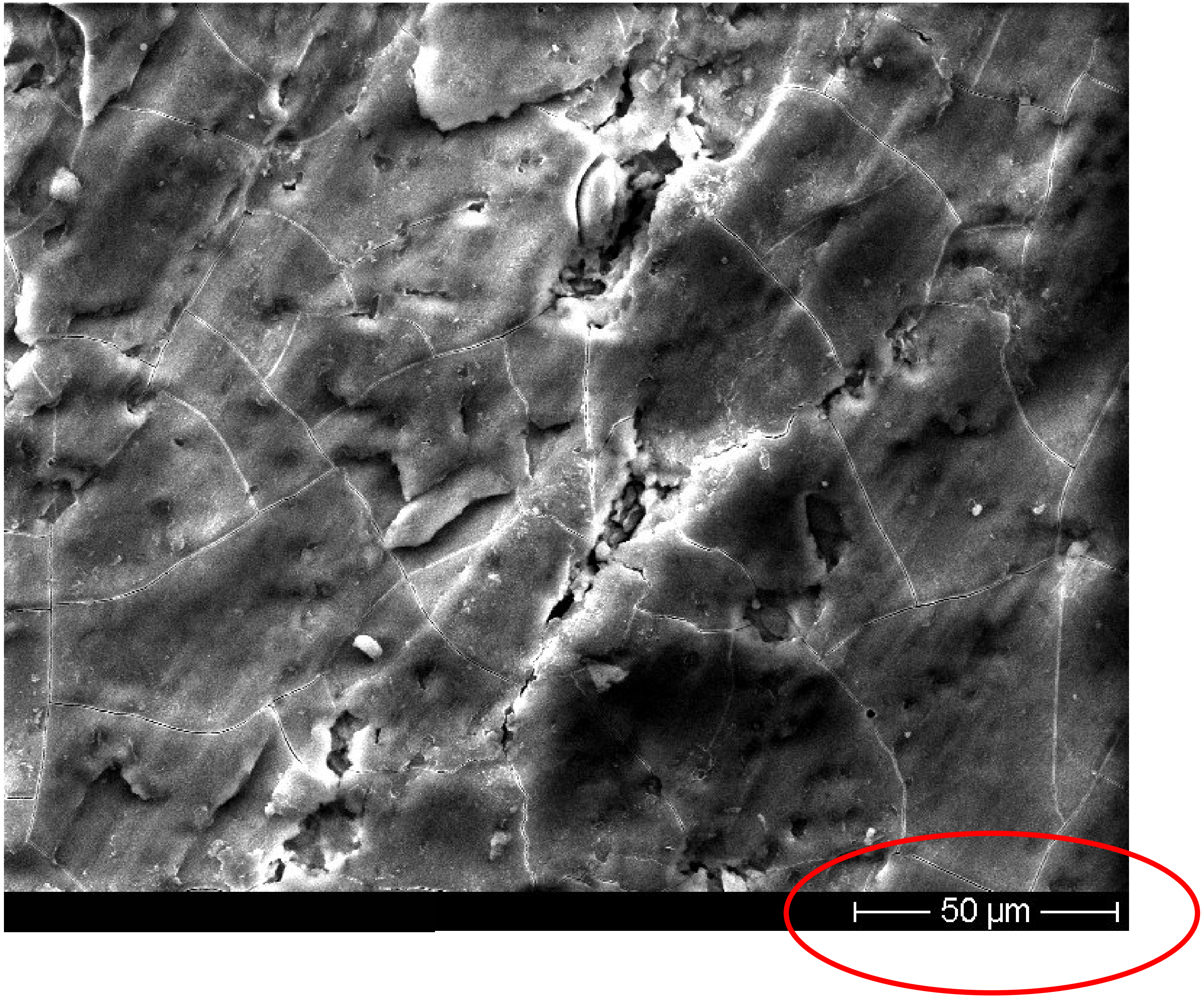
Carrier Material *Aluminium anodized*



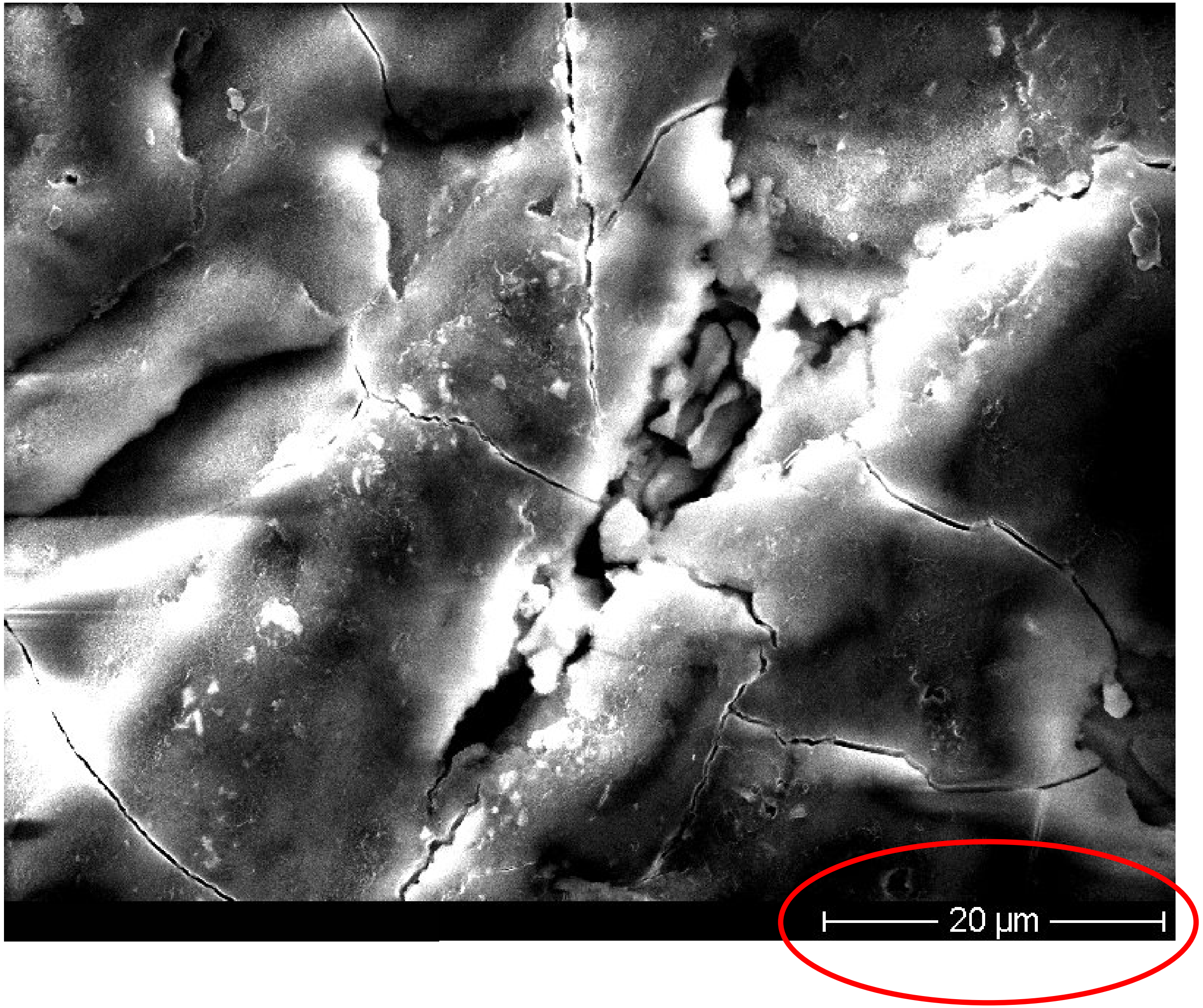
Carrier Material *Aluminium anodized*



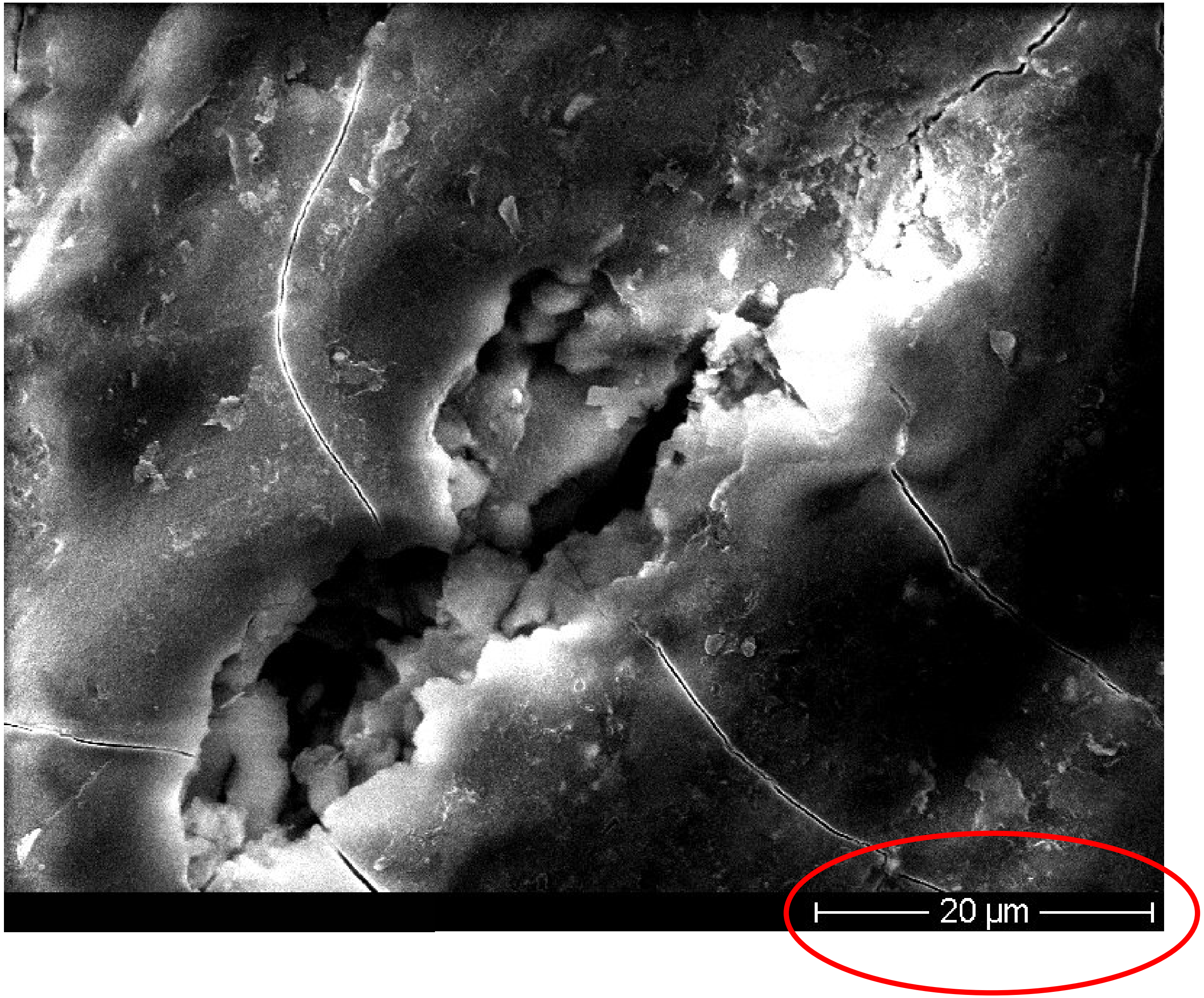
Carrier Material *Aluminium anodized*



Carrier Material *Aluminium anodized*



Carrier Material *Aluminium anodized*



Selection of Bioindicators *Carrier Material*

Unsuitable Carrier Material

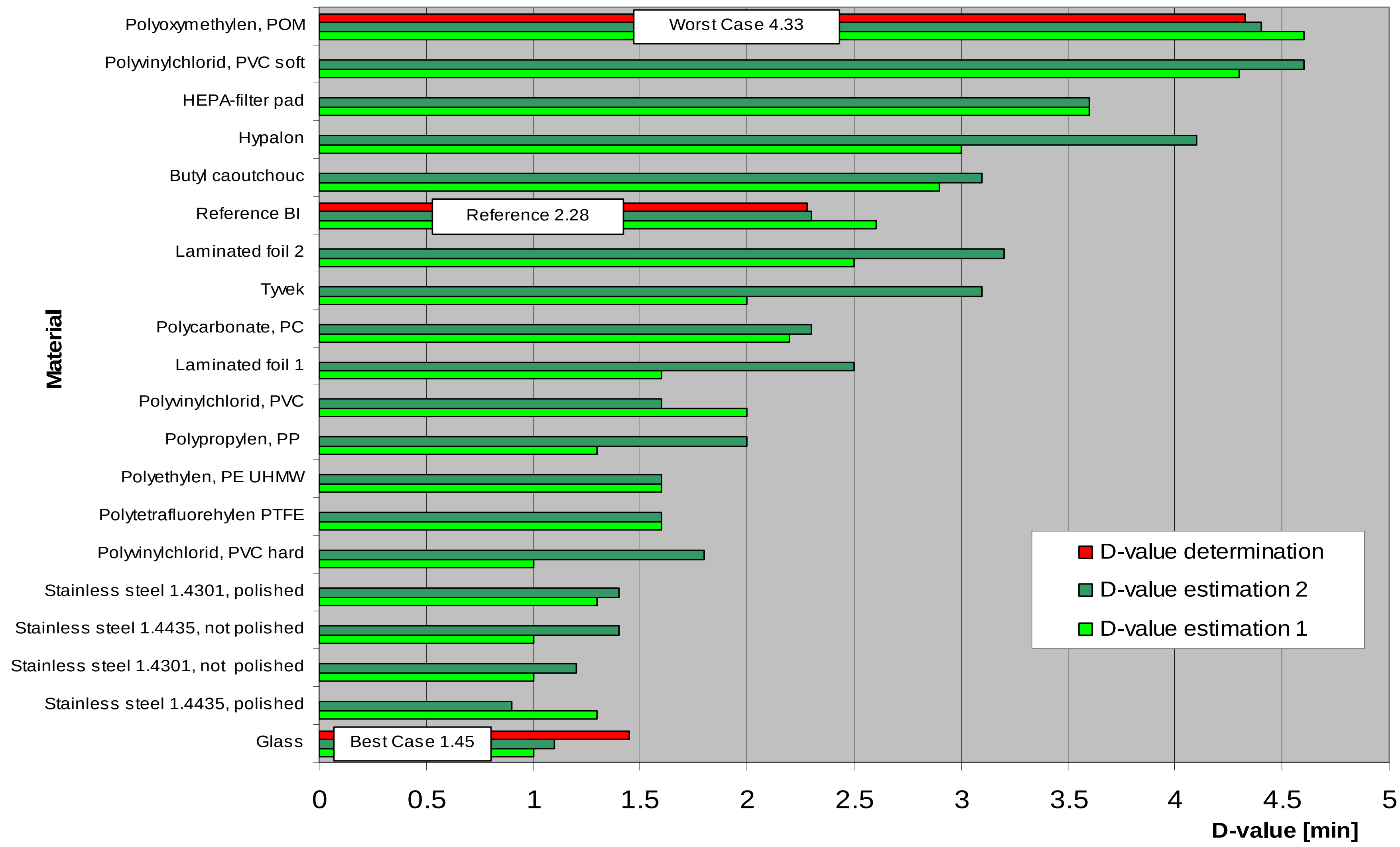
- Negative influence on the *Resistance* of Bioindicators
- Negative influence on the *Model Behavior* of Bioindicators

- Materials are *spongy* or *porous*
- Test Organism *penetrate into* those Materials
- *not or only hart* to be reached by H_2O_2 Decontamination

The *Penetration* of the Decontamination Effect of the H_2O_2 Process is

- *not ensured*
- *but also not expected*

Selection of Bioindicators *Carrier Material*



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: > 1.0×10^6
Carrier Material: Stainless Steel

\+ growth
\- no growth

Group		01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]		2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	+
Result	1	+	+	+	+	-	-	-	-	-	-	neg
	2	+	+	+	-	-	-	-	-	-	-	-
	3	+	+	+	-	-	-	-	-	-	-	

- 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

\+ growth
\- no growth

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	+
Result 1	+	+	-	-	-	-	-	-	-	-	neg
2	+	+	-	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	

- 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 $4.5x10^5$
Carrier / Package: Glasfibre / Tyvek
Spezified D-value: 1.5 [min]

+ growth
- no growth

Run 01

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	+
result	1	+	+	+	+	-	+	+	-	-	-	neg
	2	+	+	-	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-	-	

Run 02

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0	+
result	1	+	-	+	-	+	-	-	-	-	-	neg
	2	+	-	-	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-	-	

Biological Indicator for gaseous H_2O_2

Testorganism: B. stearothermophilus ATCC 7953, min 1.0 x10^6
Carrier / Package: Glasfibre / Tyvek, PE
Spezified D-value: 3.1 [min]

\+ growth
\- no growth

Run 01

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
result	1	+	-	+	+	+	+	+	-	-	+	neg
	2	+	-	+	+	+	+	+	-	-	+	-
	3	-	-	-	+	-	+	-	-	-	+	

Run 02

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0	+
result	1	+	+	-	+	+	+	+	-			neg
	2	+	+	-	-	+	-	-	-			-
	3	+	-	-	-	-	-	-	-			

Selection of Biological Indicators Steam

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

\+ growth
\- no growth

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]	10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0	+
Result 1	+	+	+	+	+	+	+	+	+	+	neg
2	+	+	+	+	+	+	+	+	+	+	-
3	+	+	+	+	+	+	+	-	-	+	

- 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

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
Result 1	+	+	+	+	-	-	-	-	-	-	neg
2	+	+	+	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	

+ growth

- no growth

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

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_2O_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

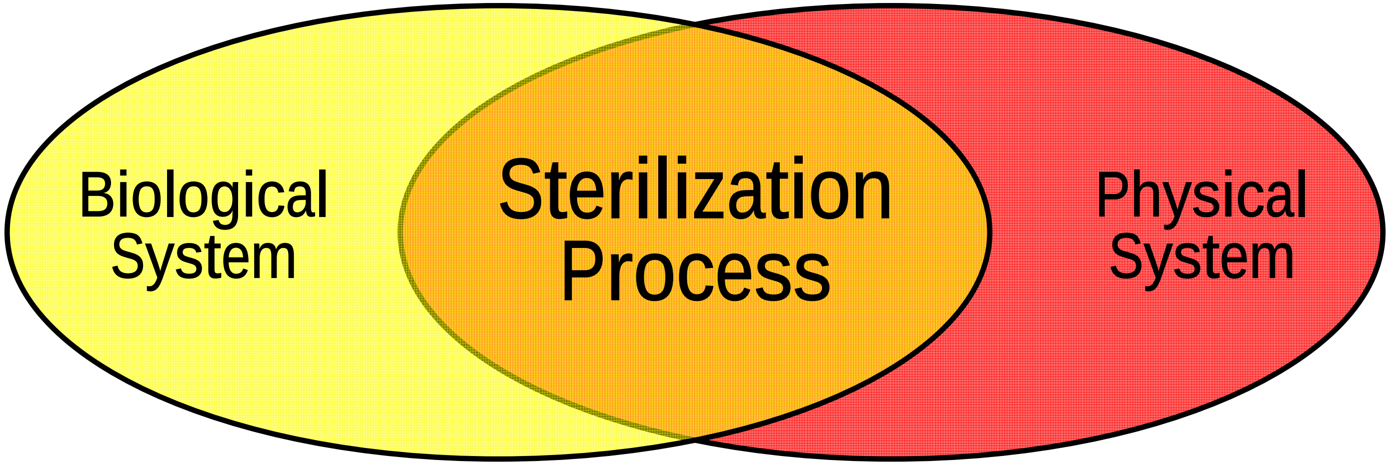
SKAN

Process Development

Biological Indicators and H_2O_2 Decontamination

Process Development

- Process Development of alternative Sterilization Method



Investigation on Germ Reducing Parameters of the H_2O_2 Decontamination Method

- *Process Development*

- Establish parameters of microbial reducing effects of H_2O_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_2O_2 Decontamination Method

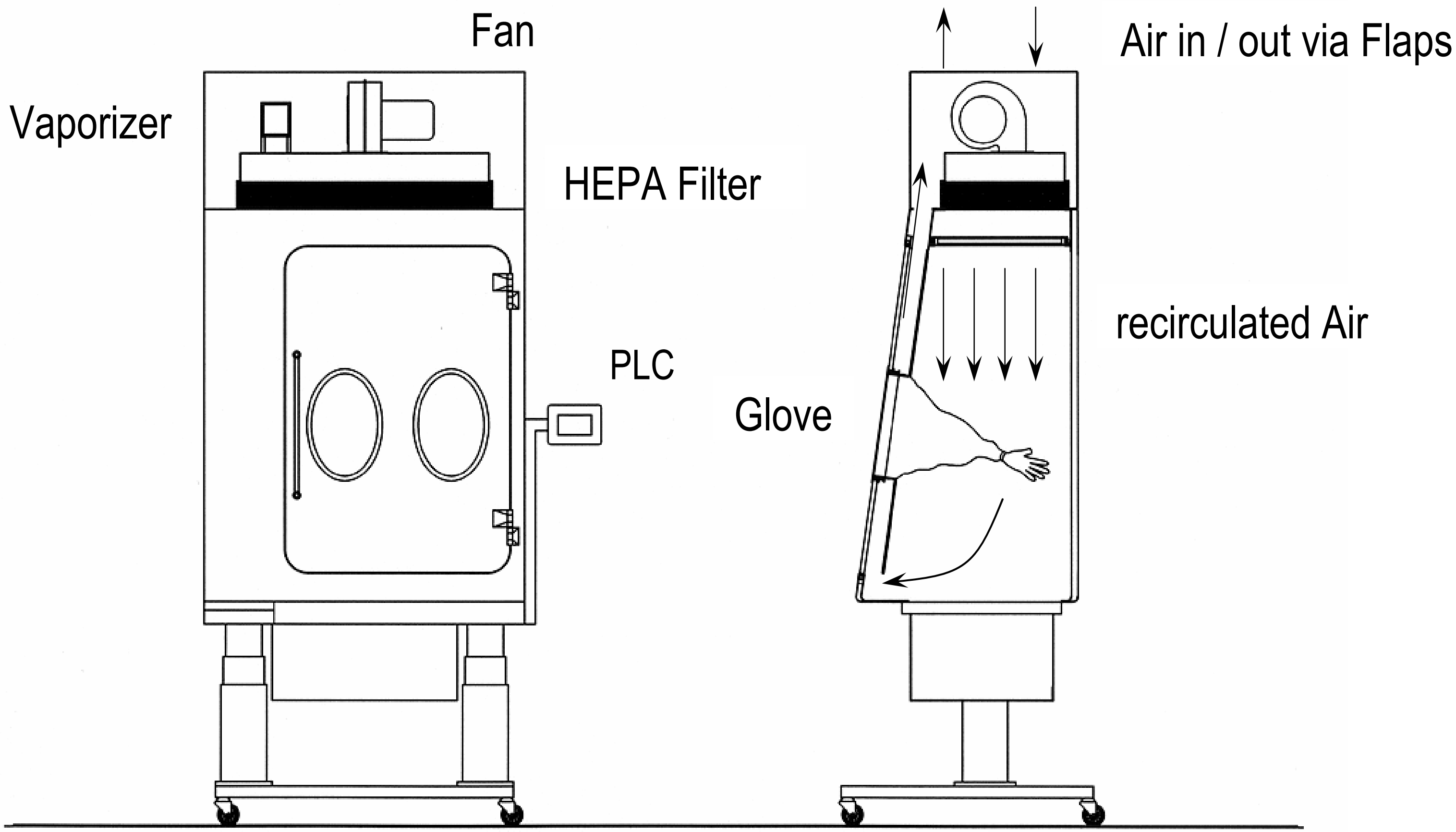
- Comprehension of the decontamination process
- The *nice and need to have* parameters for the decontamination success
- Hints for Design, Qualification and Monitoring

- Method to ***describe, develop and quantify*** H_2O_2 decontamination cycles

Investigation on Germ Reducing Parameters of the H_2O_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 m^3 (40 ft^3)

Sensors

Process

• Temperature	[°C]
• Humidity	[% rH]
• Air Velocity	[m/s]
• Pressure	[Pa]
• Mass / Balance	[g]

H_2O_2 Gas-Concentration

- Electro-chemical Sensor
- UV-Spectrometer
- IMS-Spectrometer
- NIR-Spectrometer
- Wet-chemistry Method

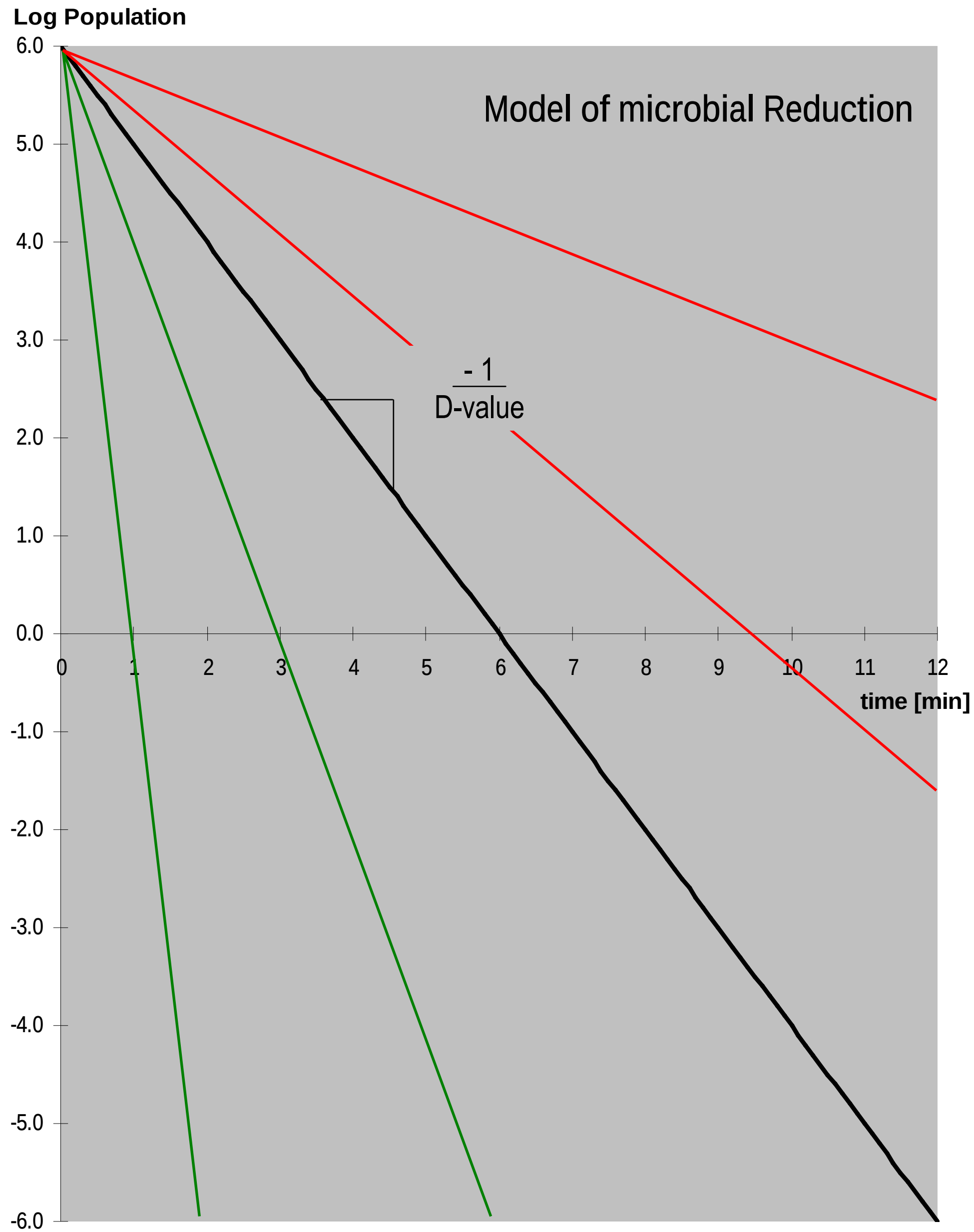
Microbiological System

- 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

exposure	01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
result 1	+	+	+	+	-	-	-	-	-	-	neg
2	+	+	+	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	

\+ growth
\- no growth

estimated D-value = 2.0 [min]

Limited Spearman Karber Method LSKM

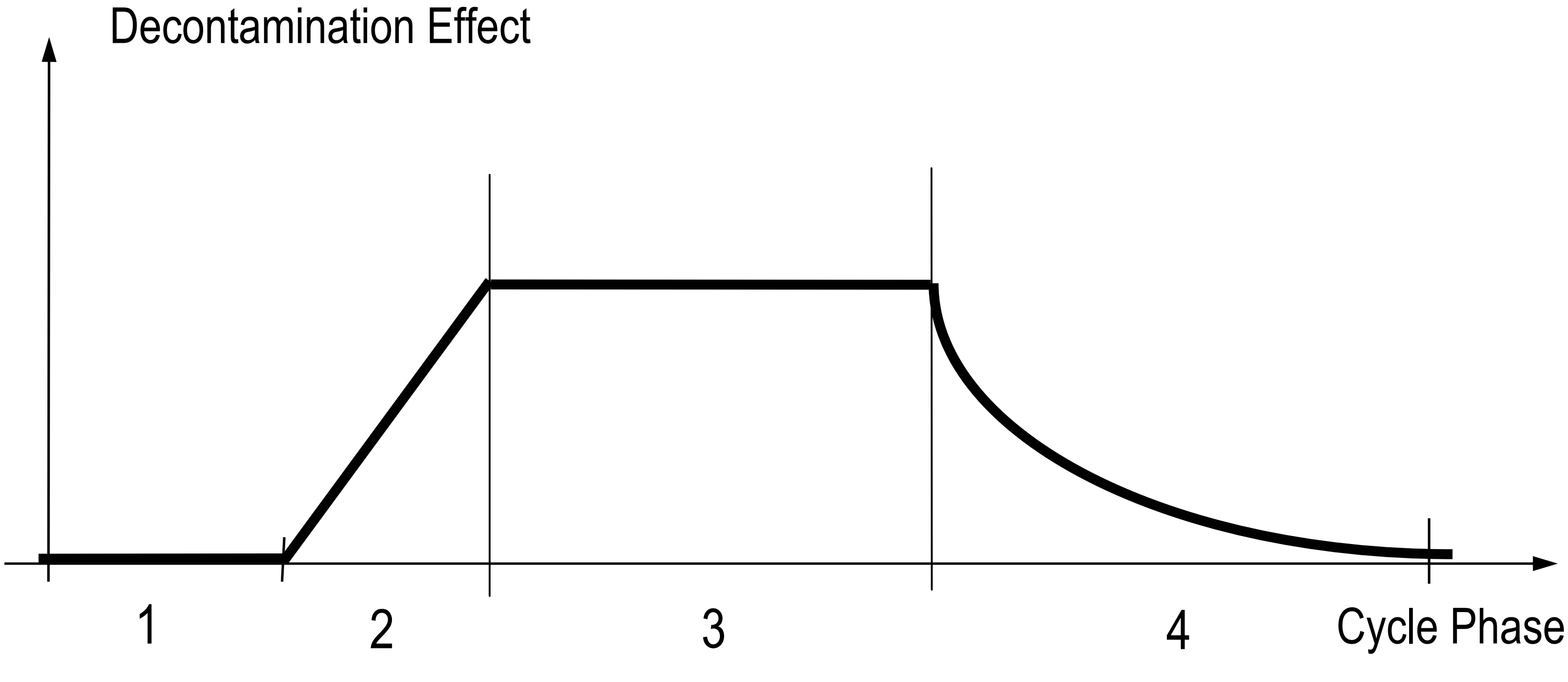
exposure	01	02	03	04	05	06	07	08	09	10	
exposure time [min]	6.0	8.5	11.0	13.5	16.0	18.5	21.0	23.5	26.0	28.5	
result 1	+	+	+	+	+	+	-	-	-	-	neg
2	+	+	+	+	+	+	-	-	-	-	-
3	+	+	+	+	-	-	-	-	-	-	-
4	+	+	+	+	-	-	-	-	-	-	pos
5	+	+	+	-	-	-	-	-	-	-	+
6	+	+	+	-	-	-	-	-	-	-	+
7	+	+	+	-	-	-	-	-	-	-	
8	+	+	+	-	-	-	-	-	-	-	
9	+	+	-	-	-	-	-	-	-	-	
10	+	-	-	-	-	-	-	-	-	-	

\+ growth
\- no growth

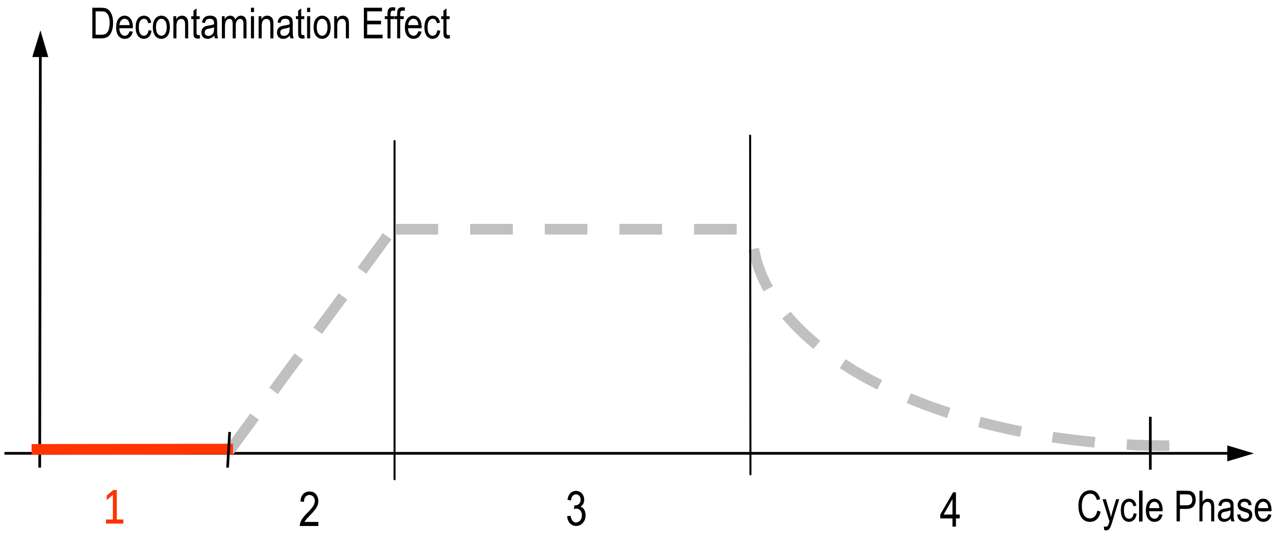
D-value = 2.06 [min]

95% CI D-value = 2.06 ± 0.22 [min]

Decontamination Cycle



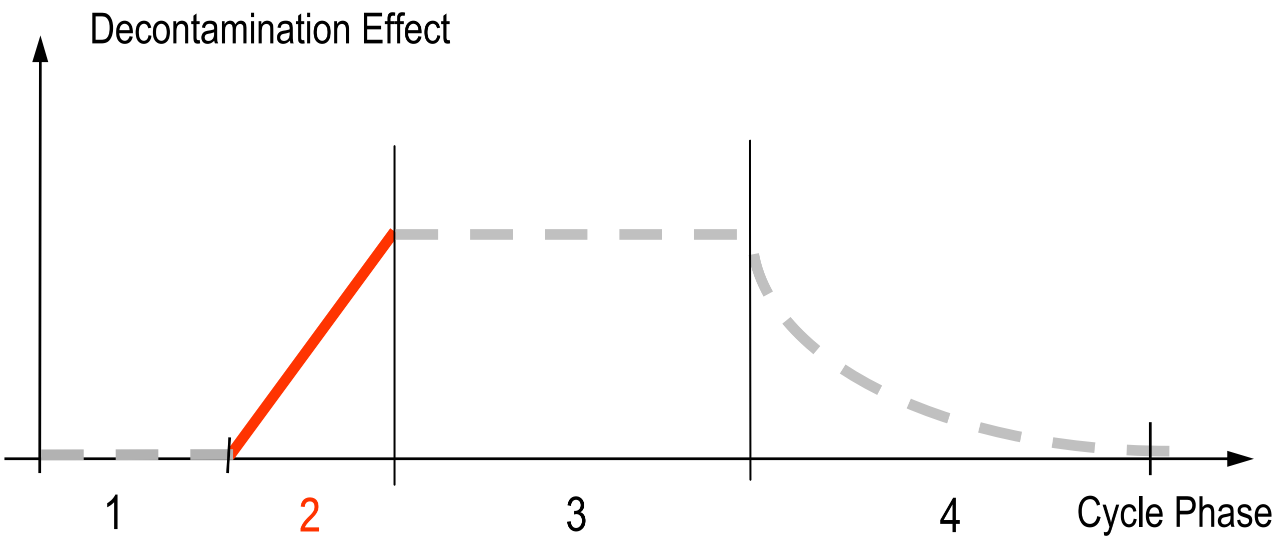
Decontamination Cycle



Phase 1: **Pre-conditioning**

to establish the initial conditions in the chamber

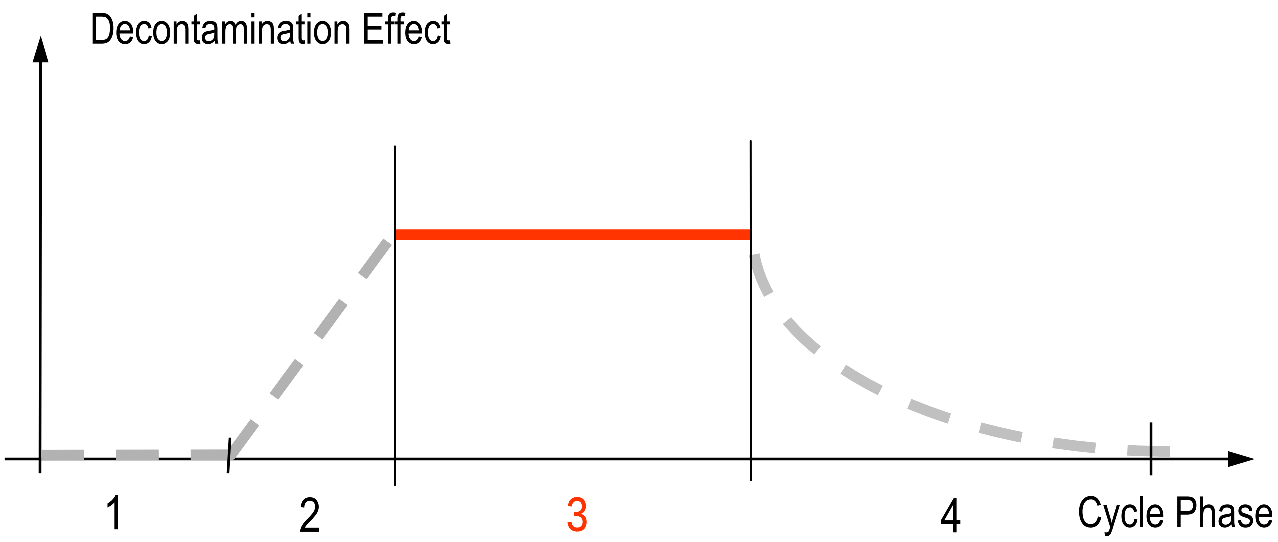
Decontamination Cycle



Phase 2: Conditioning

to establish the decontamination effect

Decontamination Cycle

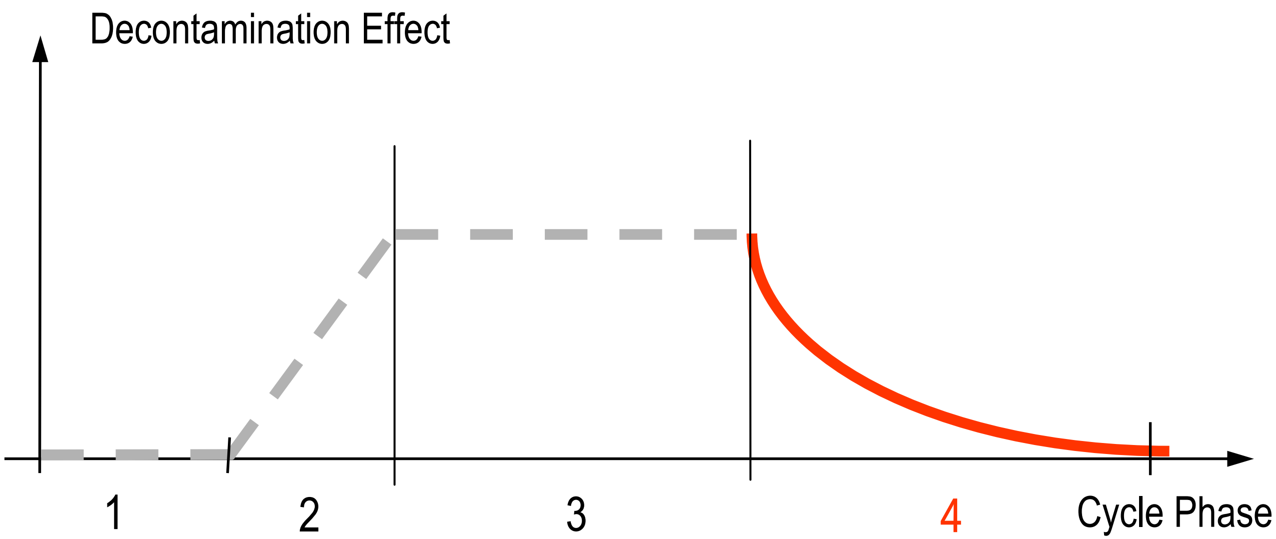


Phase 3: **Decontamination**

to maintain stable decontamination effect

to ensure the total bacterial reduction over time

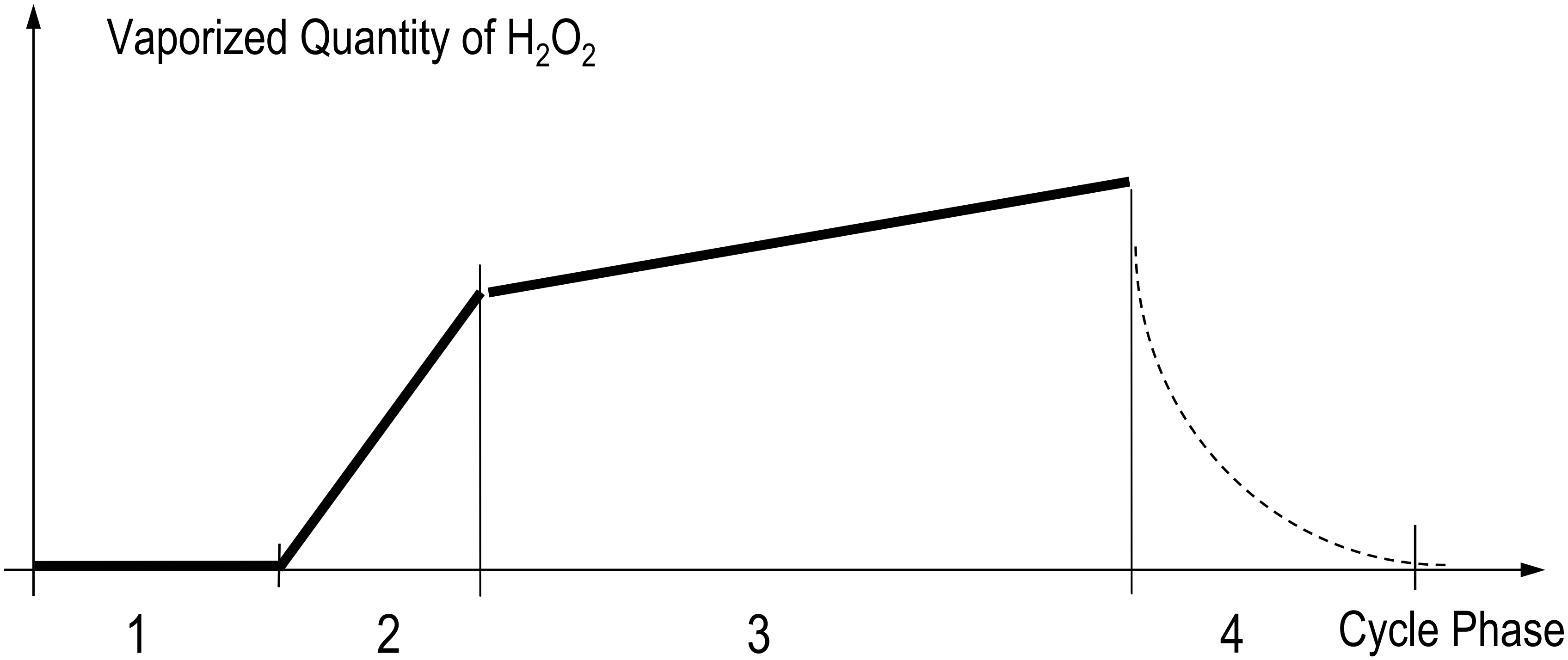
Decontamination Cycle



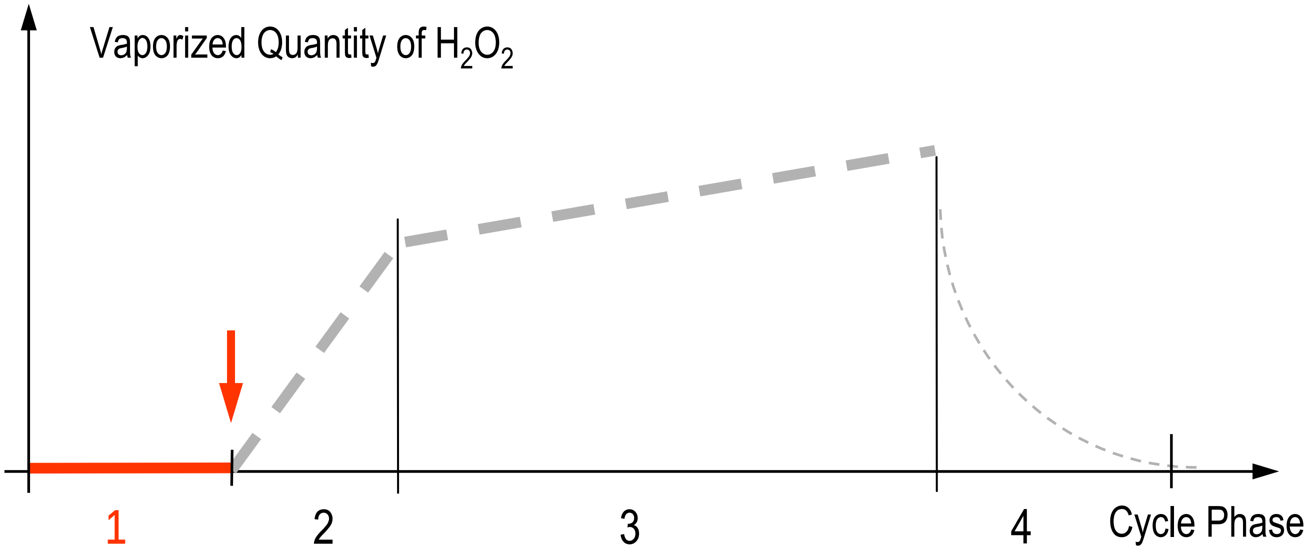
Phase 4: **Aeration**

to reach minimal residual H_2O_2 concentration

Selection of Factors



Selection of Factors

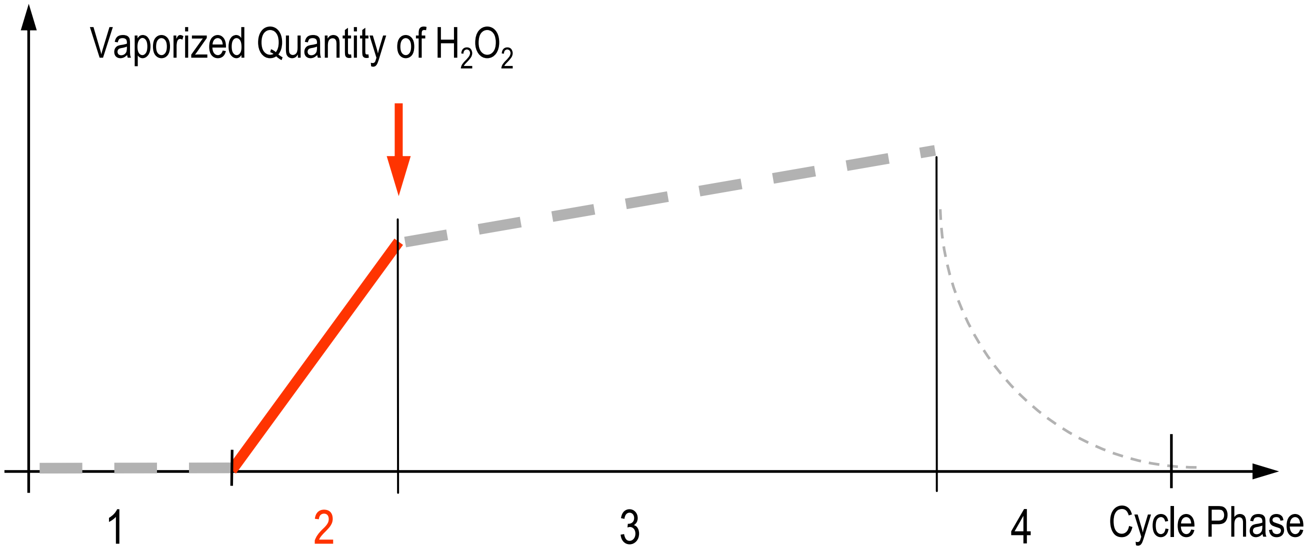


Pre-conditioning

Initial conditions in the chamber

Temperature	*[°C]*
Humidity	*[%rH]*

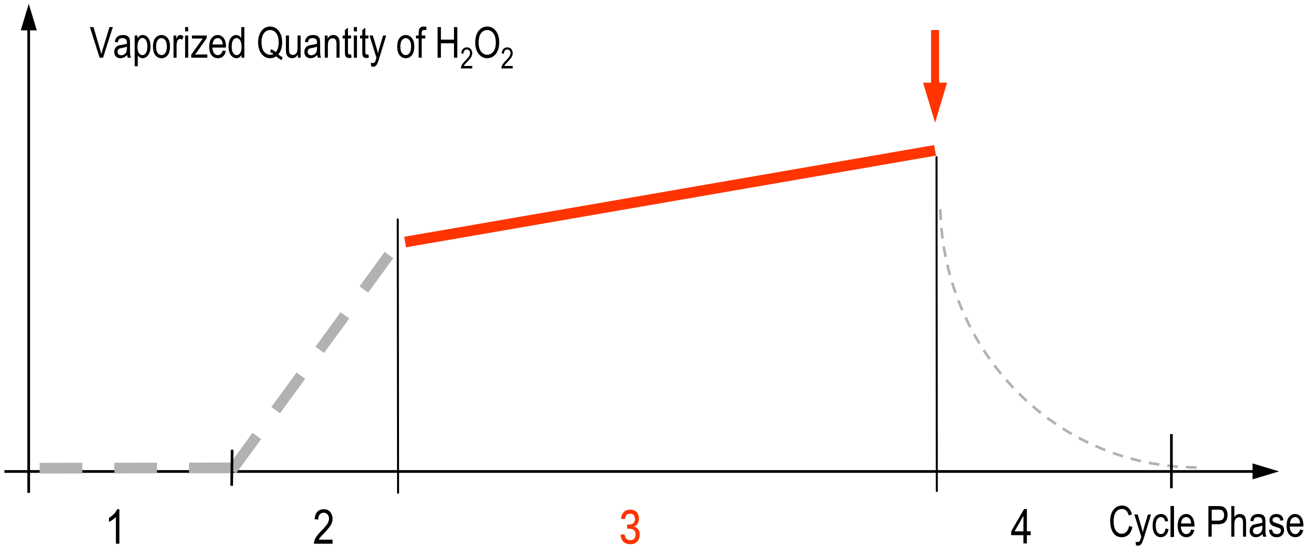
Selection of Factors



Conditioning

Vaporized initial quantitiy of *pure* H_2O_2 *Quantity* *[g/m³]*

Selection of Factors



Decontamination

Rate of continuously vaporized *pure* H_2O_2 ***Redose*** ***[%/h]***

Selection of Factors

Special

Concentration of H_2O_2 Solution | *H_2O_2* | *[%]*

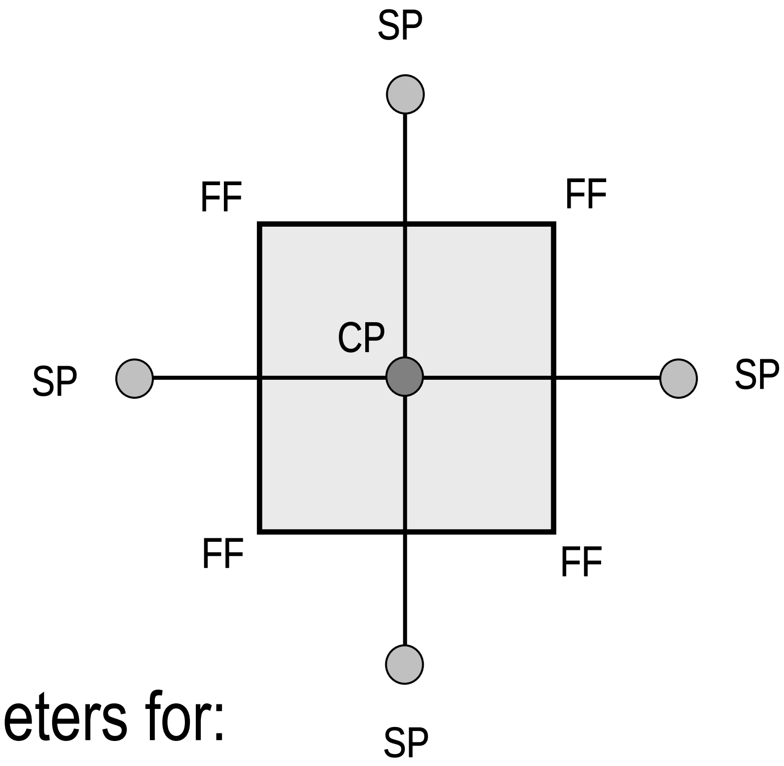
5 factors selected for the investigation

A:	*Quantity of pure H_2O_2*	*[g/m^3]*
B:	*Rate of Redose*	*[%A/h]*
C:	*Temperature*	*[°C]*
D:	*Humidity*	*[%rH]*
E:	*Concentration of H_2O_2*	*[%]*

Range of Factors

No:	Description	Unit	SP-	FF-	CP	FF+	SP+
A:	Quantity of pure H_2O_2	g/m^3	4	5	6.5	8	9
B:	Rate of Redose	% A/h	20	40	70	100	120
C:	Temperature	°C	26	30	35	40	44
D:	Humidity	% rH	6	10	15	20	24
E:	Concentration of H_2O_2	%	30	35	42.5	50	55

Design of Experiment DoE



• Fractional Factorial Plan	FF
• Centre Point	CP
• Star Point	SP

Independent Estimation of the Parameters for:

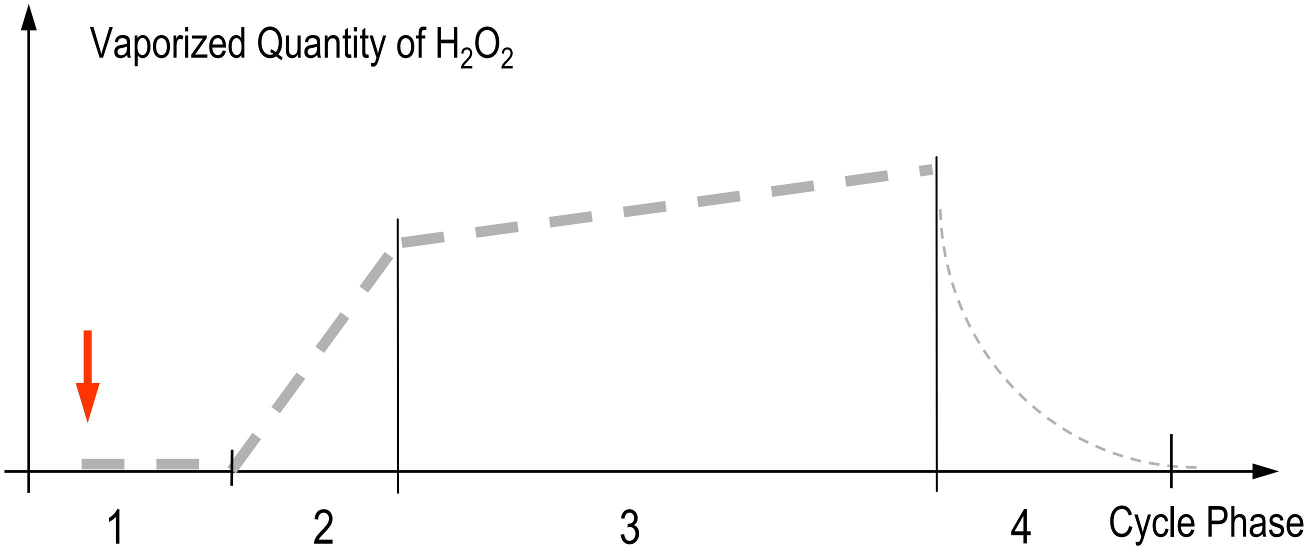
- Main Effects
- Quatratic Effects
- Interactions

- \+ 4 independent determinations of Centre Point

Range of Factors

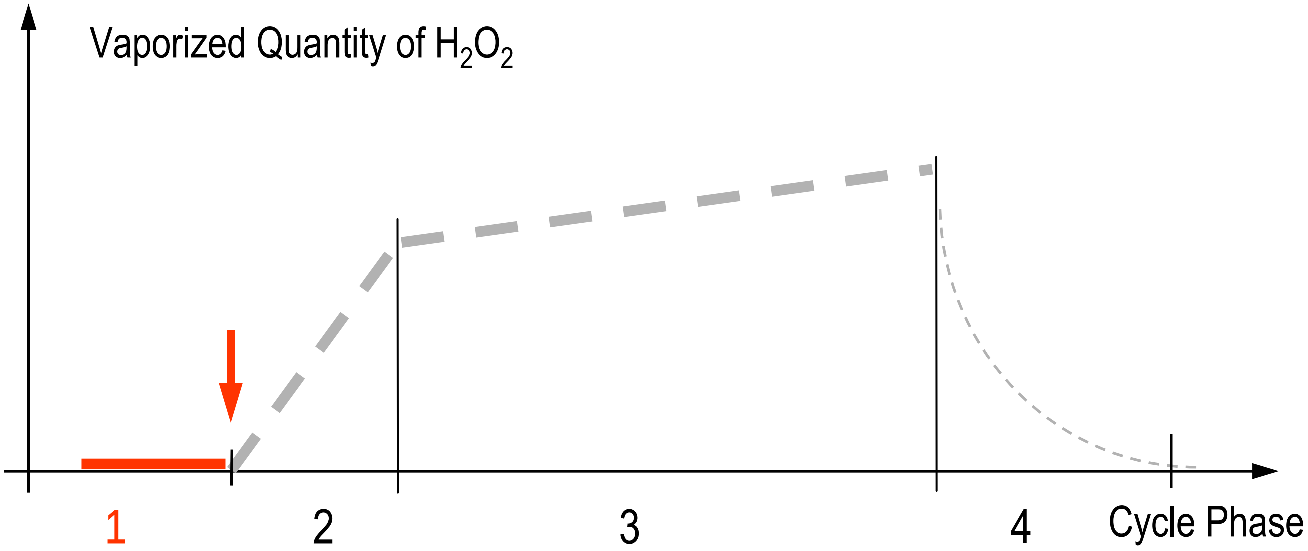
No:	Description	Unit	SP-	FF-	CP	FF+	SP+
A:	Quantity of pure H_2O_2	g/m^3	4	5	6.5	8	9
B:	Rate of Redose	% A/h	20	40	70	100	120
C:	Temperature	°C	26	30	35	40	44
D:	Humidity	% rH	6	10	15	20	24
E:	Concentration of H_2O_2	%	30	35	42.5	50	55

Test Handling



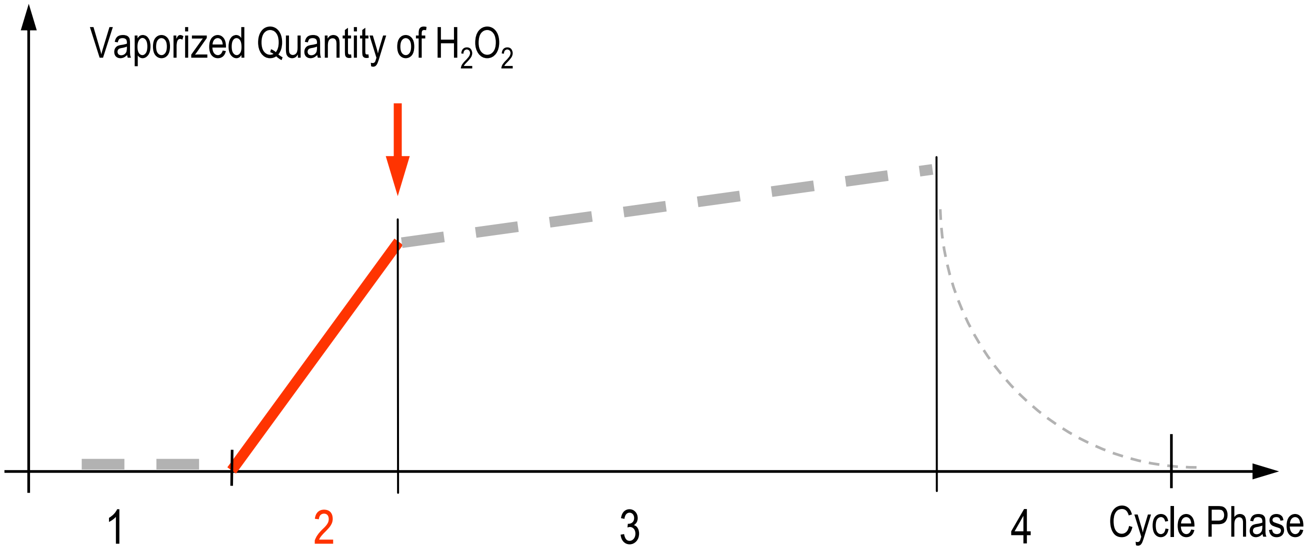
- place BI`s in the isolator gastight wrapped

Test Handling



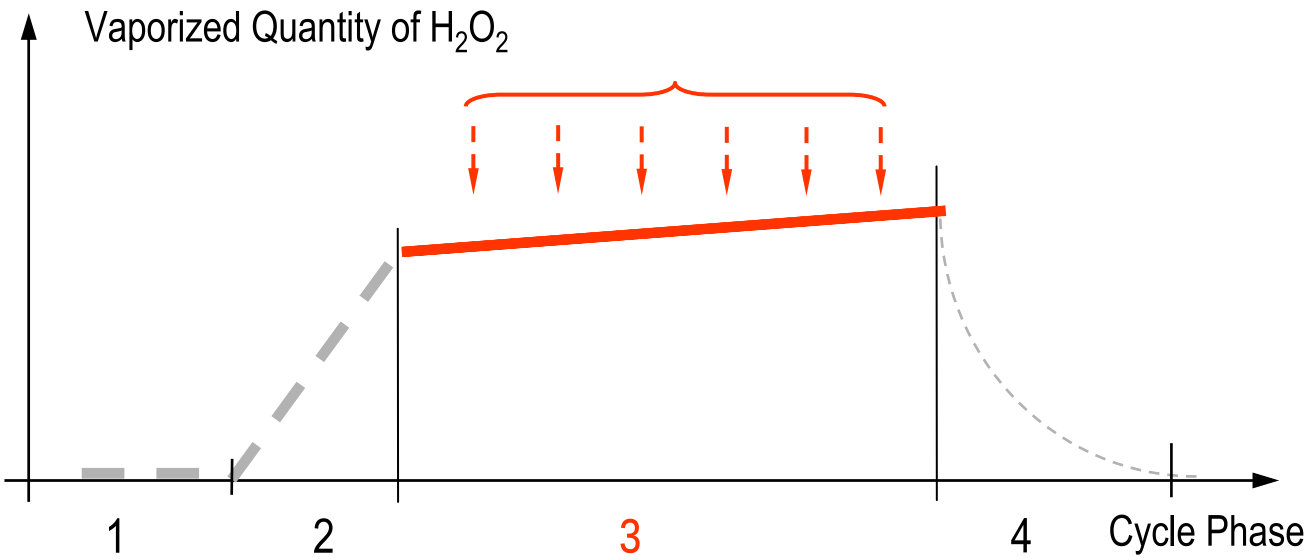
- establish required initial conditions
- start vaporizing H_2O_2 up to required quantity

Test Handling



- expose BI`s to the inactivation atmosphere
- start redosing

Test Handling



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

Results and Interpretation Centre Point

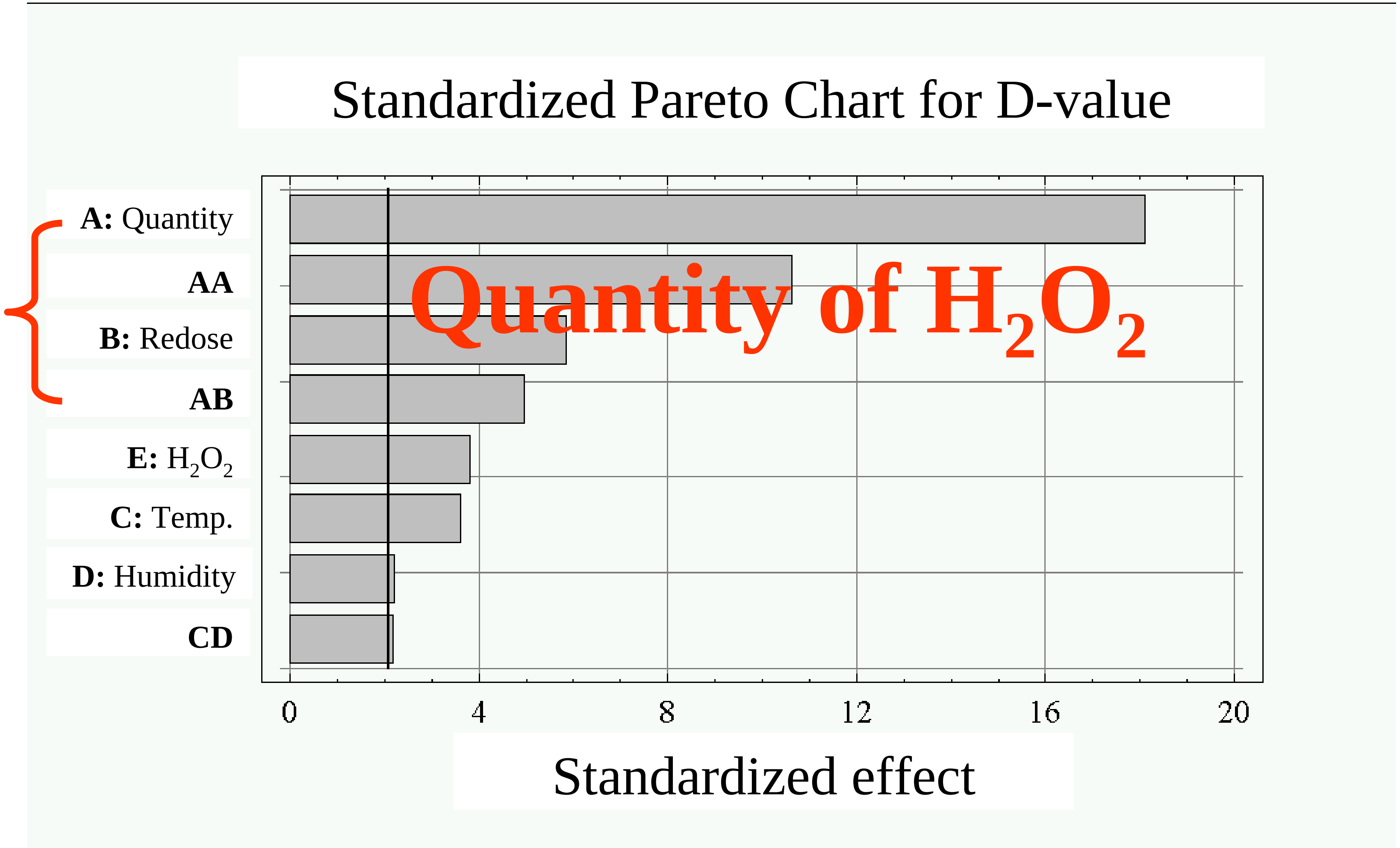
Independent Centre Point Determinations

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

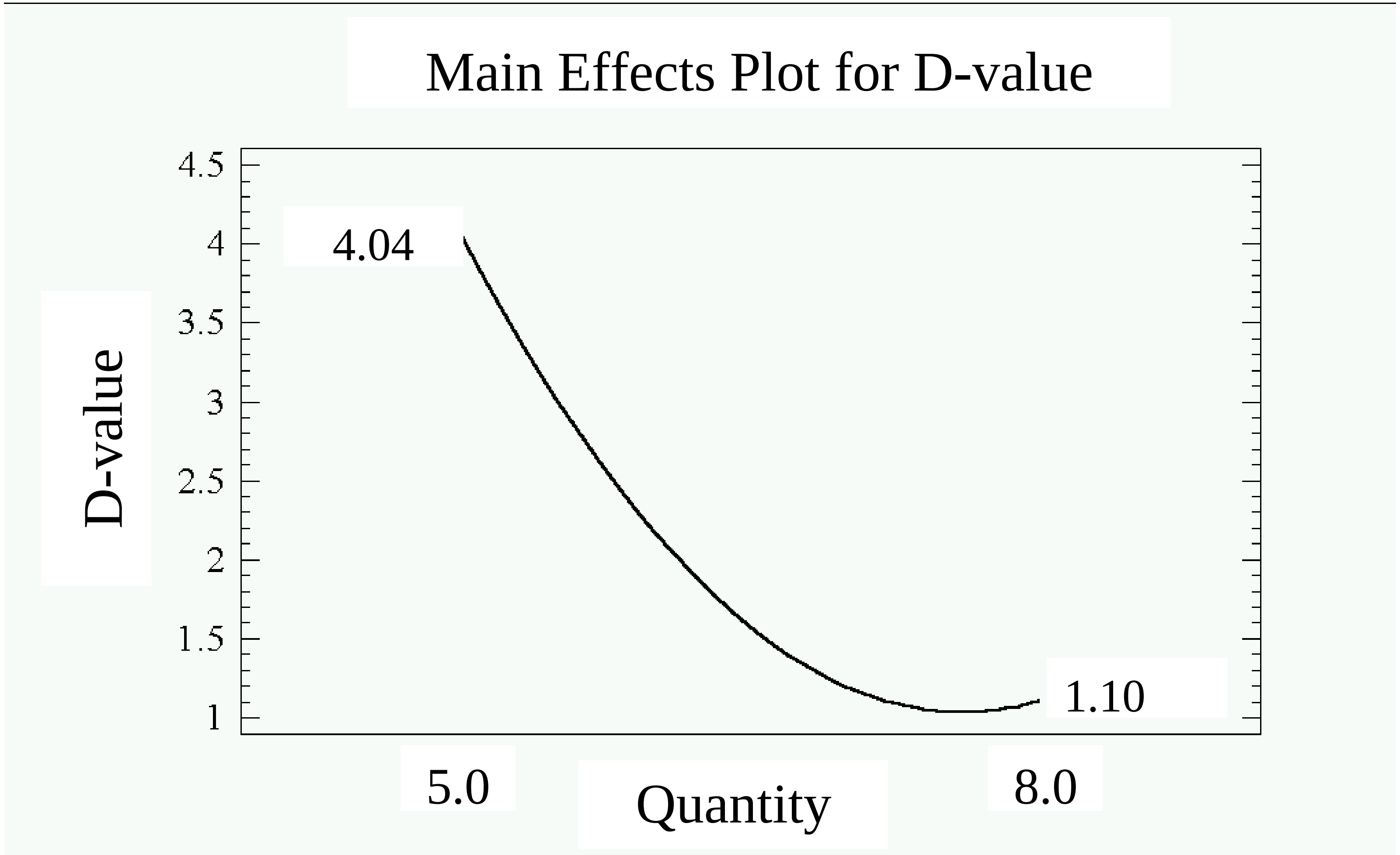
Run No.:	*D-value [min]*
1	*1.75*
2	*1.80*
3	*1.74*
4	*1.71*

• *Constant* parameters lead to a *reproducable* decontamination effect

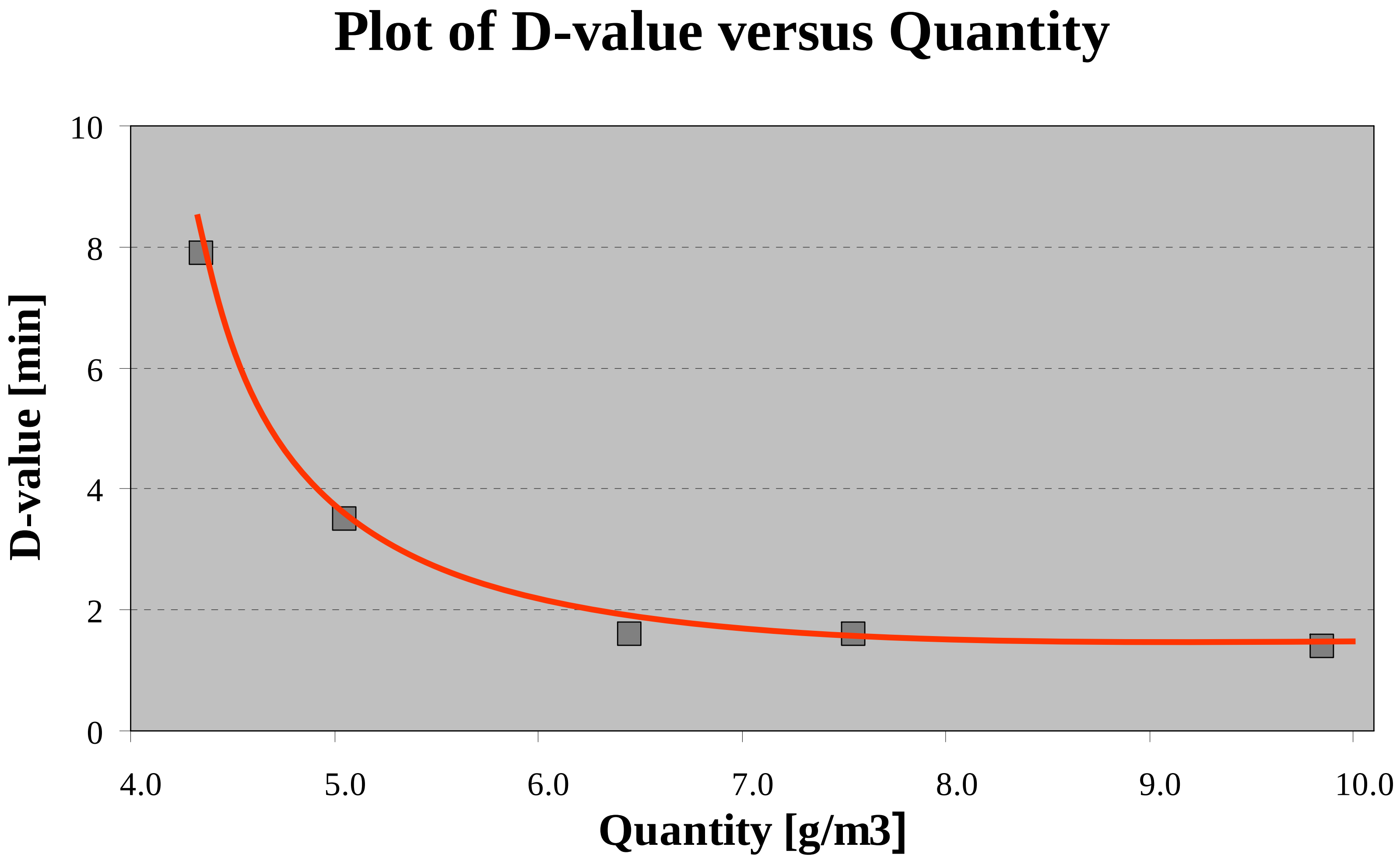
Results and Interpretation Model



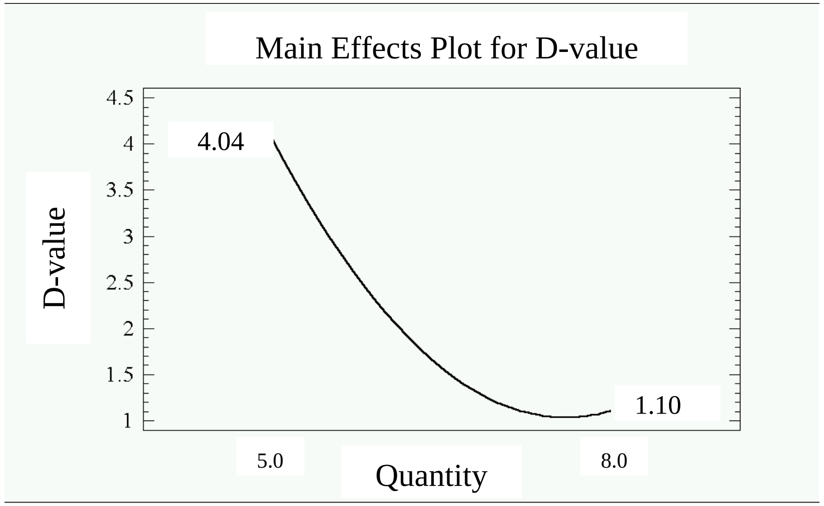
Results and Interpretation   Quantity (A)

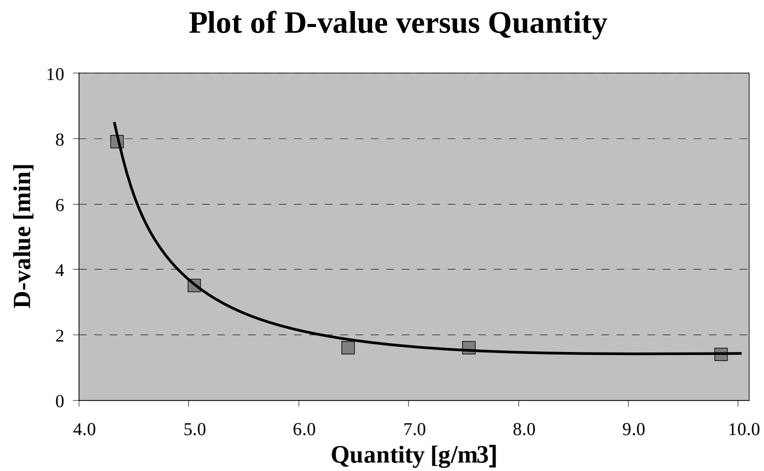


Results and Interpretation Quantity (A)



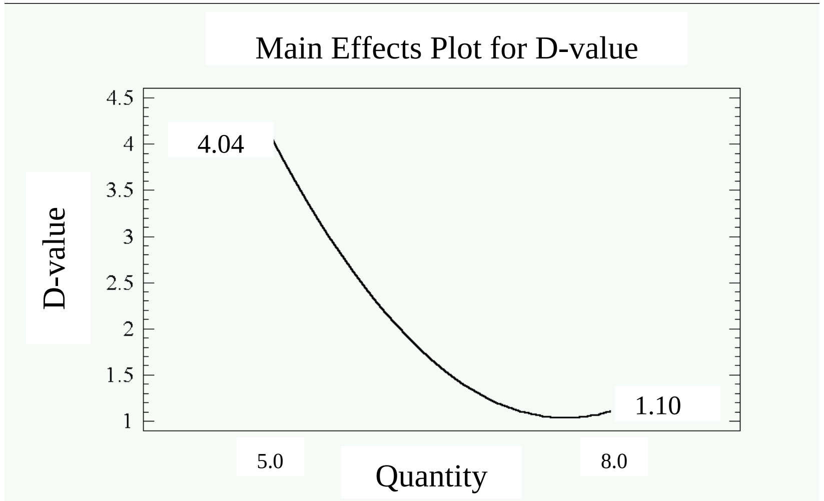
Results and Interpretation *Quantity (A)*

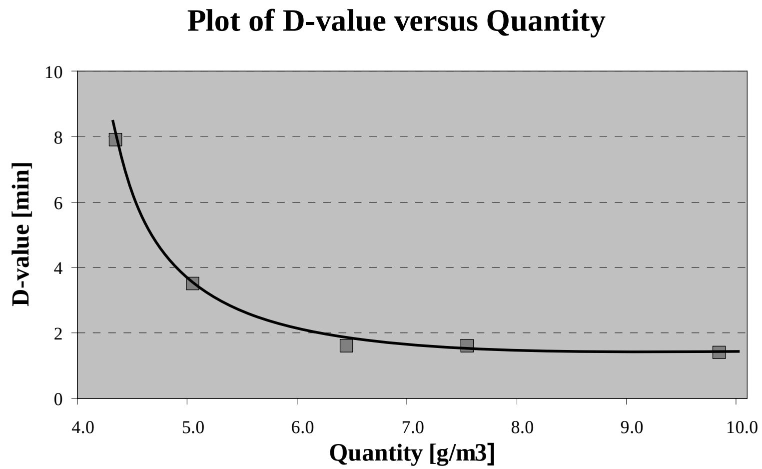




- Quantity of vaporized H_2O_2 is the *Main Effect* in decontamination process
- Nonlinear effect quadratic fit within range of model
- Asymptotic character observed with larger range than model

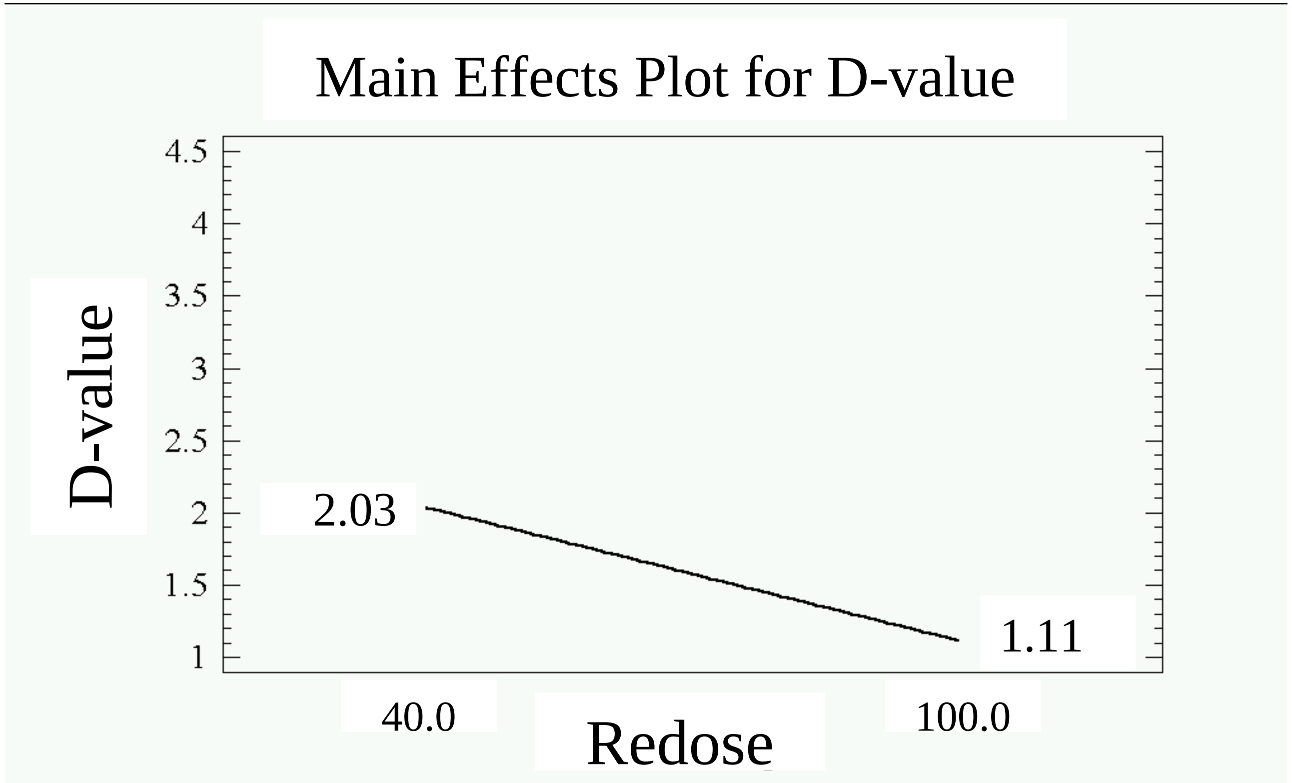
Results and Interpretation Quantity (A)





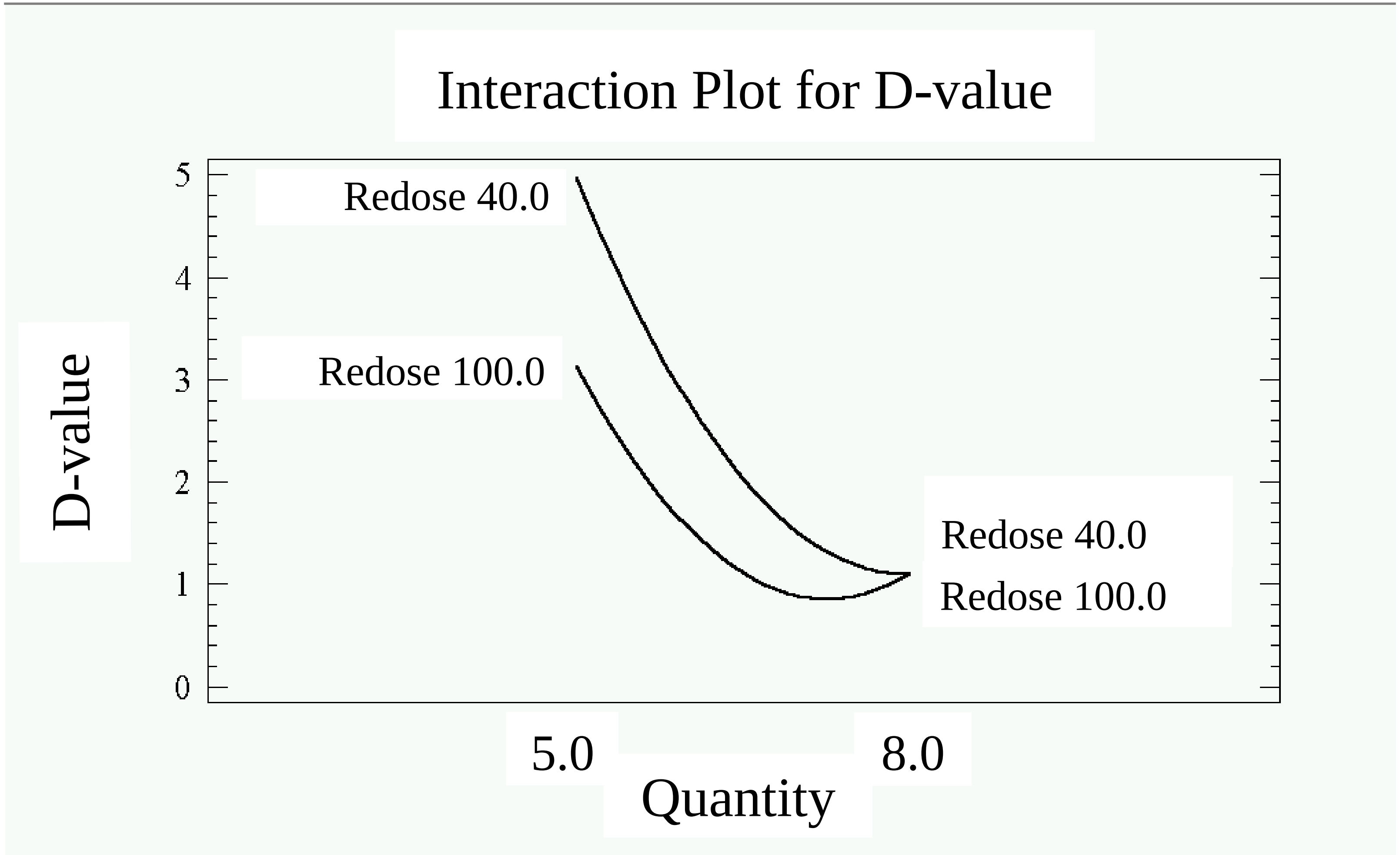
- Starting with constant initial conditions
  boost the quantity of vaporized H_2O_2
  leads to a *Steady State* in decontamination effect

Results and Interpretation Redose (B)

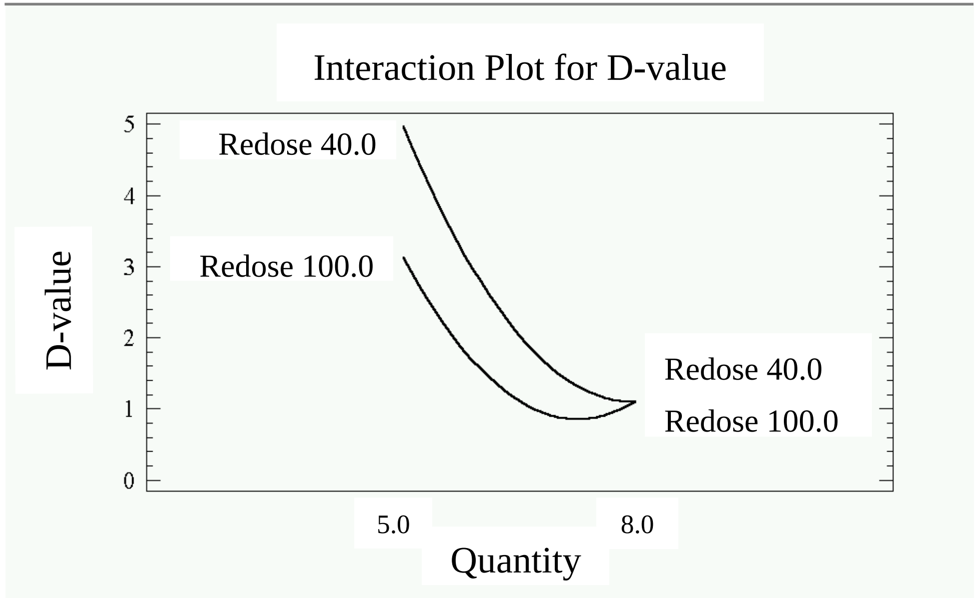


- The *higher* the rates of Redose (B) the *better* the decontamination effect

Results and Interpretation Interaction (AB)

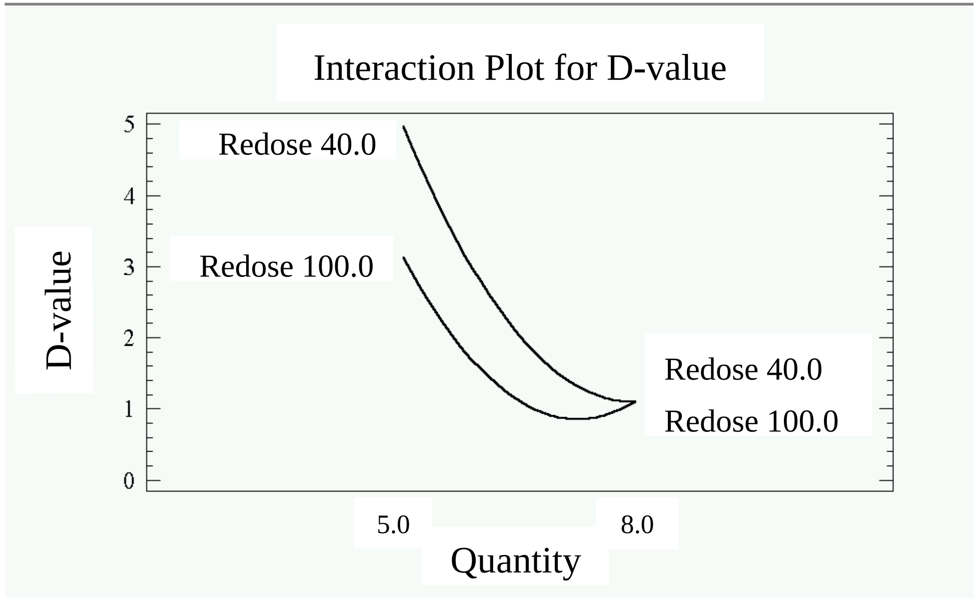


Results and Interpretation   Interaction (AB)



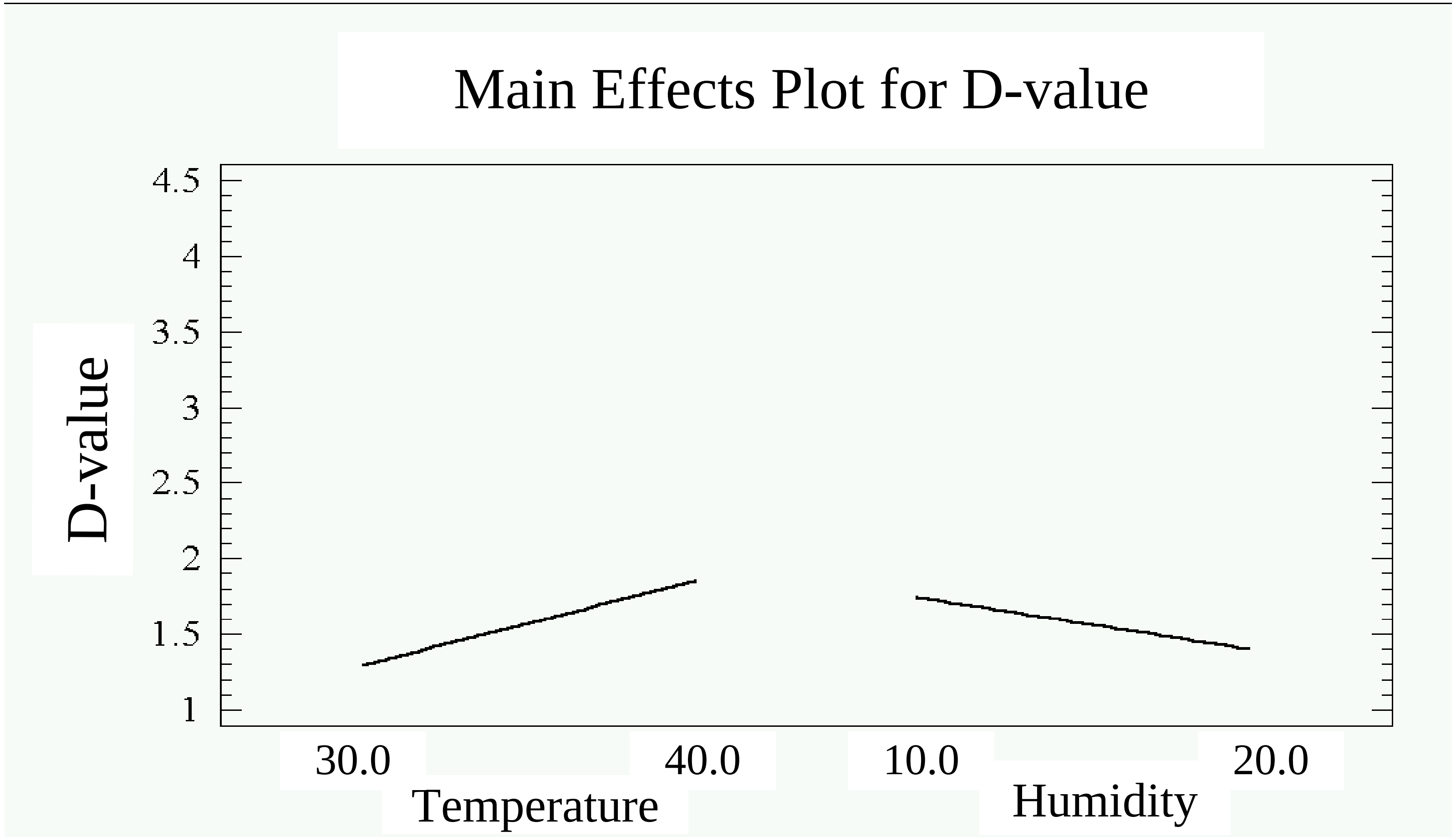
- Redose (B) loses its influence at higher values of factor Quantity (A)
- Only high rates of Redose (B) improve a low decontamination effect

Results and Interpretation *Interaction (AB)*

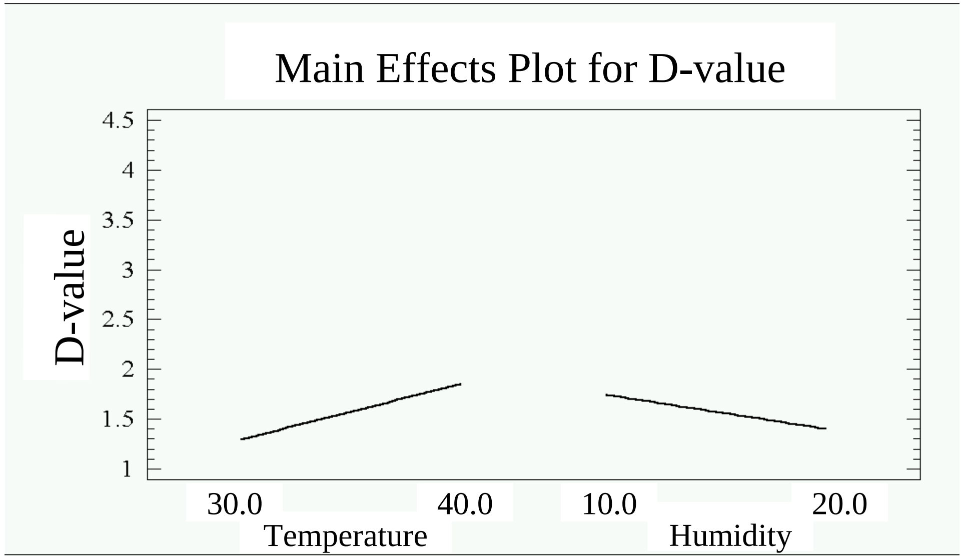


- *Stability* of decontamination effect over time depends on the *Rate of Redose*

Results and Interpretation Temperature (C) Humidity (D)

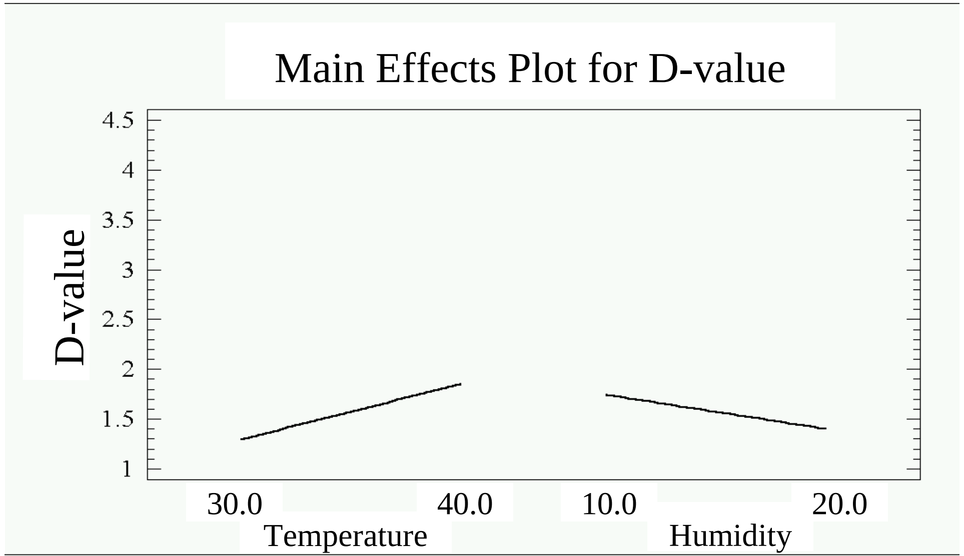


Results and Interpretation Temperature (C)



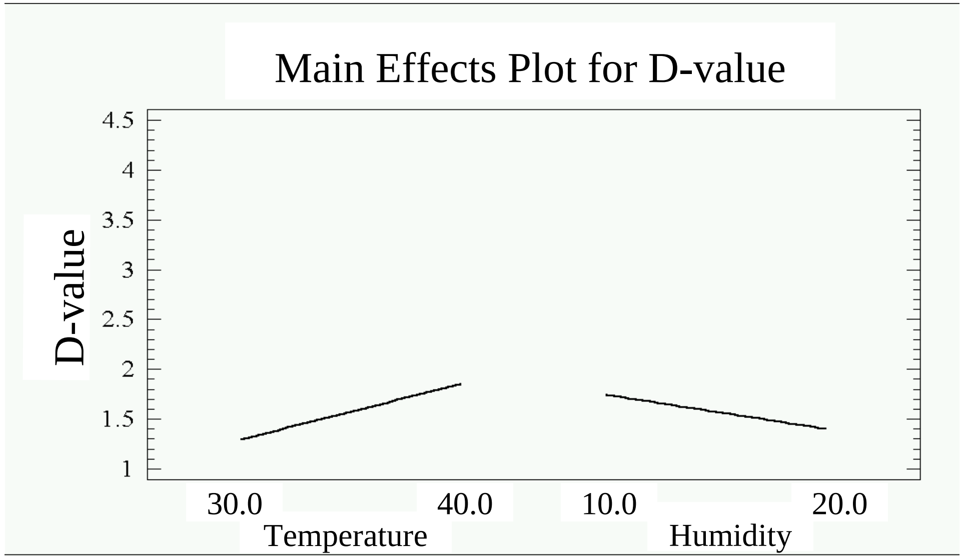
- The *lower* the Temperature (C) the *better* the decontamination effect
- Minor effect: change in D-value of only + 0.5 [min]
  within a temperature range of 10 [°C]

Results and Interpretation Temperature (C)



- *Worst Case* positions for the decontamination effect are positions with *higher temperature*

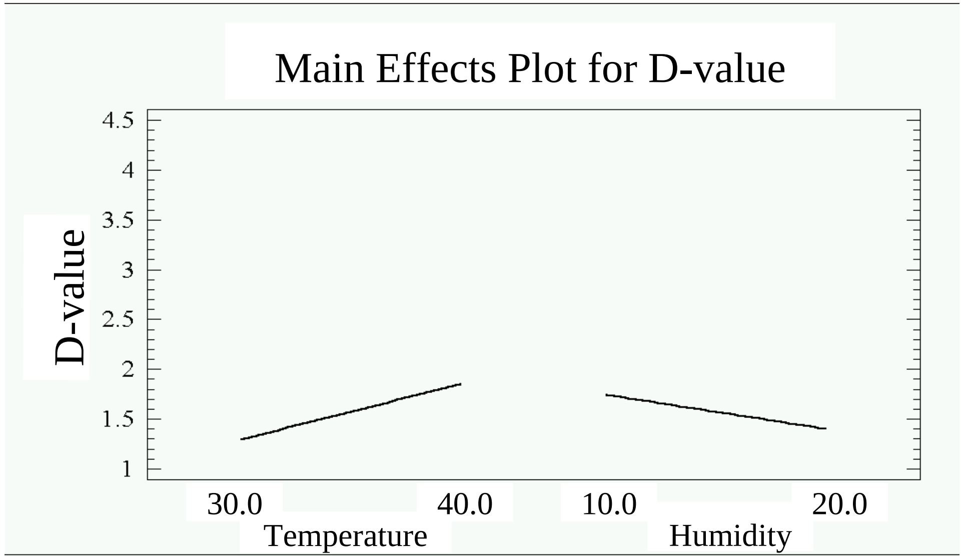
Results and Interpretation Humidity (D)



- The *higher* the Humidity (D) the *better* the decontamination effect
- Minor effect: change in D-value of only - 0.4 [min]

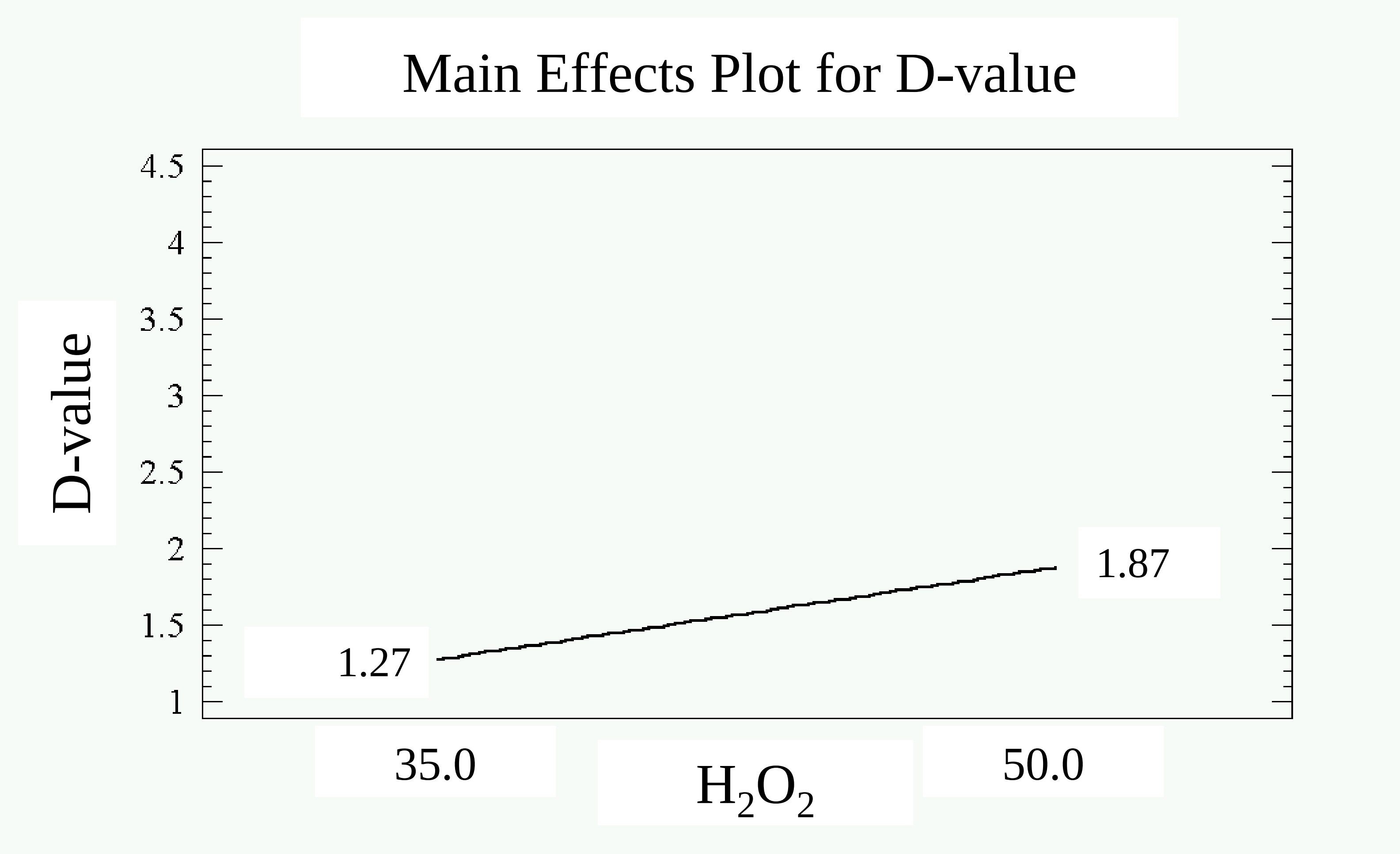
  within a humidity range of 10 [% rH]

Results and Interpretation *Humidity (D)*

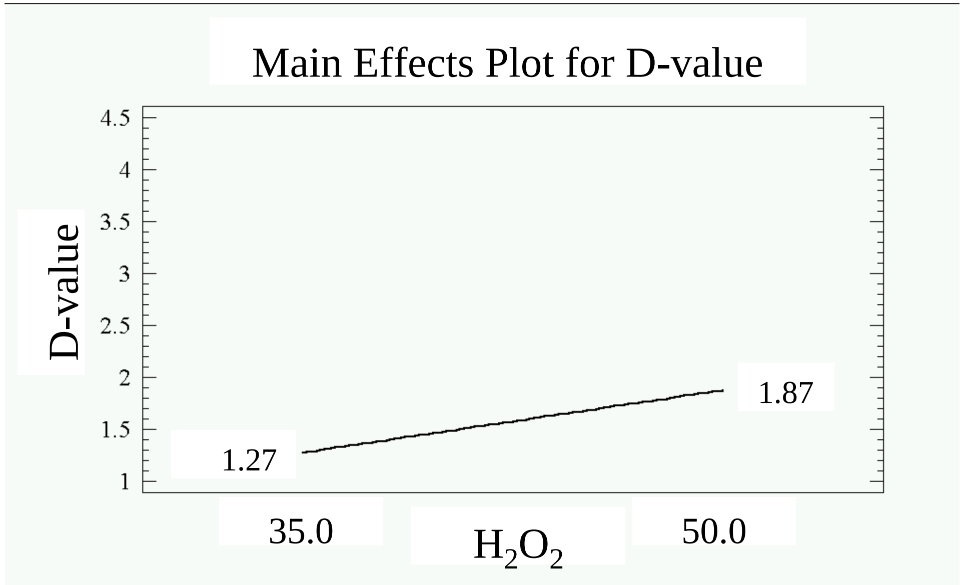


- *Worst Case* positions for the decontamination effect are positions with *lower humidity*

Results and Interpretation *H_2O_2 (E)*

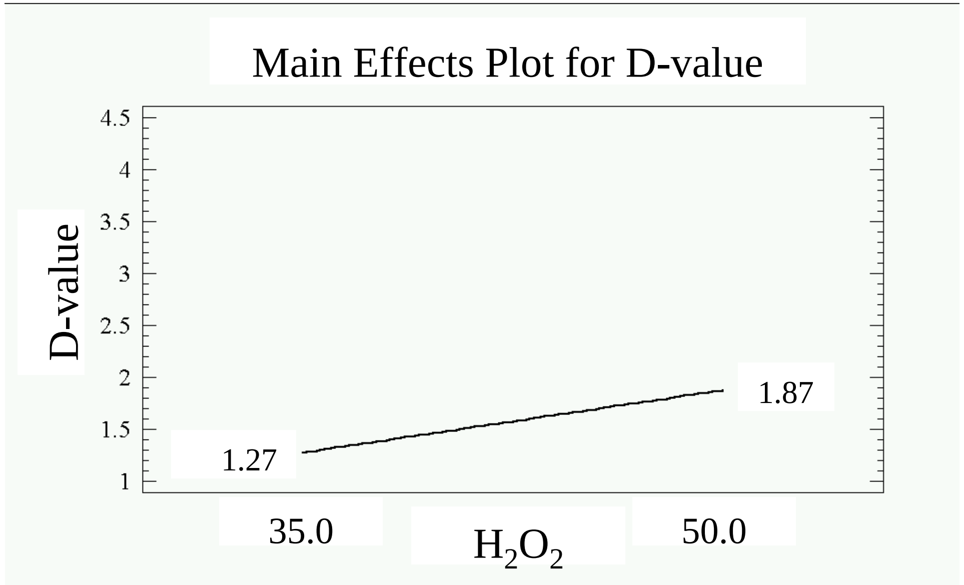


Results and Interpretation *H_2O_2 (E)*



- The *lower* the Concentration of H_2O_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 *H_2O_2 (E)*



- Vaporization of the same quantity of pure H_2O_2 produces a *better* decontamination *effect* at *lower concentrations* of the H_2O_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: H_2O_2	lower concentration	higher *saturation* of gaseous phase

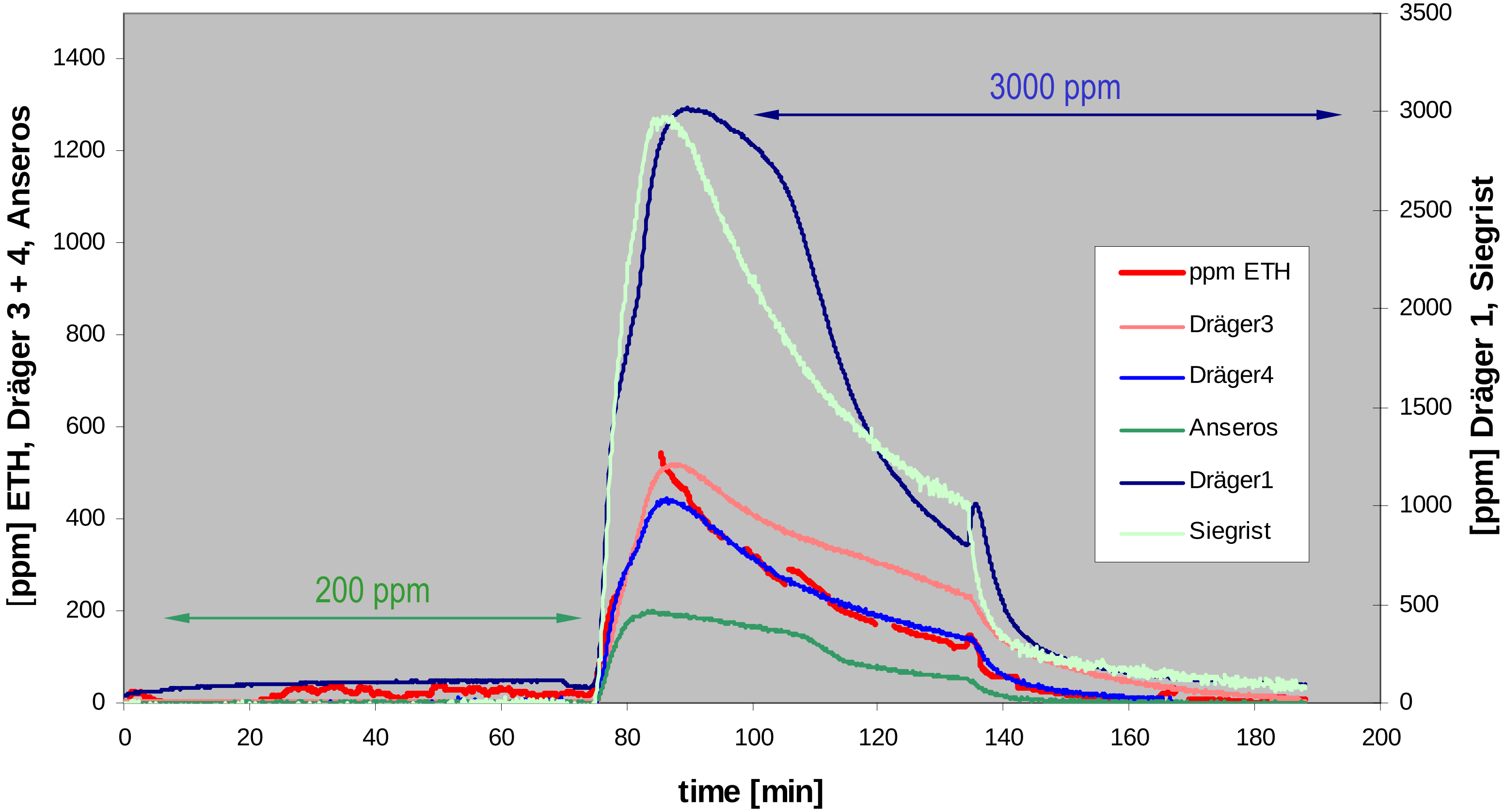
- Decontamination effect depends on *Saturation of gaseous phase*
- *"Physical Pressure" from gaseous phase to surface*

Results and Interpretation H_2O_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 *H_2O_2 Gas-Concentration*



Results and Interpretation H_2O_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_2O_2 Decontamination Cycles

Selection of Biological Indicators

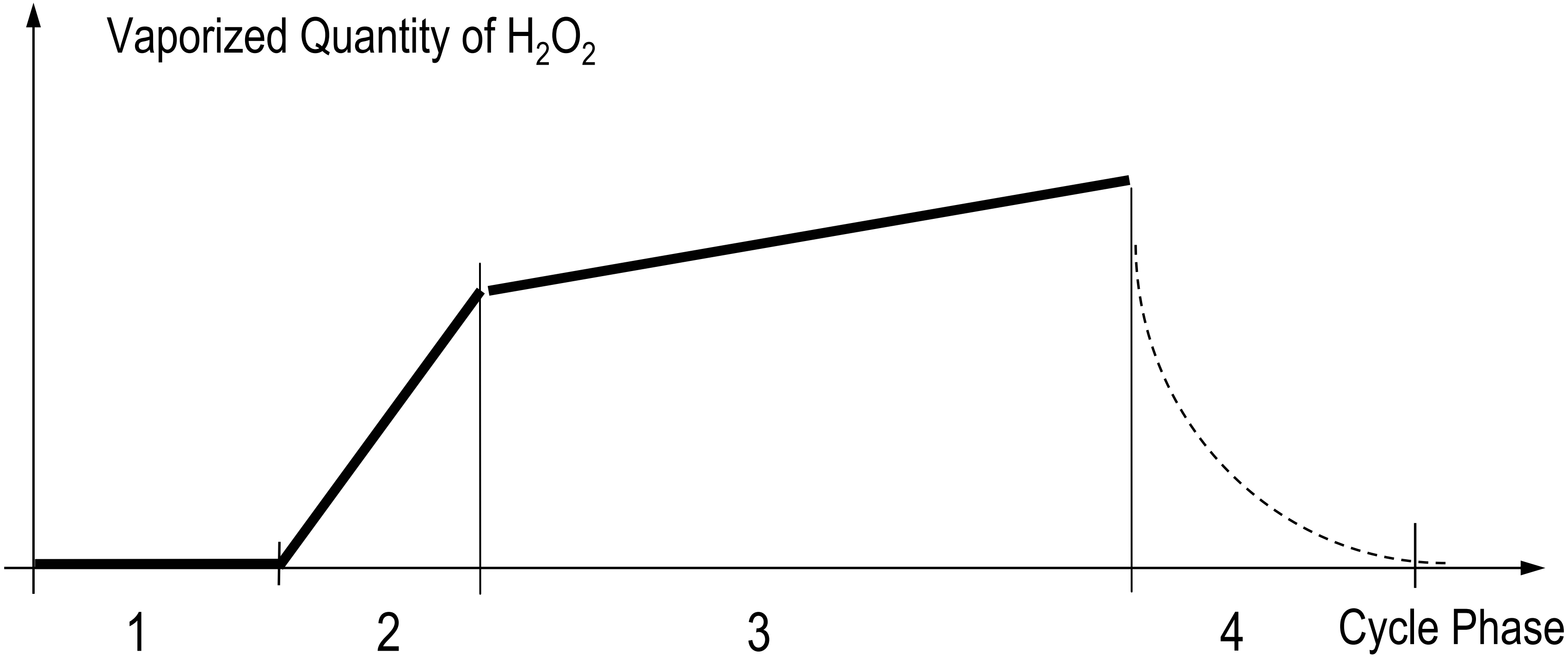
Test Organism: B. stearothermophilus
Initial Population: > 1.0 x 10^6
Carrier Material: CrNi- Stahl
Primary packaging: Tyvek

Group	01	02	03	04	05	06	07	08	09	10	pos
Exposure Time [min]	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
Result 1	+	+	+	+	-	-	-	-	-	-	neg
2	+	+	+	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	

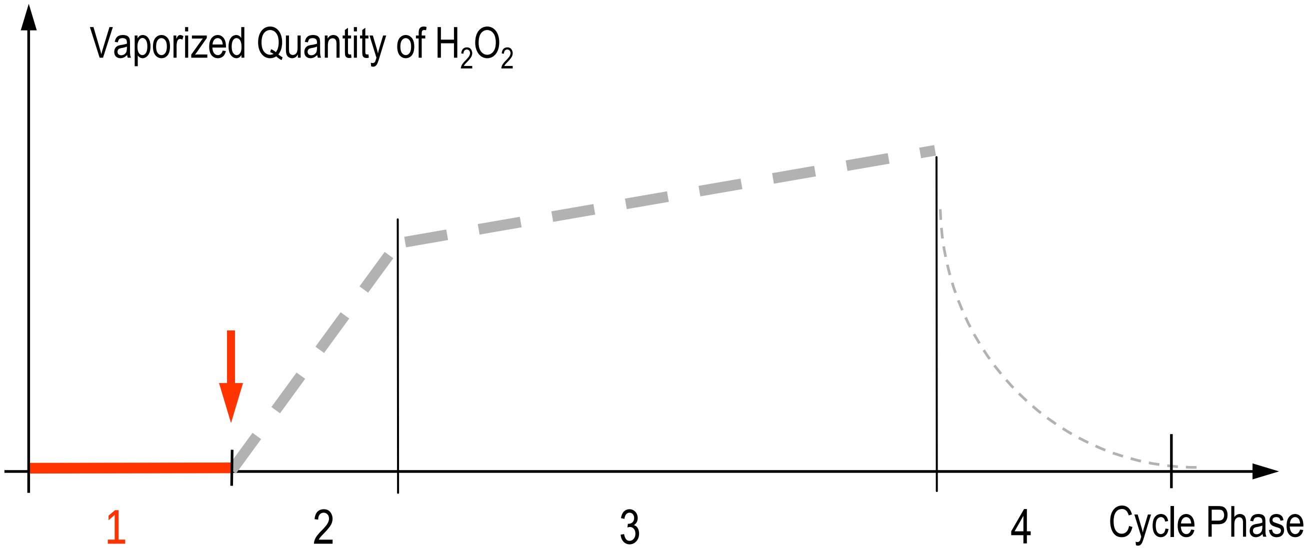
+ growth
- no growth

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

Description of Cycle *Physical*



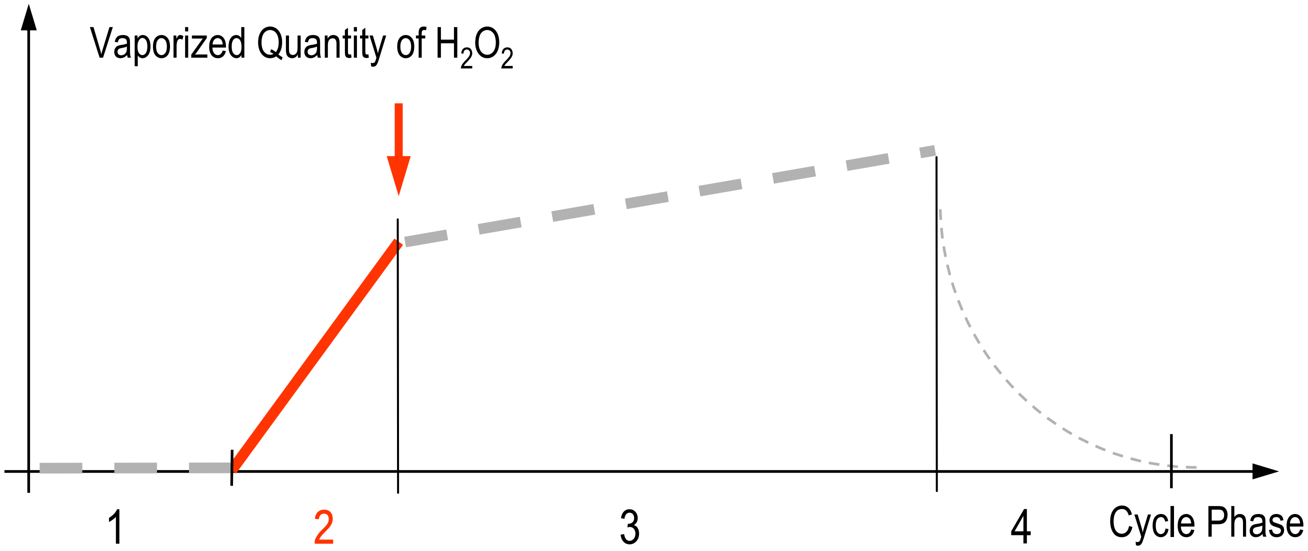
Description of Cycle *Physical*



Reproducability of Decontamination Effect

Initial conditions in chamber	*Temperature*	± 5 [°C]
	Humidity	± 5 [%rH]

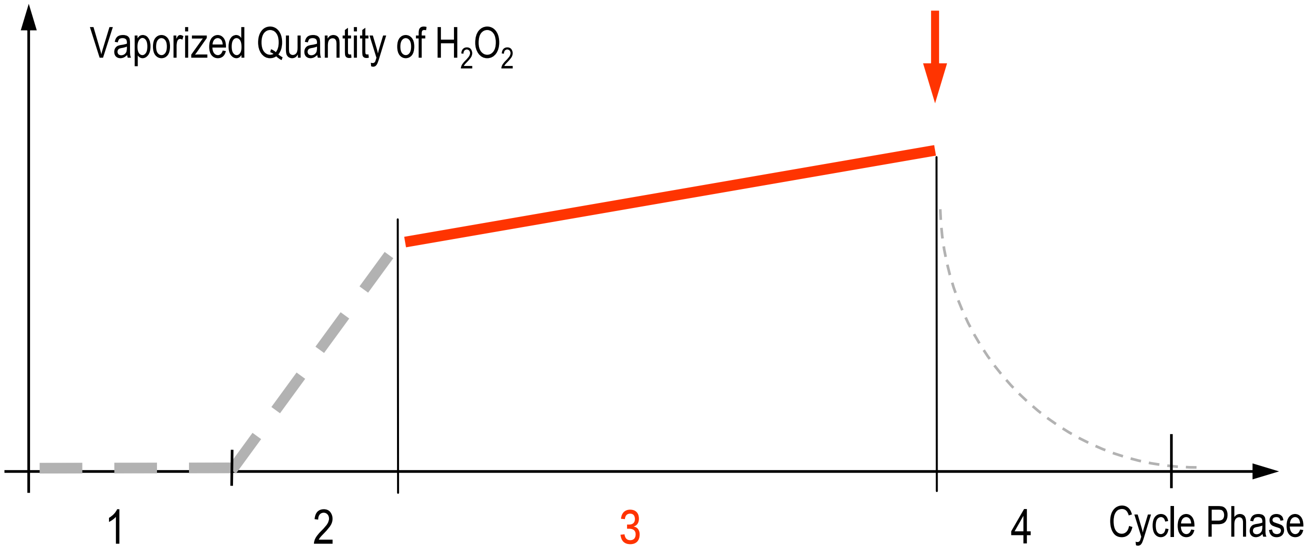
Description of Cycle *Physical*



Achieved Decontamination Effect

Quantity of initial vaporized H_2O_2 mass control, balance *[g]*

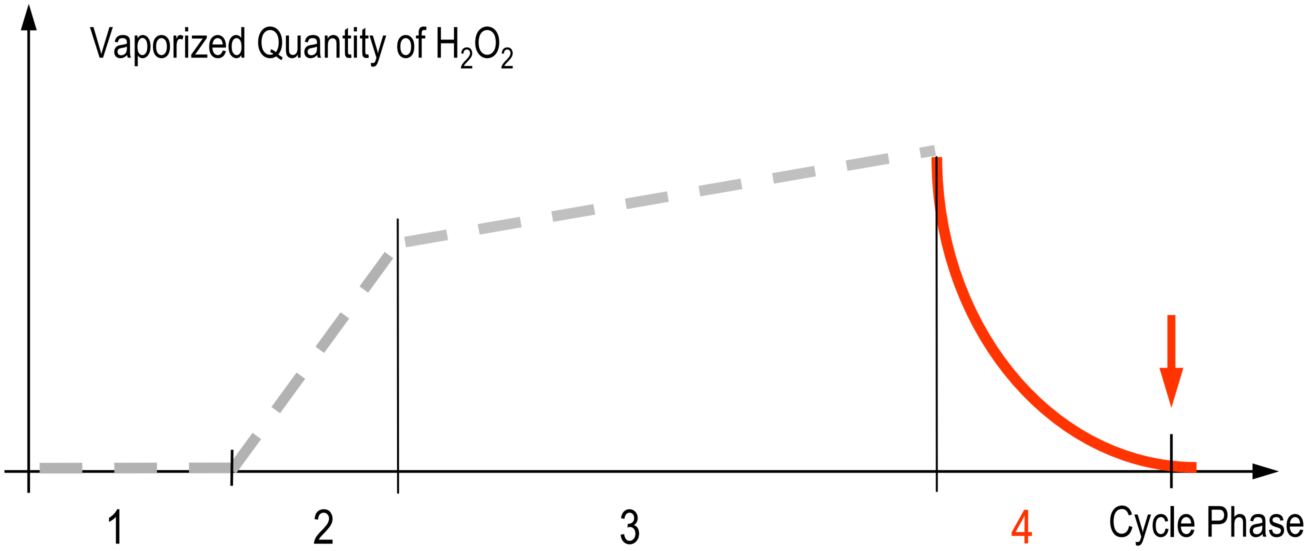
Description of Cycle *Physical*



Stability of Decontamination Effect

Rate of Redose H_2O_2 mass control, balance *[g/t]*

Description of Cycle *Physical*



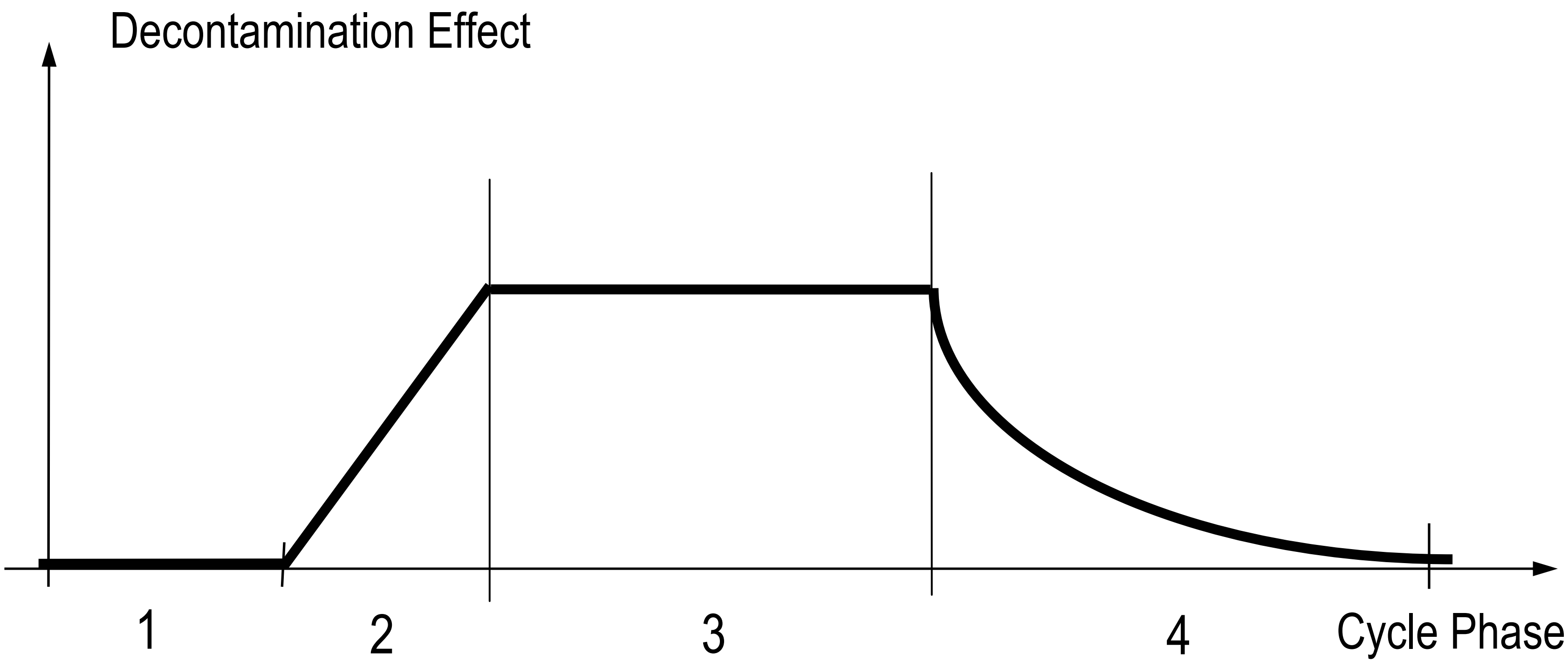
Residual Concentration of H_2O_2

Aeration curve low concentration sensor *[ppm/t]*

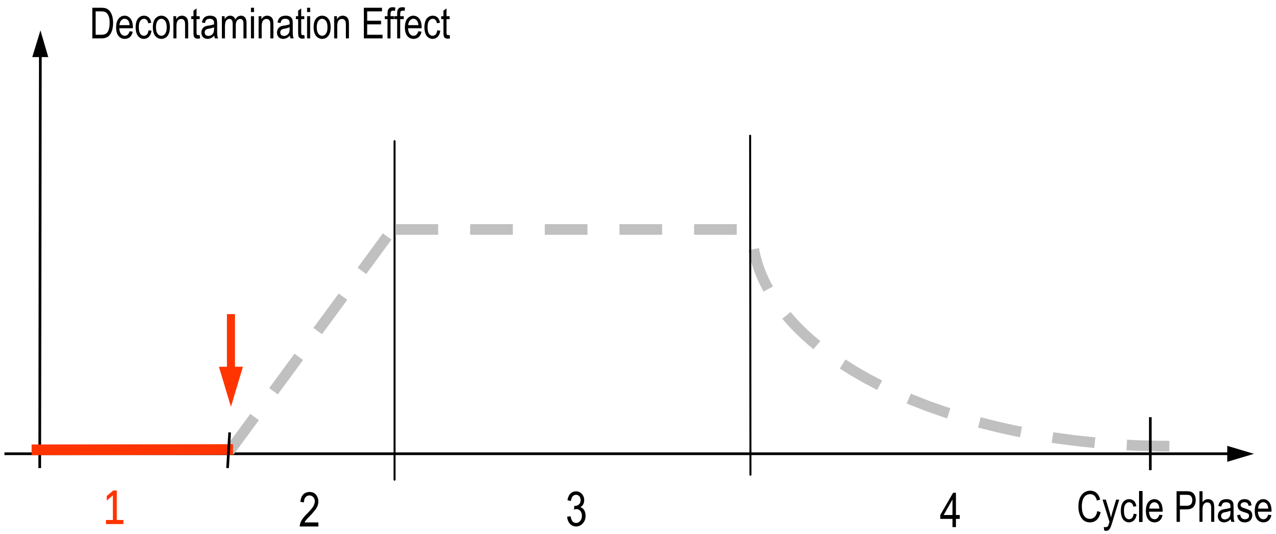
Description of Cycle Decontamination effect

- 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



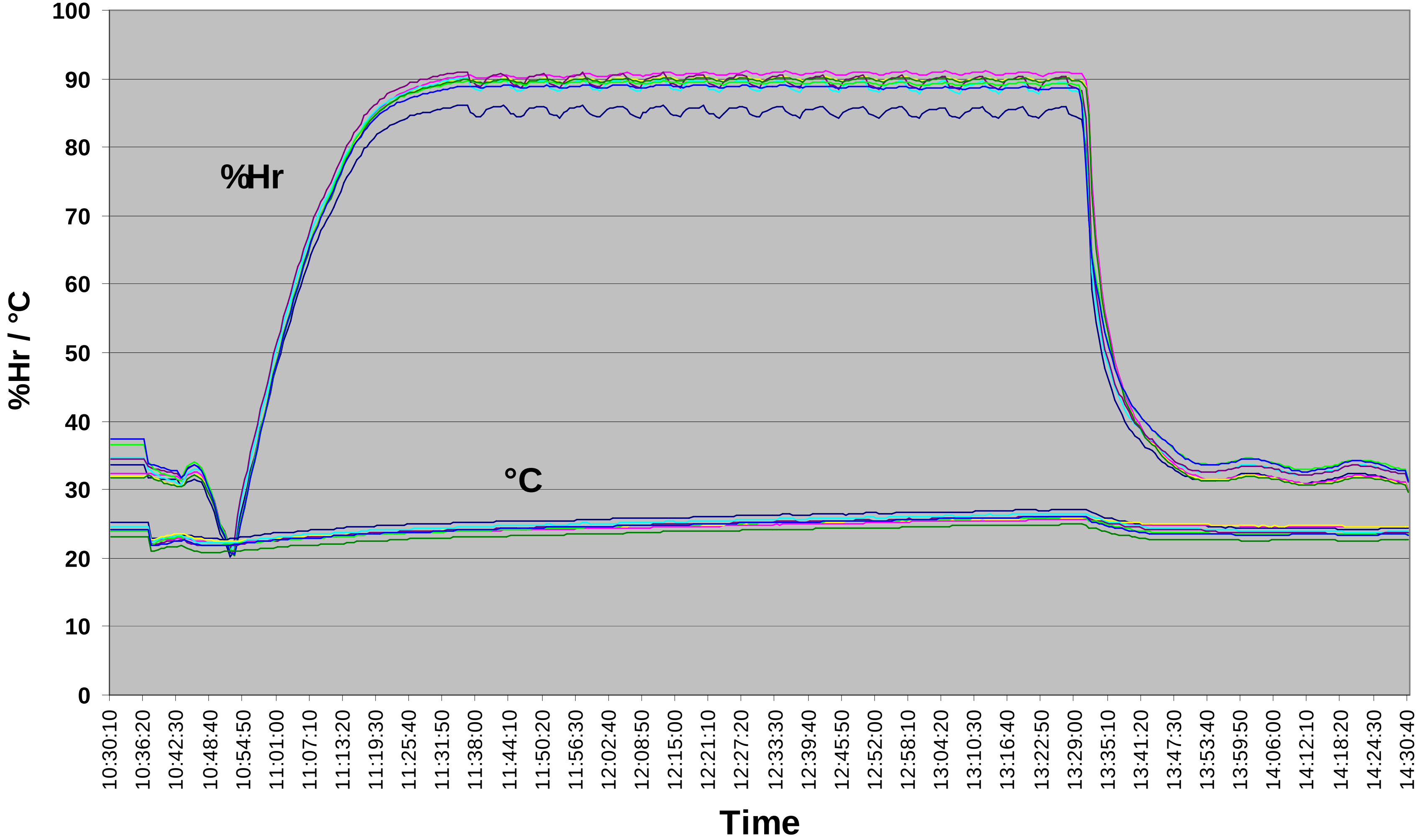
Description of Cycle   Decontamination effect



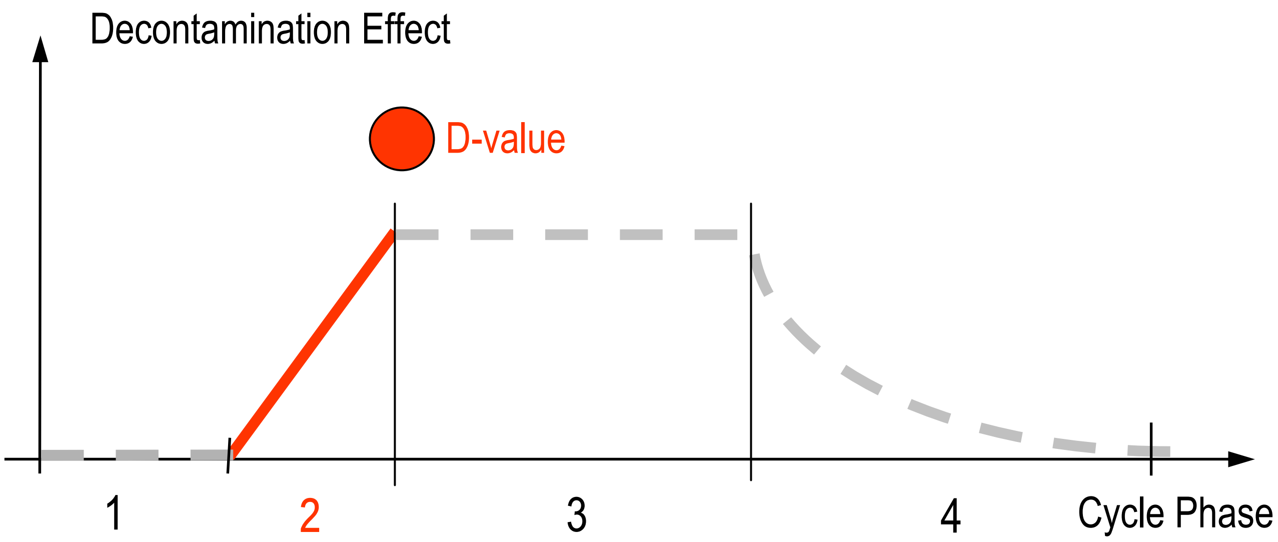
Reproducability of Decontamination Effect

Physically defined by:	*Temperature*	± 5 [°C]
	Humidity	± 5 [%rH]

Temperature and Humidity Mapping



Description of Cycle Decontamination effect



Achieved Decontamination Effect

achieved D-value ***defined*** *initial Quantity of H_2O_2 [g]*

Achieved Decontamination effect

Achieved decontamination effect

Quantity 5 [g/m^3]

\+ growth

\- no growth

exposure	01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
result 1	+	+	+	+	+	+	+	+	-	-	neg
2	+	+	+	+	+	+	-	-	-	-	-
3	+	+	+	+	-	-	-	-	-	-	

Achieved Decontamination effect

Achieved decontamination effect

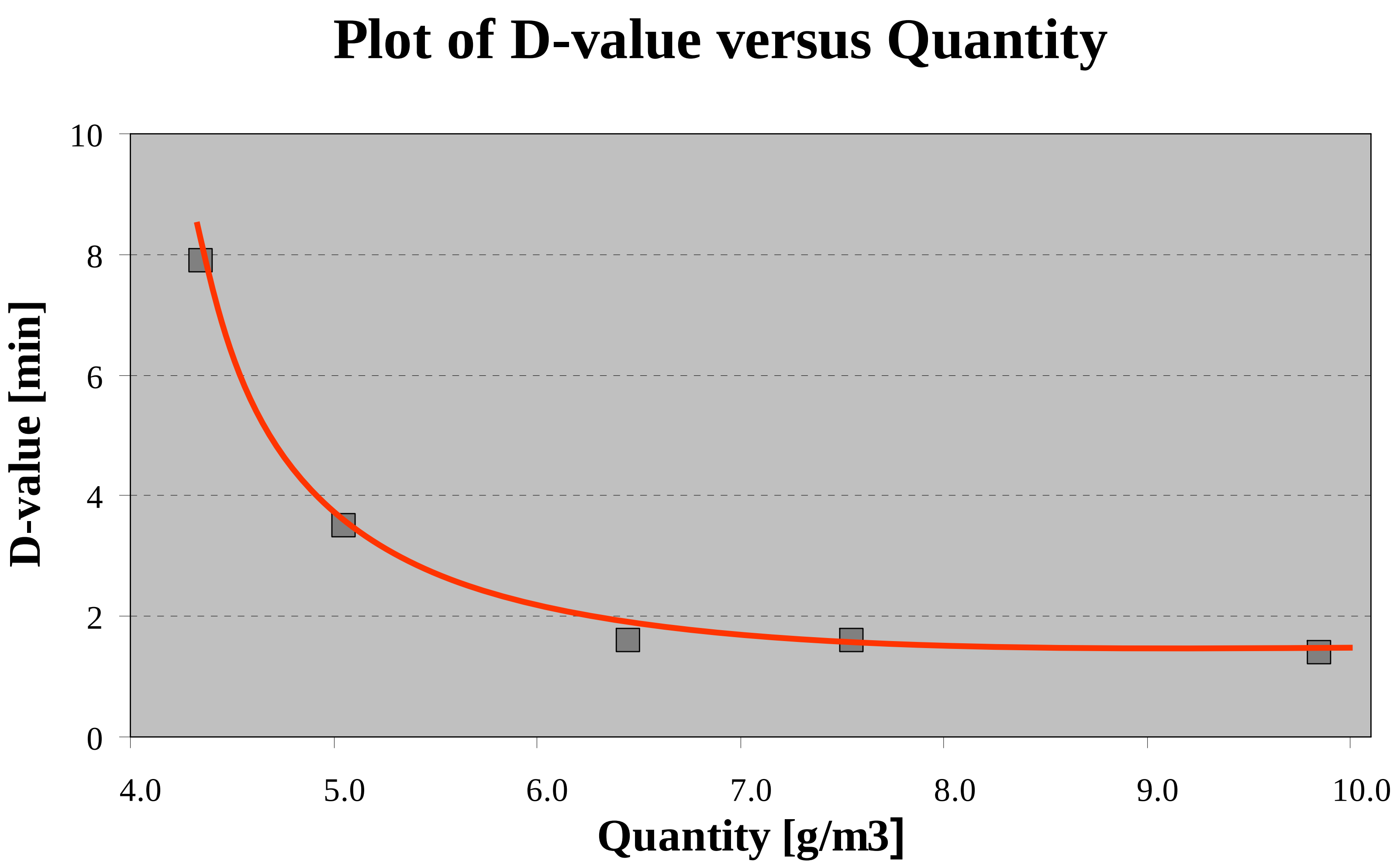
Quantity 7.5 [g/m^3]

\+ growth

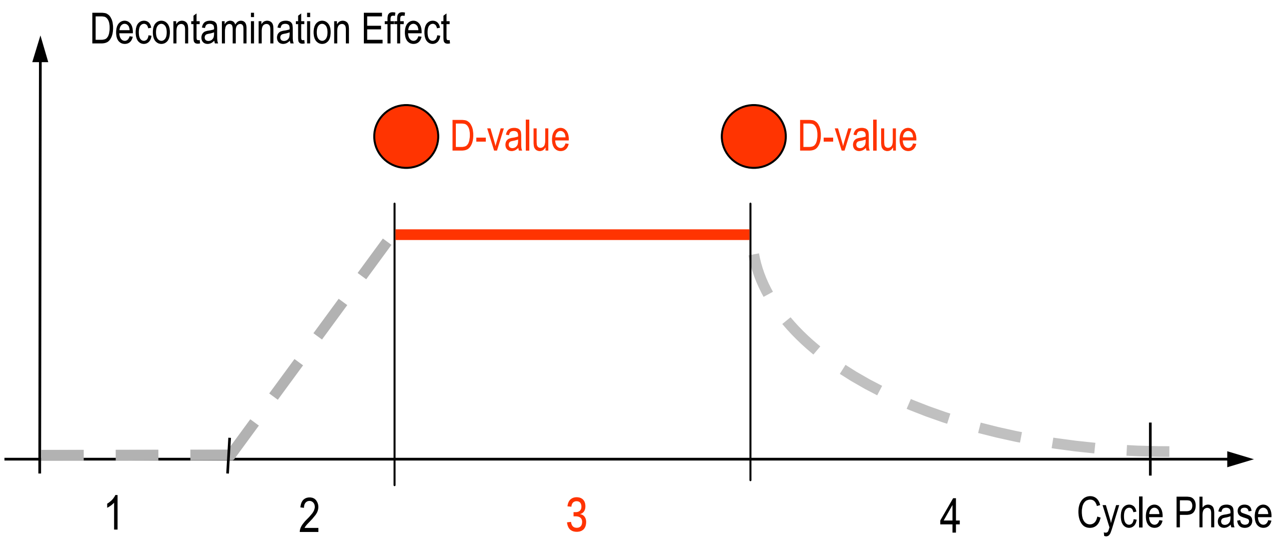
\- no growth

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	+
result	1	+	+	+	-	-	-	-	-	-	-	neg
	2	+	+	-	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-	-	

Achieved Decontamination effect



Description of Cycle *Decontamination effect*



Stability of Decontamination Effect

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

Stability of Decontamination effect

Rate of Redose 25 [%A/h]

\+ growth
\- no growth

LSKM 1, exposition 5 [min] after conditioning

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		7.5	10.0	12.5	15.0	17.5						+
result	1	+	-	-	-	-						neg
	2	+	-	-	-	-						-
	3	-	-	-	-	-						

LSKM 2, exposition 30 [min] after conditioning

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		7.5	10.0	12.5	15.0	17.5						+
result	1	+	+	+	+	+						neg
	2	+	+	+	+	+						-
	3	+	+	+	+	+						

Stability of Decontamination effect

Rate of Redose 100 [%A/h]

+ growth
- no growth

LSKM 1, exposition 5 [min] after conditioning

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		7.5	10.0	12.5	15.0	17.5						+
result	1	+	-	-	-	-						neg
	2	+	-	-	-	-						-
	3	+	-	-	-	-						

LSKM 2, exposition 30 [min] after conditioning

exposure		01	02	03	04	05	06	07	08	09	10	pos
exposure time [min]		7.5	10.0	12.5	15.0	17.5						+
result	1	+	-	-	-	-						neg
	2	+	-	-	-	-						-
	3	-	-	-	-	-						

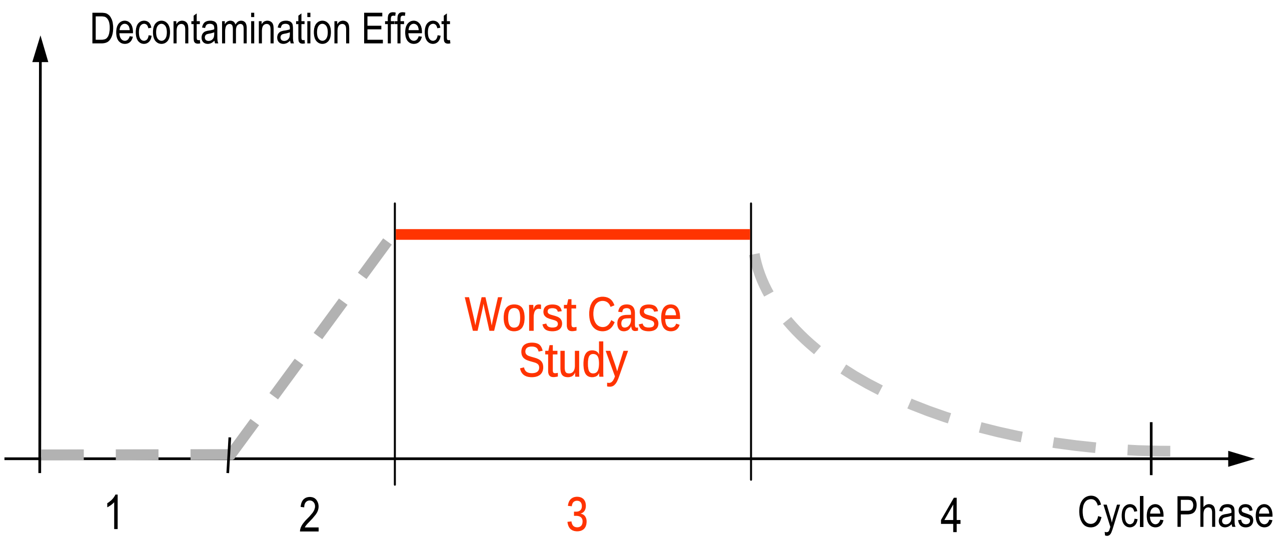
Description of Cycle Worst Case Positions

Definition of Worst Case Positions, (BI- Positions)

• Air Flow and Distribution	Smoke Tests
• Temperature, Humidity Distribution	Deco-Process
• Chemo Indicator Mapping	qualitative
• deeply consider the Process	Risk Analysis

Extreme Point of Application *no Assumptions*

Description of Cycle *Decontamination effect*



Duration of Decontamination Phase

Worst Case Study ***defined*** *D-value Worst Case*

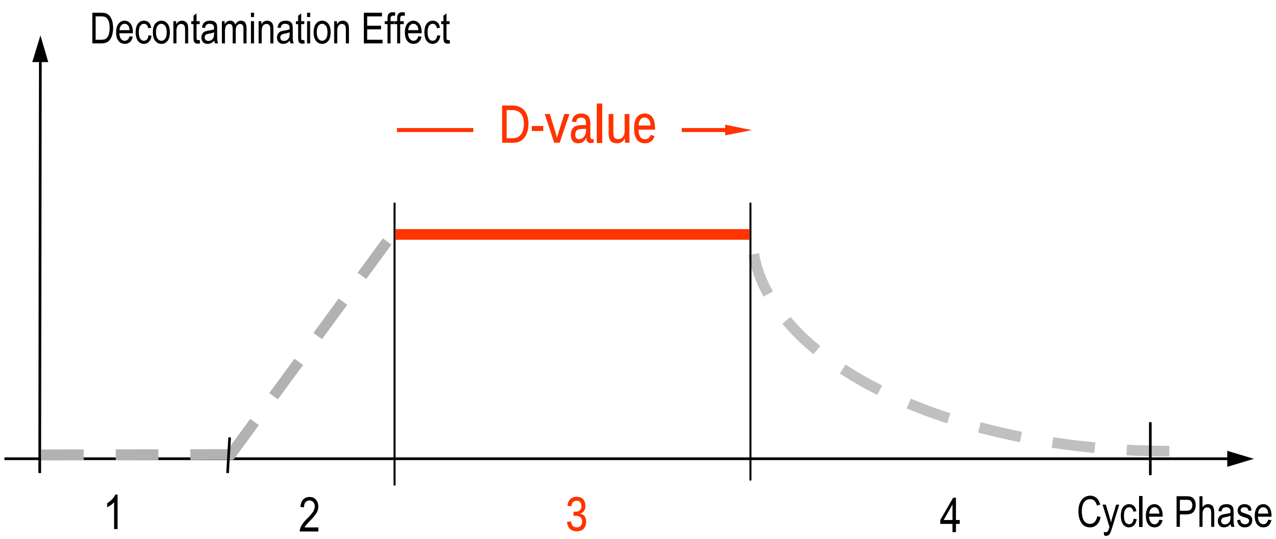
Duration of Decontamination [t]

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*



Required log Reduction

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

H_2O_2 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

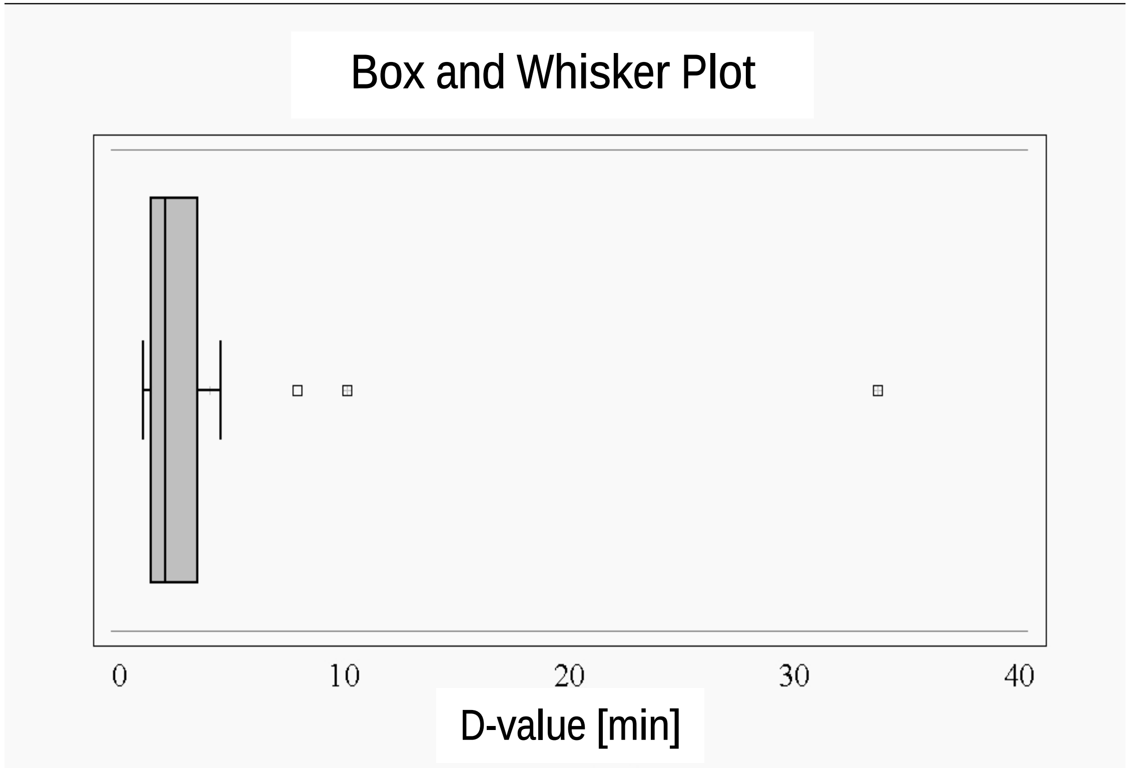
- Materials used in isolator — as construction materials
as disposables in routine work
- Inoculate with min $1.0x10^6$ spores of B. stearothermophilus
- Determination of D- value and Reactive Pattern
- Compare the results with commercially available BI
- Transfer results to different Production Isolator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

- Conclusion “HOW to handle the Material Question”

Materials Study Results

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

Materials Study *Summary of Result*



Significantly higher D-value for the various *aluminium* samples

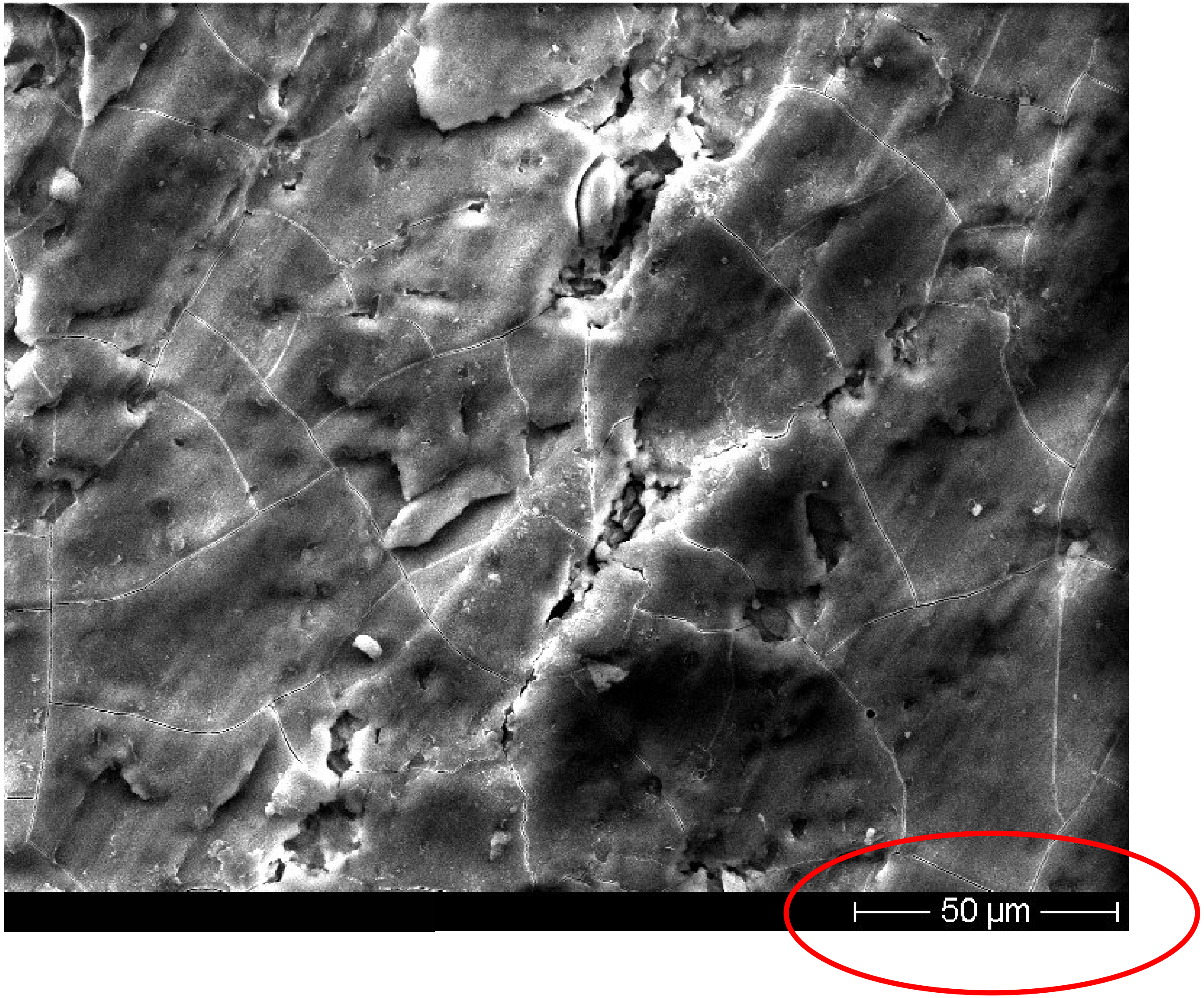
Materials Study *Interpretation Aluminium*

- Extremely *porous surface* structure
- Suspension was *absorbed into surface*
- H_2O_2 decontamination is ***not able*** to *penetrate into a surface*

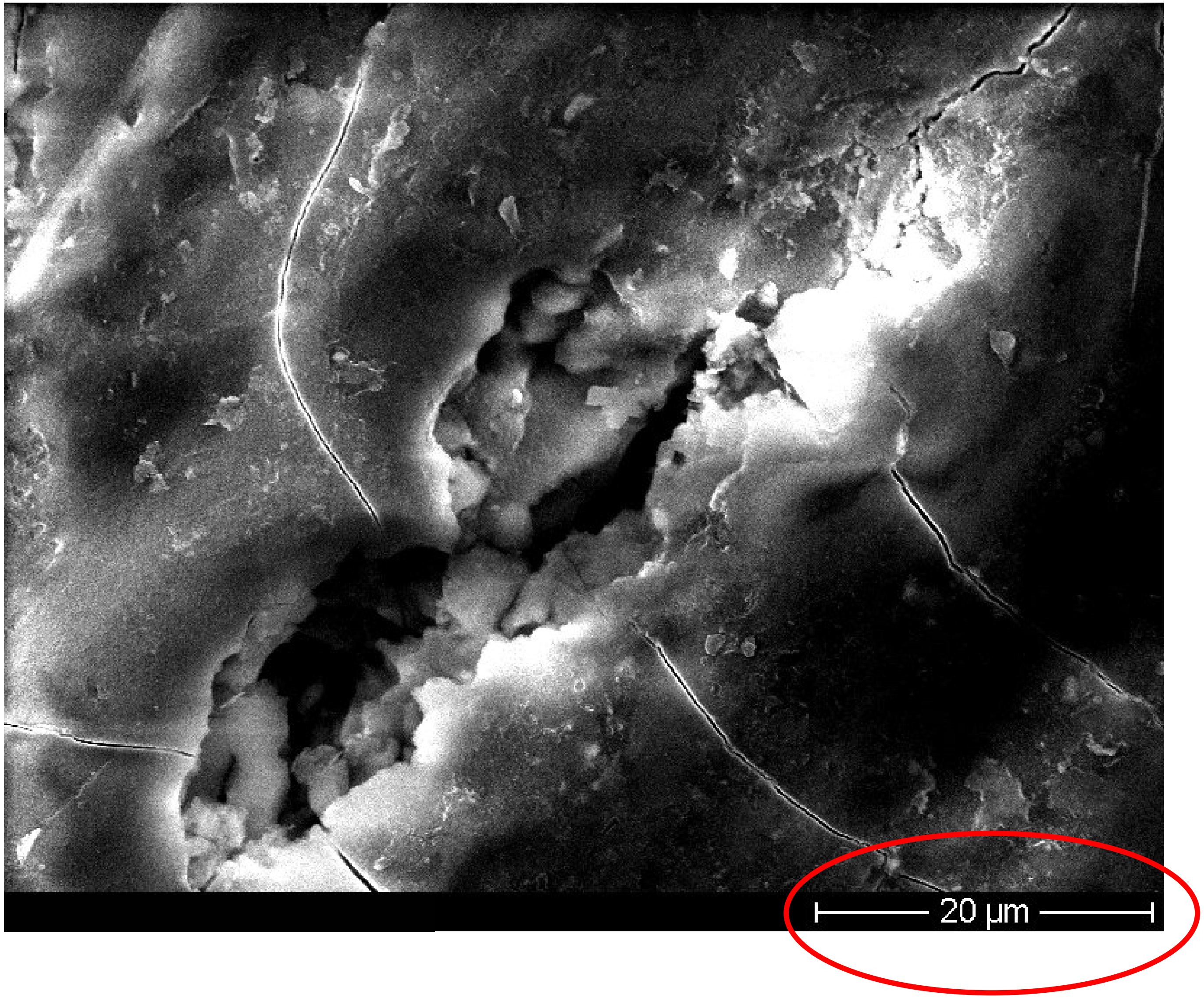
→ *No* or only a *bad* inactivation effect

→ *Not suitable for H_2O_2 decontamination*

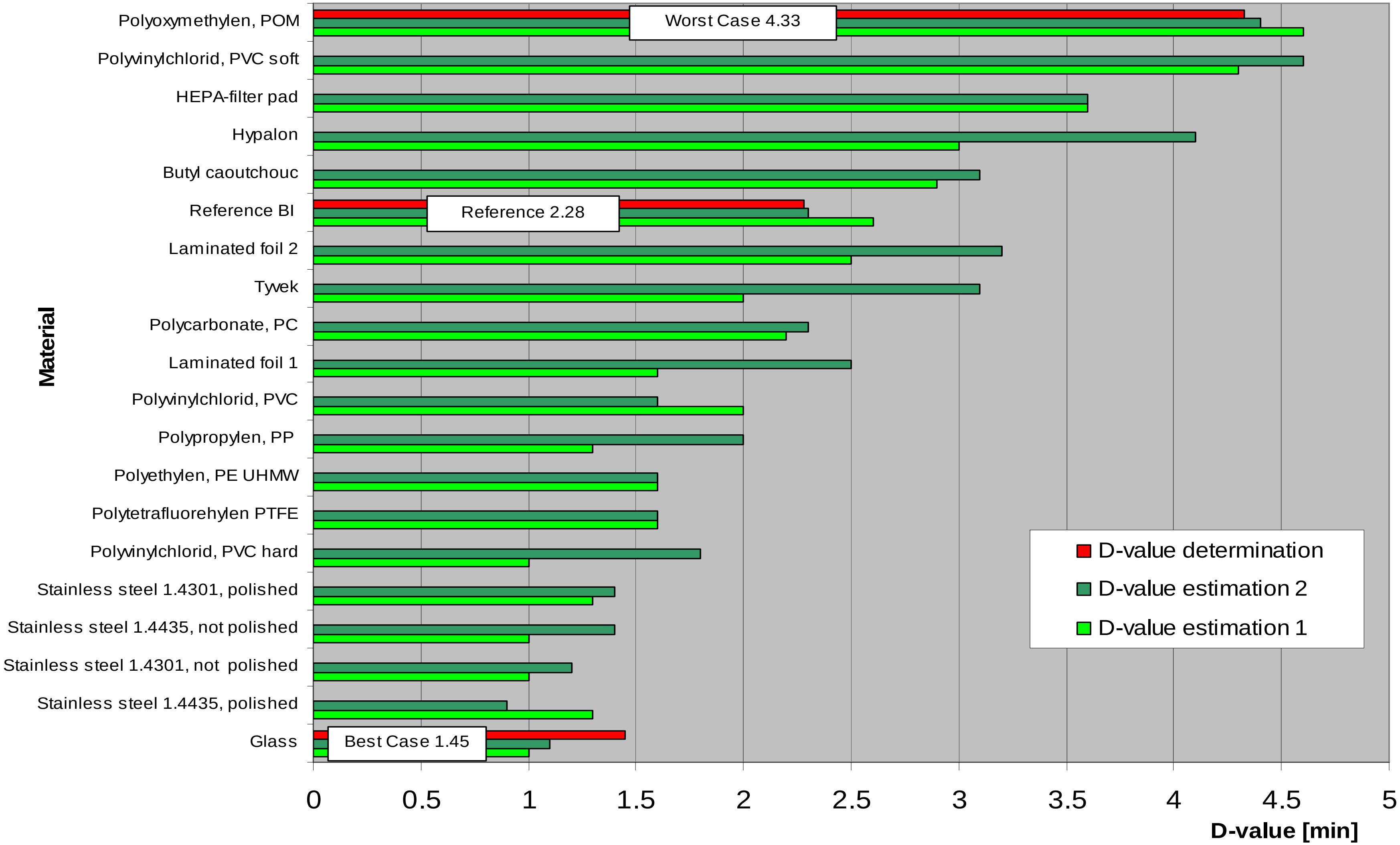
Carrier Material *Aluminium anodized*



Carrier Material *Aluminium anodized*



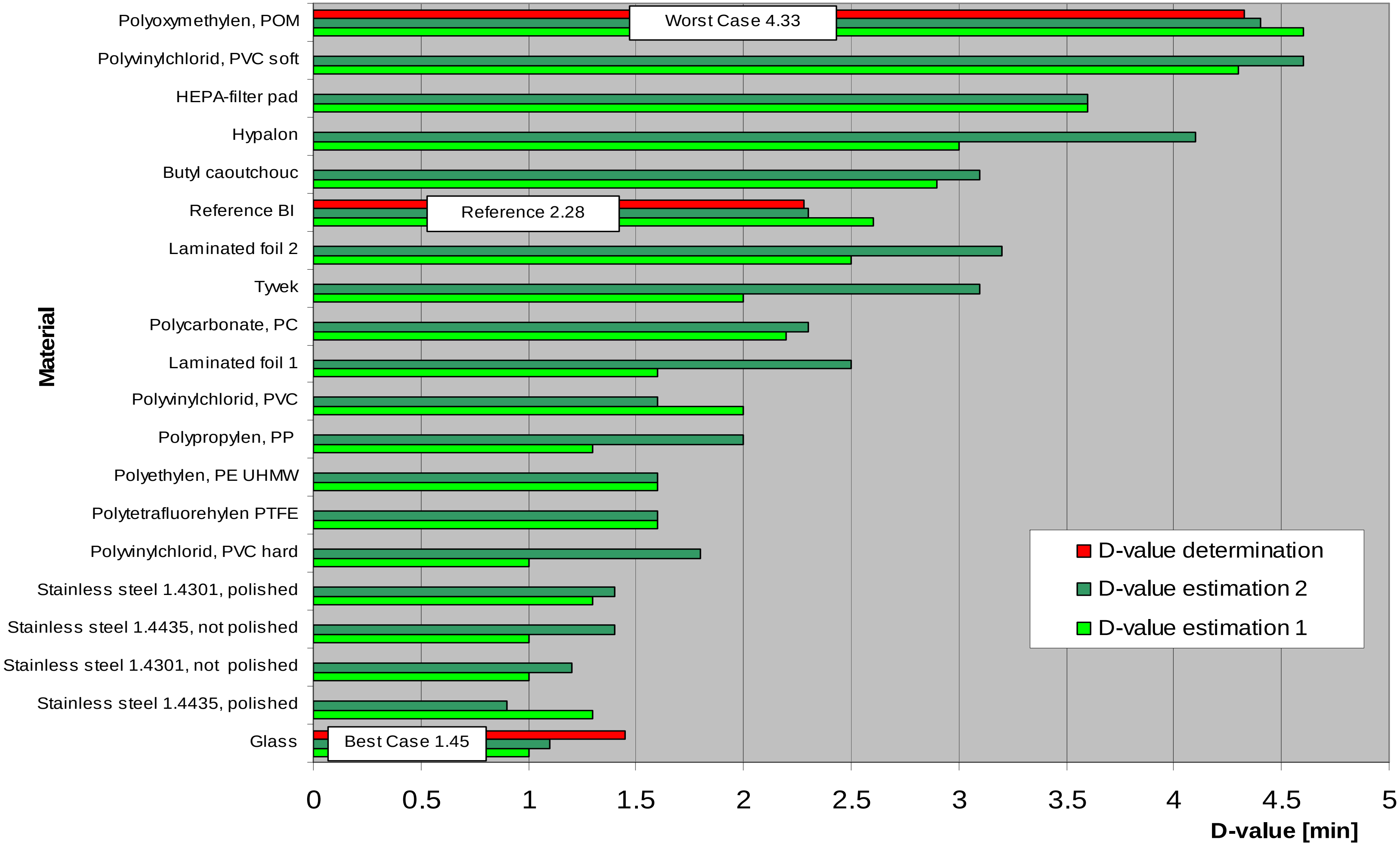
Materials Study Results



Materials Study	*Transferability*
• 1 x Reference Isolator	1,4 m^3
• 2 x Sterility Test Isolator	2,2 m^3
• 5 x Filling Isolator	9,0 m^3
• 2 x Material Pass Through	15,0 m^3

- IQ / OQ finished
- Cycle development based on commercial BI`s finished
- Initial condition and cycle parameter defined

Materials Study *Results*

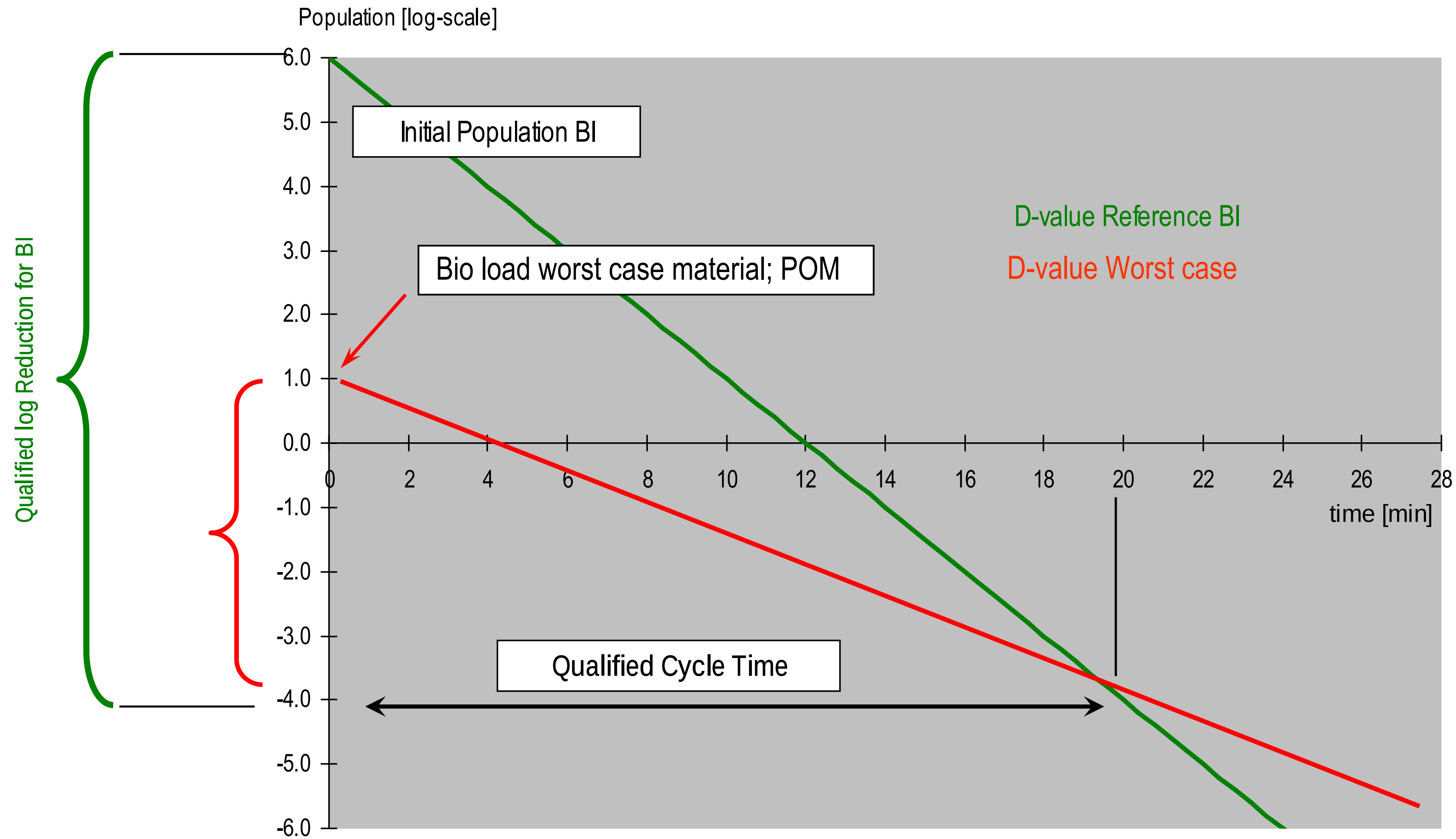


Materials Study *Transferability*

- 1 x Reference Isolator 1,4 m^3
- 2 x Sterility Test Isolator 2,2 m^3
- 5 x Filling Isolator 9,0 m^3
- 2 x Material Pass Through 15,0 m^3

	D-value [mins] Reference Isolator	D-value [min] Serility Test Isolator	D-value [min] H_2O_2 Material Pass Through	D-value [min] Filling Isolator
B. stearothermophilus on Glass	1.45 ± 0.07	1.41 ± 0.04	0.96 ± 0.05	1.18 ± 0.20
Biological Indicator Reference	2.28 ± 0.06	2.22 ± 0.06	1.62 ± 0.08	1.72 ±0.07
B. stearothermophilus on POM	4.33 ± 0.20	4.00 ± 0.30	3.38 ± 0.16	3.41 ± 0.23

Materials Study *Implementation*



Materials Study *Summary*

- Reproducible *different resistances* on *different carrier materials*
- Some Materials *seems* to bee *not suitable* for H_2O_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_2O_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

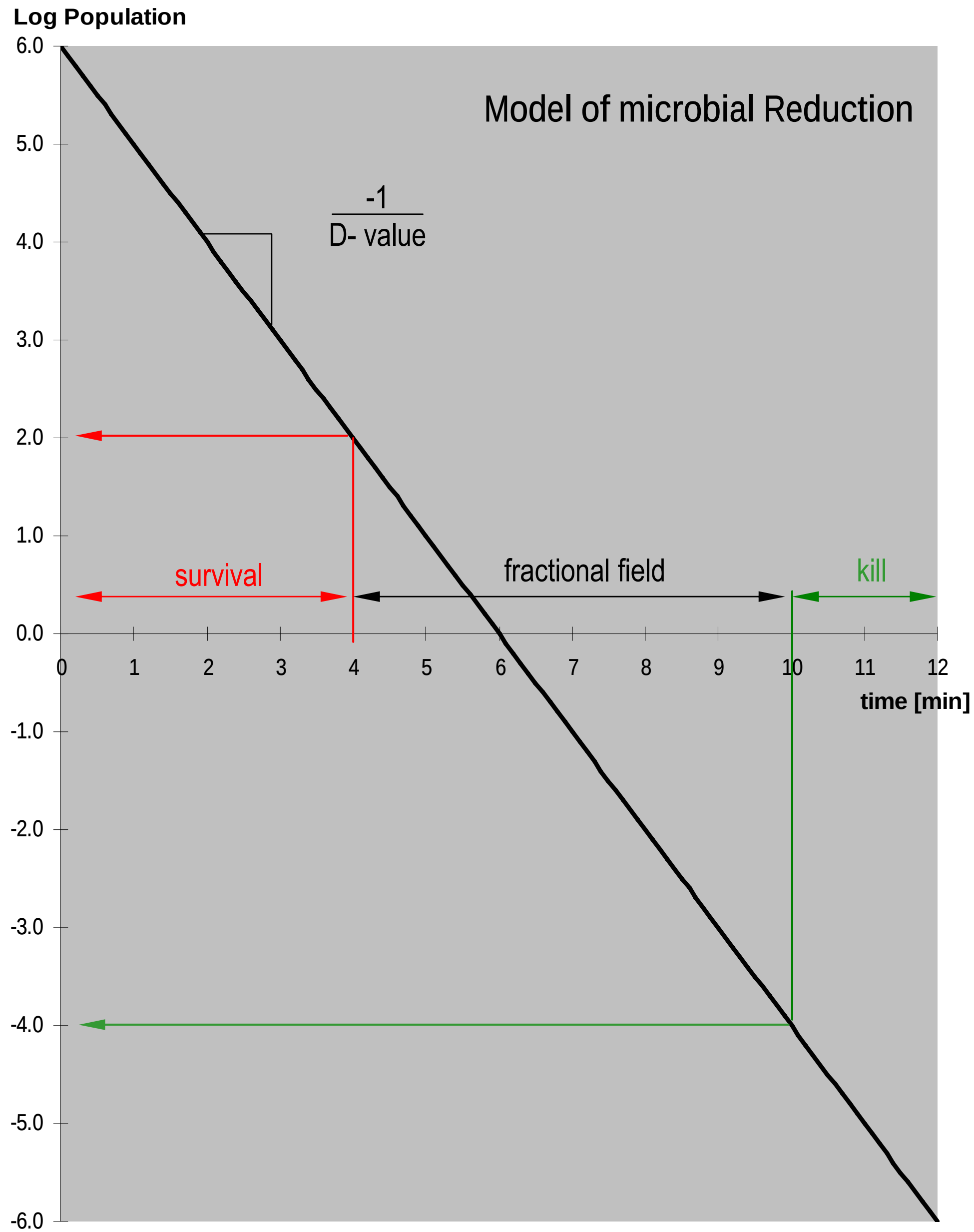
Regulatory Requirements; FDA Aseptic Guideline

"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"

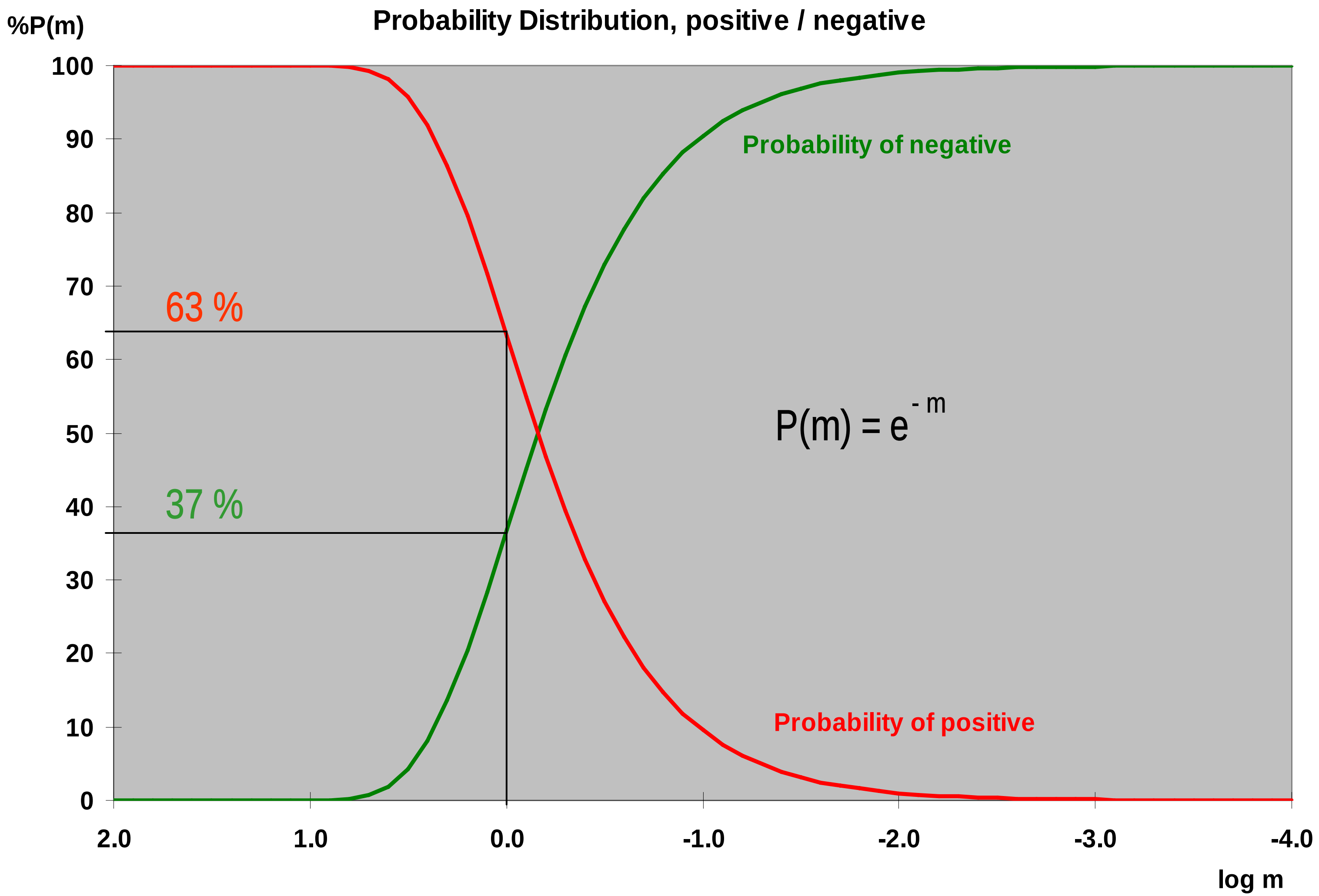
Microbiological System

Model of Microbial Reduction

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



Microbiological System



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 10^4 spores per unit, while fraction negative studies are suitable for BIs with a population of 10^5 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 *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 — BI Quality Control
- 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

Volker Sigwarth
Skan AG
Switzerland

Process Development