Suitability of Different Construction Materials for Use in Aseptic Processing Environments Decontaminated with Gaseous Hydrogen Peroxide

BEATRIZ UNGER,[1] UTA RAUSCHNABEL,[1] BERTHOLD DÜTHORN,[1] VOLKER KOTTKE,[2] CHRISTIAN HERTEL,[2] AND JOHANNES RAUSCHNABEL[1]

[1]Robert Bosch GmbH, Blaufelder Str. 45, 74564 Crailsheim, Germany, Phone: +49 7951 402-471, Fax: +49 711 811-5180358, E-mail: beatriz.unger@de.bosch.com; [2]University of Hohenheim, 70599 Stuttgart, Germany

ABSTRACT: The purpose of this study is to examine the behavior of different materials towards the microbial inactivation kinetic of gaseous hydrogen peroxide. Samples of 49 materials potentially used in aseptic processing environments were inoculated with 10^6 spores of Geobacillus stearothermophilus ATCC #12980 and exposed to defined periods using a reproducible hydrogen peroxide bio-decontamination cycle. The inactivation characteristic of each material was investigated by means of repeated D-value calculations. The results demonstrate that different materials show highly variable performance regarding the inactivation pattern of spores on each particular surface. Not only the chemical composition of the material but also differences in manufacturing processes and surface treatments were found to have an effect on the resistance of the test organisms. From the data obtained it is concluded that some correlation exists between the calculated D-values and roughness as well as wettability of the materials. Best- and worst-case materials were identified, and the dependence of specific decontamination characteristics on material properties was investigated. It is suggested to integrate studies regarding the inactivation characteristics of incorporated materials into the construction process of new aseptic processing systems bio-decontaminated with hydrogen peroxide.

KEYWORDS: Aseptic processing, Bio-decontamination, Construction material, D-value, Filling machine, Gaseous hydrogen peroxide, Geobacillus stearothermophilus, Inactivation kinetic, Isolator, Material surface, Resistance, Spores.

Introduction

Various parenteral drugs and other pharmaceutical formulations that are labeled as sterile cannot be terminally sterilized. These products must be processed and filled in an aseptic environment to reduce contamination from particles and microorganisms (1–5). Physical and aerodynamical barriers provide this required controlled space by separating the aseptic process from the surrounding area.

Barrier technology, which started in the 1980s (6), is widely used in the pharmaceutical industry today (7). However, before a filling operation can take place, the interior of the isolator has to be bio-decontaminated to eliminate viable bioburden. A common sporicidal process is to generate gaseous hydrogen peroxide that is transferred into the aseptic process area and reacts with contaminants on the surfaces (2, 3, 8). Figure 1 presents a schematic diagram of an exemplary decontamination cycle with hydrogen peroxide consisting of four main phases. The preparation phase I includes preconditioning of the isolator temperature and humidity as well as heating of the ductwork. During the conditioning phase II, hydrogen peroxide is injected at a high rate in order to achieve the desired level of peroxide inside the isolator. The main kill takes place during the bio-decontamination phase III. After completion of the sporicidal phase, the hydrogen peroxide is removed from the isolator during the following aeration phase IV.

The materials of construction of the isolator and filling machine that are exposed during the decontamination cycle have to be mechanically and chemically compatible with the intended process and the sporicidal agent used. Previous studies (9, 10) suggest that the materials also have an impact on the resistance of particular microbial spores to the sporicidal process applied. Consequently, the choice of materials of construction for the aseptic environment provides the basis for a successful bio-decontamination process. This fact is also reflected in the following quotations from the Food and Drug Administration (FDA) *Guidance for Industry* (11): *"As in any aseptic processing design, suitable materials should be chosen based on durability, as well as ease of cleaning and decontamination";* and *"if various isolator materials are thoroughly evaluated during cycle development, a firm might consider placing more focus on*

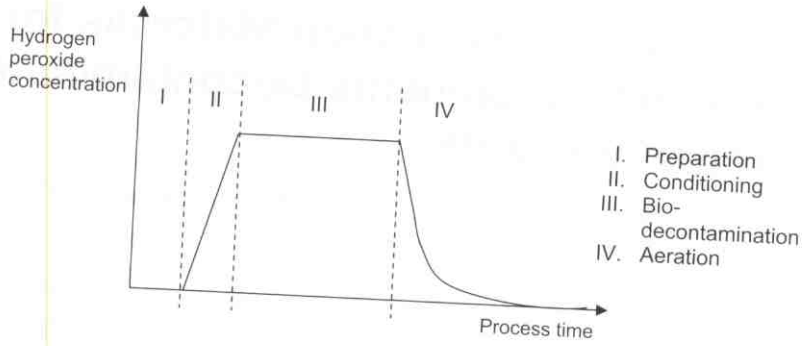


Figure 1

Schematic course of hydrogen peroxide level during bio-decontamination.

material texture and porosity during validation of the decontamination process."

This study systematically investigated the suitability of different materials for bio-decontamination with gaseous hydrogen peroxide. Therefore, typical materials of construction used in filling lines and isolators were chosen. Additional materials used in aseptic handling or that are of special interest were also included in the study. In total, 49 materials were tested. D-value calculations were performed for every material in order to detect differences in the resistance of the spores on various surfaces. Biological indicators were prepared with carriers made from these materials for this purpose. For maximum challenge, the carriers were inoculated with six logs of spores of *Geobacillus stearothermophilus* American Type Culture Collection (ATCC) #12980. This microorganism demonstrates a high resistance against gaseous hydrogen peroxide and is commonly used for validating gaseous hydrogen peroxide processes in the pharmaceutical industry (3, 5). The material indicators together with commercially available reference indicators made of stainless steel were subjected to a standardized bio-decontamination cycle using gaseous hydrogen peroxide. After defined exposure time intervals during the plateau phase of the bio-decontamination cycle, the materials were removed from the isolator and incubated in growth promotion medium. D-values were calculated from the results using the fraction-negative method (monitoring of growth or no growth is carried out proportionally to the number of samples tested). In this paper we describe in detail how the study was performed. The results are reported and compared to available data from previous studies (9). Within the scope of the survey, additional investigations were performed regarding surface characteristics of the material carriers, the impact of inoculation quality on the results, and variations in the cycle performance.

Materials and Methods

Study Design

The investigations performed during the study were divided into two parts. During the main study, the D-value of each material was analyzed three times using a first, a second, and a third sample set. The method of repeated D-value determination ensured an individual adaptation of the sampling window to the survival pattern of every material. Furthermore it enhanced the statistical evaluation of the derived data. In the first decontamination set, three samples of each material were removed from the isolator at each exposure interval. During the second and third decontamination sets, five indicators of each material were removed in parallel. The additional investigations addressed research on material characteristics and the quality of the bio-decontamination cycle.

In every decontamination run, adjacent to the test material biological indicators a highly standardized biological indicator with stainless steel coupon was included as reference. Therefore, the impact of variations in the sporicidal cycle on the material D-values could be quantified. To exclude effects of varying resistance for different spore lots, both kinds of biological indicators, test material and reference ones, were inoculated with spores derived from the same spore suspension.

Bio-decontamination System

Isolator

For determination of the D-values, the biological indicators were exposed to a bio-decontamination cycle at

TABLE I
Cycle Parameters of the Standard Bio-decontamination Cycle

Cycle Parameter	Value
Temperature (start of conditioning phase)	25 °C
Relative humidity (start of conditioning phase)	≤20%
Injection rate during conditioning	25 g/min
Total injection during conditioning	175 g
Duration of conditioning	7 min
Injection rate during bio-decontamination	9 g/min
Total injection during bio-decontamination	720 g
Duration of bio-decontamination	80 min

defined positions inside an isolator. A Bosch rigid wall single-pane positive pressure isolator with an internal volume of 7.5 m^3 was used for the study. The bio-decontamination system was operated as an open-loop system with unidirectional air flow. In the center of the isolator a perforated table was used for the exposure of the biological indicators. Homogeneity of the hydrogen peroxide concentration in the exposure area was checked and verified during a prestudy. Interventions inside the isolator were done over glove ports. A validation port was included for removal of the indicators after selected exposure periods.

Gas Generator

For the sporicidal cycle, liquid hydrogen peroxide had to be vaporized by the "*SafeVAP*" gas-generator from Bosch, which was integrated into the air handling of the isolation system. The vaporized hydrogen peroxide was carried with a flow of dry fresh air into the barrier system above the high-efficiency particulate air (HEPA) filters, and a continuous gas stream was conveyed to the exposure area of the biological indicators.

Sporicidal Agent

Hydrogen peroxide aqueous solution 35%, medical extra-pure Ph Nord, sourced from Merck KgaA, Darmstadt, Germany, was used.

Cycle Parameters

For the investigations a fully validated decontamination cycle was used. The applied cycle parameters are described in Table I.

Hydrogen Peroxide Concentration

Hydrogen peroxide and water concentrations in the isolator atmosphere were recorded during every cycle with a near-infrared (NIR) spectrophotometer from Guided Wave Incorporated (Rancho Corvo, CA). Additionally, the hydrogen peroxide concentration was monitored by an electrochemical sensor (Analytical Technology Inc., Collegeville, PA) incorporated inside the barrier system. A combined humidity and temperature sensor was applied to validate the humidity data from the NIR spectrophotometer. Figure 2 shows the typical course of a bio-decontamination cycle recorded during the study with the NIR probe. During the conditioning phase, the hydrogen peroxide concentration increases within 7 min to the desired concentration of approximately 850 ppm. During the bio-decontamination phase, when the biological indicators are exposed and removed, the concentration is kept stable and only inactivated peroxide is replaced. The water concentration decreases slowly from 9000 ppm to 5500 ppm. All decontamination cycles conducted throughout the study were operated without visible condensation inside the isolator.

Biological Indicators

Manufacturing of Biological Indicators

The biological indicators were manufactured by Apex Labs (Sanford, NC) from material samples provided by Bosch. The standard control and the material carriers were inoculated with ≥1.0 x 10^6 spores of *Geobacillus stearothermophilus* ATCC #12980. All carriers were inoculated with the same spore suspension. The indicators were wrapped in 1073B medical grade Tyvek.

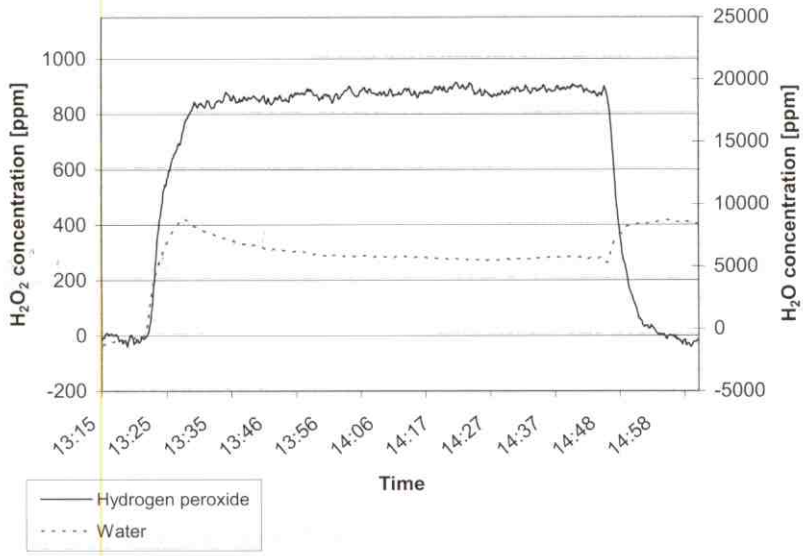


Figure 2

Hydrogen peroxide and water concentration during bio-decontamination cycle.

Carrier Materials

Standard Control Carriers: For reference purpose, commercially available biological indicators manufactured from the same lot of spore suspension with disc-shaped stainless steel carriers sourced from Apex Labs were carried through all sets of investigations. The main function of the standard controls is to reflect the behavior of a highly standardized indicator available in consistent quality. The sporicidal effect of every cycle is monitored by means of the standard control D-values.

Material Carriers: The materials were selected for testing according to their potential presence and relevance in aseptic processing environments. Factors like the size of the exposed surface and probability of potential product contact were taken into account. The majority of the materials were original construction materials from filling machines and isolators. In addition, some aluminum materials with different surface finishes were specially manufactured for the study. The list was completed with additional materials which are associated with handling in aseptic environments or which were being evaluated for potential use. A complete list of the investigated materials and their possible place of installation is presented in Table II. All materials were cut into pieces sized 10 mm x 10 mm with a thickness of 5 mm. The surfaces were then inoculated with the spore suspension and wrapped in Tyvek by Apex Labs.

Handling and Culture Conditions

Exposure of Biological Indicators

The biological indicators were attached to stainless steel bars with the inoculated side on top. To ensure a defined exposure, the bars were kept in boxes. The boxes have been proven impermeable to peroxide during a prestudy. (The prestudy consisted of measurement of hydrogen peroxide concentration inside of the container during a standard bio-decontamination cycle. The sensor used was a PAC III, Dräger for H_2O_2 concentrations from 0 to 20 ppm.) The indicators were not exposed to killing conditions until the beginning of the bio-decontamination phase. During this cycle period, steady-state conditions concerning the hydrogen peroxide concentration are maintained by a low injection rate (see Figures 1 and 2). In a preparatory study, the optimum procedure for an even exposure of all indicators was established. It was determined best to have all indicators lying with the same orientation on a rack somewhat above the transport inside the isolator.

Transfer of Biological Indicators

During the plateau phase, the biological indicators were removed from the isolator at defined time intervals through an integrated validation port. Afterwards they were aseptically transferred into test tubes with autoclaved medium under controlled conditions. The

rocessing of the indicators was done directly after emoval from the isolator.

Culture Conditions

The indicators were cultured in the incubator at 55 °C or 7 days. Test tubes were inspected for growth daily nd the results were recorded. Positive and negative ontrols were used during each incubation procedure.

According to the *United States Pharmacopoeia (USP)* 27 Chapter Soybean Casein Digest Broth was used for culturing and consisted of the following ingredients:

- 17.0 g Pancreatic Digest of Casein
- 3.0 g Papaic Digest of Soybean Meal
- 5.0 g Sodium Chloride
- 2.5 g Dibasic Potassium Phosphate
- 2.5 g Dextrose
- 1000 mL Purified Water

Calculation of D-values

The D-value is defined as the time (in minutes) of exposure at given killing conditions, for example, temperature, which causes a one log or 90% reduction of the population of a specific microorganism (5, 11). In this paper the fraction-negative approach of the Holocomb-Spearman-Karber Procedure (HSKP) was used for the estimation of the D-values (14). The reduced number of samples removed from the sporicidal cycle at each exposure time was shown to be both acceptable for an estimation of the D-value and for determining the inactivation pattern of biological indicators with unknown resistance (15). The results are valid for the particular combination of decontamination system and materials tested during the study.

Additional Studies

Bacteriostasis

Traces of residual hydrogen peroxide absorbed into carriers during decontamination or inhibitory ingredients in the materials themselves may prevent surviving spores from growing. To ensure that no false negative results are included in the D-value data, a bacteriostasis study according to ISO/DIS 11138-1 was conducted for all tested materials and the standard controls (14). Blank, uninoculated material pieces were prepared and packed into Tyvek as they were in the material study. Half of the blank, wrapped carriers were subjected to the standard decontamination cycle used throughout the entire study. Soybean Casein Digest Broth was allocated to test tubes and preheated to the culturing temperature. After 80 min exposure, the carriers were discharged from the isolator and aseptically transferred into the culture medium, two carriers in each tube. Identical wrapped blank carriers which had not been decontaminated were also placed into test tubes. Additionally, positive and negative controls of the growth-promotion medium were processed. The test tubes were put into the incubator for 2 h to elute potential inhibitory substances from the material carriers into the medium. Afterwards, less than 100 suspended spores of *Geobacillus stearothermophilus* ATCC #12980 were added to all test tubes except the negative controls. The samples were cultured at 55 °C for the validated time period and then checked for growth. In addition, a colony count analogous to the determination of recovery rates stated below was conducted for the standard control samples. Defined aliquots of the positive controls and the samples containing the H_2O_2-exposed standard controls were sampled and compared to each other.

Recovery Rates

The recovery rates of all biological indicators were determined according to regulations and following the detailed instructions of the manufacturer (14, 16). The indicators were suspended in sterile fluid and the spores were removed and separated with the help of ultrasonic waves. After diluting the samples they were subjected to a heat shock procedure. Defined aliquots of the spore suspension were plated with liquid agar and incubated for 48 h at 55 °C in an incubator. Colonies were counted and a recovery rate calculated for every batch of biological indicators.

Variance in Resistance of Biological Indicators

Standard control biological indicators of the same lot were divided into three batches and subjected to the identical sporicidal cycle. Groups from every batch were removed from the isolator at the same time after defined periods of exposure. All indicators were

TABLE II
Overview of the Tested Materials

ID No.	Material Name	Material Name Abbreviation	Potential Use of Material
1	Stainless Steel 1.4404 brushed	S.S. 1.4404 brushed	Main construction material
2	Stainless Steel 1.4404 polished	S.S. 1.4404 polished	Construction material interior
3	Stainless Steel 1.4404 e-polished	S.S. 1.4404 e-polished	Construction material interior
4	Stainless Steel 1.4404 passivated	S.S. 1.4404 passivated	Air ducts
5	Stainless Steel 1.4435 brushed	S.S. 1.4435 brushed	Supporting elements
6	Stainless Steel 1.4301 e-polished	S.S. 1.4301 e-polished	Construction profiles
7	Polyoxymethylene	POM	Size parts
8	Aluminum type A, untreated	Alu A, untreated	Not used, manufactured for study
9	Polyethylene ultra high molecular weight	PE-UHMW	Size parts
10	Polyethylenetherephtalate	PETP	Size parts
11	Polyvinylidenefluoride	PVDF	Screw caps
12	Ethylenepropylediene	EPDM	Gaskets/O-rings
13	Silicone caoutchouc	VMQ	Gaskets/O-rings
14	Chlorosulfonepolyethylene (Hypalon)	CSM	Glove material
15	Polyvinylchloride transparent	PVC transparent	Glove material
16	Polyamide 12GF 30	PA 12GF 30	Glove ports
17	Polyetheretherketone black	PEEK black	Sliding devices
18	Polyvinylchloride black	PVC black	Cables
19	Polyetheretherketone nature	PEEK nature	Size parts, sliding devices
20	Polycarbonate	PC	Temporary windows
21	Polyvinylchloride soft	PVC soft	Hoses
22	Polyester mesh	PE mesh	CG air diffuser cloth
23	Fluorocarbon elastomer	FPM	O-rings
24	Polyvinylchloride hard	PVC-U	Cables
25	Aluminum type B, untreated, Rz 25	Alu B, untreated, Rz 25	Not used, manufactured for study
26	Aluminum type B, anodized 20 µm, Rz 25	Alu B, anodized 20 µm, Rz 25	Not used, manufactured for study
27	Aluminum type B, anodized 100 µm, Rz 25	Alu B, anodized 100 µm, Rz 25	Not used, manufactured for study
28	Aluminum type C, untreated, Rz 25	Alu C, untreated, Rz 25	Not used, manufactured for study
29	Aluminum type C, anodized 20 µm, Rz 25	Alu C, anodized 20 µm, Rz 25	Not used, manufactured for study
30	Aluminum type C, anodized 100 µm, Rz 25	Alu C, anodized 100 µm, Rz 25	Not used, manufactured for study
31	Aluminum type C, 150-µm coating, Rz 25	Alu C, 150-µm coating, Rz 25	Not used, manufactured for study
32	Aluminum type C, anodized 20 µm, Rz 10	Alu C, anodized 20 µm, Rz 10	Not used, manufactured for study
33	Spring stainless steel	Spring Steel	Springs
34	Stainless steel hard chrome-plated	S.S. hard chrome-plated	Conveyor belt
35	Light barrier body	Light barrier body	Cover of light barrier electronics
36	Polytetrafluoroethylene (PTFE) coating	PTFE coating	Low friction surface
37	Silicone for sealing	Silicone sealing	Sealing

TABLE II
(continued)

ID No.	Material Name	Material Name Abbreviation	Potential Use of Material
38	Silicone hose Type A	Silicone hose A	Hose of filling system
39	Silicone hose Type B with Platinum catalyst	Silicone hose B (Pt)	Hose of filling system
40	Nitrile butadiene caoutchouc	NBR	Gaskets/O-rings
41	Polyamide hose	PA hose	Pneumatic hoses
42	Polypropylene	PP	Size parts
43	Polyethylene fiber Tyvek	Tyvek	Packaging material
44	Polyurethane hose	PU hose	Pneumatic hoses
45	Polyurethane belt	PU belt	Conveyor belt
46	Ceramic based on zircon oxide	Ceramic zircon oxide	Pump material
47	Ceramic based on aluminum oxide	Ceramic aluminum oxide	Pump material
48	Glass fiber HEPA filter	HEPA	Filter
49	Glass	Glass	Window pane

ID no.: Identification number of each material.

treated equally and D-values were calculated for every batch. The objective of the test was to find out in which order of magnitude the resistance of the indicators shows variation.

Sporicidal Effect at Run Time of Decontamination Cycle

To detect fluctuation in the sporicidal effect of the decontamination cycle over time, three D-values of standard controls were determined in the same run (15). The biological indicators were enclosed in a box non-permeable to hydrogen peroxide until start of the particular exposure. The first group was subjected to the hydrogen peroxide from minute 0 to 20 of the bio-decontamination phase. The second and third groups were exposed during minute 25 to 40 and 50 to 70, respectively. D-values were calculated for every group of biological indicators.

Artifact Study

The objective of the test was to determine if detectable inoculation effects, like the formation of multi-layers, occurred for the 10^6-inoculated samples in comparison to carriers inoculated with fewer spores. Carriers of the chosen materials were inoculated by Apex Labs with 10^4 and 10^6 spores following the same procedure applied for the preparation of the biological indicators used in the main study. For the artifact study, the same lot of spores that had been previously used for the main study was employed. The 10^4 and 10^6 biological indicators were then exposed to the same standard decontamination cycle that had been used in the main study. D-values were determined twice for the 10^4- and the 10^6-inoculated samples of the four materials. Standard controls were also used during the decontamination cycle to monitor the sporicidal effect.

Microscopy

Carriers of every material were analyzed in blank and inoculated status with an Axioskop 2 MAT microscope from Zeiss and photographically documented with a Canon Powershot G5 camera. The materials were investigated with magnification factors of 50x, 200x, and 1000x.

Scanning Electron Microscopy (SEM)

Spreading, layer formation and interaction of material surface with spore inoculation were investigated in detail using a LEO 1530 Gemini scanning electron microscope with an acceleration voltage of 10 kV. Non-conductive materials were coated with an atomic carbon layer in a CRESSINGTON Carbon Coater 208 prior to measurement. The magnification factor varied from 50x to 5000x.

TABLE III
Calculated D-Values for the Tested Materials

ID No.	Material	Estimated D-Values in Min			Resistance of Spores on Material Surface*		
		1st Set	2nd Set	3rd Set			
1	S.S. 1.4404 brushed	1.6	1.6	2.6	low		
2	S.S. 1.4404 polished	c.n.p.	0.9	2.8	low		
3	S.S. 1.4404 e-polished	c.n.p.	2.5	3.1		medium	
4	S.S. 1.4404 passivated	0.6	c.n.p.	1.0	low		
5	S.S. 1.4435 brushed	0.4	0.4	0.6	low		
6	S.S. 1.4301 e-polished	0.6	0.8	0.8	low		
7	POM	1.4	1.5	3.1	low		
8	Alu A, untreated	1.1	c.n.p.	1.3	low		
9	PE-UHMW	2.1	2.9	2.0		medium	
10	PETP	2.1	1.6	2.6		medium	
11	PVDF	1.1	0.7	1.1	low		
12	EPDM	0.6	0.5	1.4	low		
13	VMQ	3.4	c.n.p.	c.n.p.			high
14	CSM	0.9	1.1	1.9	low		
15	PVC transparent	2.4	3.1	2.3		medium	
16	PA 12GF 30	2.1	3.5	2.8		medium	
17	PEEK black	2.9	2.2	2.3		medium	
18	PVC black	0.9	1.1	1.4	low		
19	PEEK nature	1.4	2.3	2.3		medium	
20	PC	0.9	1.4	c.n.p.	low		
21	PVC soft	1.1	0.4	1.1	low		
22	PE mesh	1.4	1.0	1.6	low		
23	FPM	c.n.p.	c.n.p.	c.n.p.			high+
24	PVC-U	3.9	4.1	4.7			high
25	Alu B, untreated, Rz 25	1.1	1.3	1.7	low		
26	Alu B, anodized 20 µm, Rz 25	1.9	1.6	2.0	low		
27	Alu B, anodized 100 µm, Rz 25	2.1	2.8	0.8	low		
28	Alu C, untreated, Rz 25	1.1	2.6	2.0	low		
29	Alu C, anodized 20 µm, Rz 25	3.4	3.8	c.n.p.			high
30	Alu C, anodized 100 µm, Rz 25	3.1	c.n.p.	2.9		medium	
31	Alu C, 150-µm coating, Rz 25	c.n.p.	1.3	2.1	low		
32	Alu C, anodized 20 µm, Rz 10	c.n.p.	c.n.p.	c.n.p.			high+
33	Spring steel	0.4	0.4	0.4	low		
34	S.S. hard chrome-plated	0.4	0.2	0.4	low		
35	Light barrier body	1.4	1.3	1.1	low		
36	Polytetrafluoroethylene (PTFE) coating	0.6	1.6	c.n.p.	low		
37	Silicone sealing	0.4	0.4	0.4	low		
38	Silicone hose A	0.6	0.5	0.4	low		
39	Silicone hose B (Pt)	1.6	1.6	0.8	low		
40	NBR	3.6	5.2	4.4			high
41	PA hose	3.1	4.1	3.8			high
42	PP	2.1	2.0	2.6		medium	
43	Tyvek	c.n.p.	3.8	3.4			high

TABLE III
(continued)

ID No.	Material	Estimated D-Values in Min			Resistance of Spores on Material Surface*		
		1st Set	2nd Set	3rd Set			
44	PU hose	c.n.p.	7.4	7.1			high
45	PU belt	4.6	4.4	3.4			high
46	Ceramic zircon oxide	c.n.p.	0.7	0.8	low		
47	Ceramic aluminum oxide	0.4	0.4	0.5	low		
48	HEPA	0.4	1.6	0.5	low		
49	Glass	0.4	c.n.p.	2.3	low		

* Spore resistance: low for average D-value < 2 min; medium for average D-value 2 to 3 min; high for average D-value > 3 min; high + for materials where no complete kill was achieved in set 1, 2, and 3 after 80 min of exposure.
ID no.: Identification number of each material.
c.n.p. = calculation of D-value not possible.

Contact Angle

During the inoculation procedure, spores suspended in 40% ethanol solution were pipetted onto the surface of the materials by the manufacturer of the indicators. To examine the interaction of the fluid with the particular surface, a measurement of the contact angle of a 40% ethanol dilution was conducted with the Krüss DSA10 Drop Shape Analyzer. Prior to the measurement, the carriers were prepared in the same way as the inoculated carriers before application of the spores. An accurate volume of the test liquid was dosed onto the sample at a defined rate to determine the contact angle. A video system positioned horizontally to the sample recorded the drop profile. The contour of the sessile drop was automatically identified and the digitized data was characterized by a mathematical equation. In this way, a three-phase contact angle between material surface, surrounding air, and the test liquid was determined. The contact angle was averaged over 10 measurements taken for each material.

Roughness

Roughness of the materials was measured optically with a NanoFocus® μSurf® confocal scanning microscope from NanoFocus AG. The samples are scanned in the z-direction with light from an external light source. Reflected light from defocused planes is suppressed, and only the light reflected from the focused height level is detected by the CCD chip. Afterwards the surface roughness parameters Ra and Rz were calculated from the recorded data. Ra is the arithmetical mean deviation of the roughness profile; Rz is the average height of roughness profile: peak-to-valley height (12).

Results

Spore Resistance

D-values for Materials: For the biological indicators manufactured from the different materials, a D-value determination was conducted three times: set 1, set 2, and set 3. The results obtained are summarized in Table III. According to their average D-value, the materials were classified into three groups with surfaces where the spores showed low, medium, and high resistance against the gaseous hydrogen peroxide.

For most materials, at least one D-value could be calculated. Only for fluorocarbon elastomer (FPM) (ID 23) and Aluminum type C, anodized 20 μm (ID 32) with a roughness of Rz 10 no complete inactivation of the spores could be achieved even after 80 min of exposure to the sporicidal agent. For silicone caoutchouc (VMQ) (ID 13), only one D-value of 3.4 min was obtained. Polyurethane (PU) hose and belt (ID 44 and 45), polyvinylchloride hard (PVC-U) (ID 24), and nitrile butadiene caoutchouc (NBR) (ID 40) were identified as "worst-case materials" with average D-values exceeding 4 min.

Among the materials tested, stainless steel hard-chrome-plated (ID 34) showed the lowest estimated D-value. Furthermore, silicone sealing (ID 37), spring

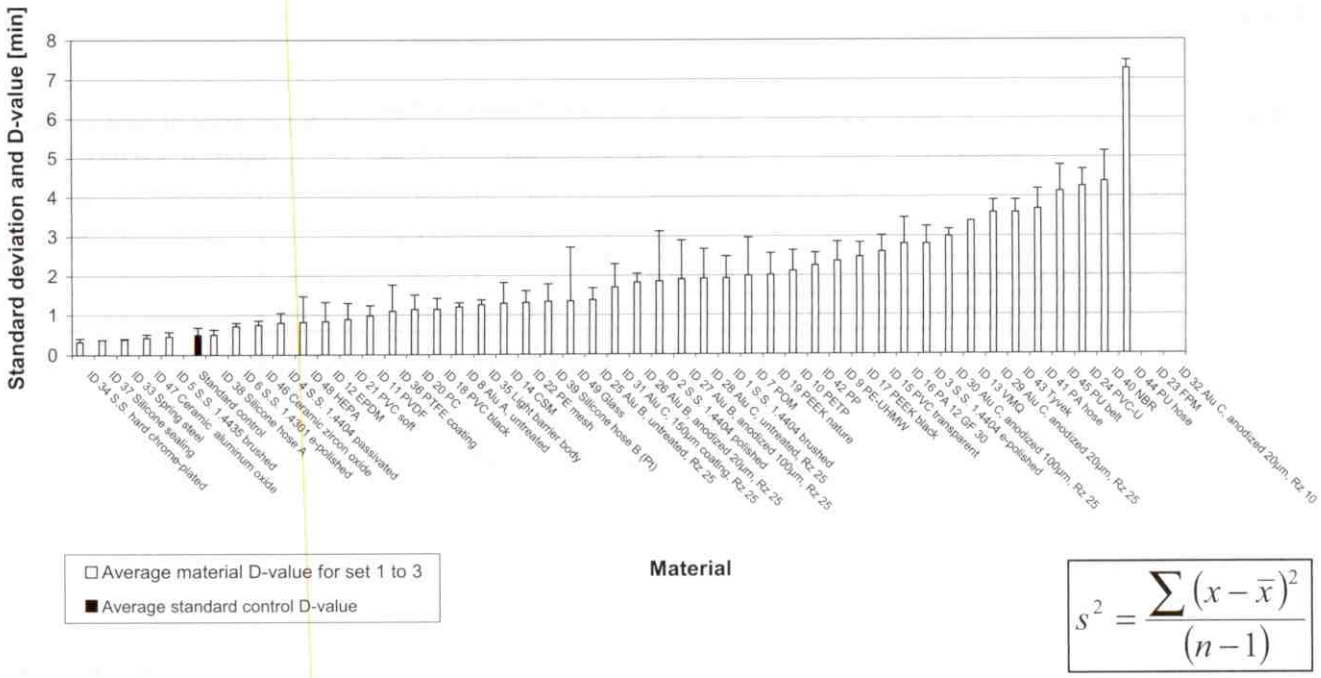


Figure 3

Average D-value and standard deviation for materials and controls.

steel (ID 33), ceramic aluminum oxide (ID 47), and stainless steel brushed (ID 5) showed very good results, with average D-values below 0.5 min. No remarkable trend for differences in the suitability of plastics and metal-based materials could be observed. In both material categories, both lower and higher D-values appeared.

Polyoxymethylene (POM) was found to have one of the highest D-values out of a range of 23 tested materials in the previously published literature (9). In this study, the tested POM quality (ID 7) could be decontaminated within a reasonable time and presented good model behavior. The estimated D-values are 1.4 min, 1.5 min, and 3.1 min. The variation in between the single D-values was 49% and herewith higher than for most of the other materials, but tolerable compared to 37% variation in the standard control D-values (see Figure 3). Other materials with a high variation concerning the estimated D-values are PVC soft (ID 21), aluminum type B, anodized 100 µm (ID 27), ethylenepropylenediene (EPDM) (ID 12), polytetrafluoroethylene (PTFE) coating (ID 36), stainless steel polished (ID 2), HEPA filter (ID 48), and glass (ID 49). The remaining materials show reproducible and consistent D-value estimations across the three cycles.

In literature it is reported that different aluminum samples showed, when compared to other materials, noticeable high D-values with inadequate model behavior (9). To learn more about the reasons for this behavior, several aluminum grades were tested for their inactivation characteristics in this study. The results revealed that some types of aluminum can be decontaminated within reasonable time. Only two of the tested aluminum grades, the Alu C anodized 20µm with roughness Rz 10 (ID 32) and the Alu C anodized 20 µm with a roughness Rz 25 (ID 29), showed excessively high D-values and bad model behavior. The other types of aluminum tested showed average D-values below 3 min. This suggests that the manufacturing process and surface treatment of the aluminum have a high impact on the aluminum's ability to be decontaminated with vaporized hydrogen peroxide.

D-values for Standard Controls: To monitor the sporicidal effect during the whole study, standardized control biological indicators were included in every decontamination cycle and removed from the isolator together with the material biological indicators. The calculated D-values for the standard controls are diagrammed in Figure 4. Together with

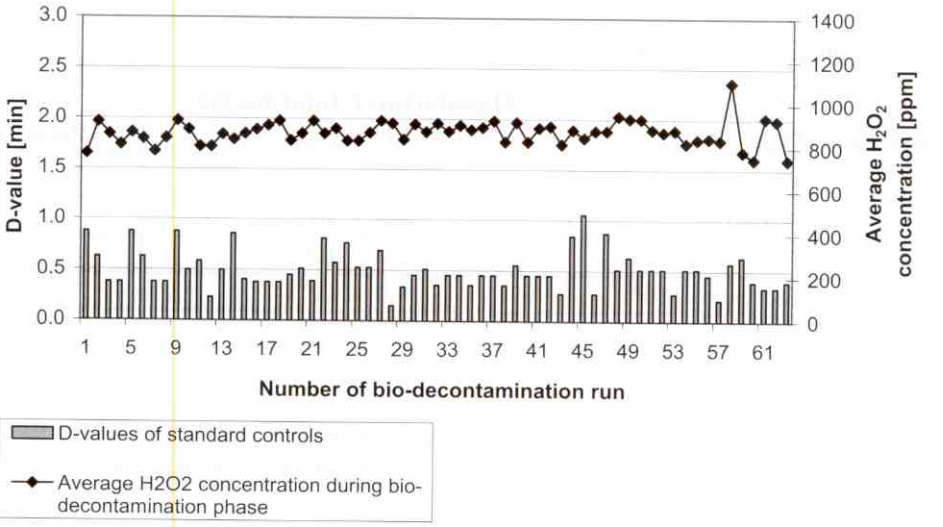


Figure 4

Average hydrogen peroxide concentrations during bio-decontamination phase and D-values of standard controls.

the D-values, the average NIR-measured H_2O_2 concentrations for the bio-decontamination phase of each sporicidal cycle are plotted. Table IV summarizes statistical parameters for the standard control D-values. In the test for normality the null hypothesis "the standard control D-values have a normal distribution" could not be rejected for a significance level of $\alpha = 10\%$ (17). In the following, the D-values of the standard controls and subsequently the material D-values are inferred to be normal distributed.

As shown in Figure 4, the concentration of hydrogen peroxide in the gas phase during exposure was reproducible within the accuracy of the measurement technique and averaged 870 ppm. With a correlation of the standard control D-values and the hydrogen peroxide concentration near zero, no assignment of the data could be found. Even for the highest concentration value (run number 58), no notably low D-value could was observed.

An additional experiment was conducted based on parallel, triple D-value determinations for standard control biological indicators from the same lot in the same cycle. The resulting estimates for the D-values of 0.39 min, 0.57 min, and 0.63 min confirmed that the indicators themselves produce a basic variation of around 24%.

TABLE IV
Statistical Returns for the Standard Control D-Values

Mean	0.50 min
Standard deviation	0.19 min
Variation	37%
Range	0.90 min
Minimum	0.15 min
Maximum	1.05 min
Count	63

Decontamination Cycle

A D-value study with standard controls removed from the isolator at three consecutive time intervals was performed to ensure the stability of the sporicidal effect during the cycle. The estimated D-values were 0.33 min for the 0–20-min sampling window, 0.51 min for 25–45-min sampling window, and 0.51 min for the 50–70-min sampling window. These results are in the range of the standard control D-values derived from the cycles 1 to 63 (see Figure 4). Besides that, the mean D-values for the three consecutive tests (0.53 min) matches very well the mean D-value of the 63 standard control D-values (0.50 min; see Table IV). Consequently, no doubt arises about the stability of the conditions for bacterial inactivation throughout the cycle.

TABLE V
Recovery Rates for Biological Indicators

Indicator Type	Manufacturer Information (CFU/carrier)	Recovery Rate (%)
Material indicator (main study)	2.5×10^6	58.4
Material indicator (main study)	2.5×10^6	83.6
Material indicator (artifact study, 10^6)	2.7×10^6	62.1
Material indicator (artifact study, 10^4)	4.0×10^4	89.7
Standard control indicator	2.6×10^6	84.2

Additional Studies

Recovery Rates

The inoculation level of all material type biological indicators as specified by the manufacturer was verified in parallel to the D-value investigations. As shown in Table V, the recovery rates obtained ranged from 58.4 to 89.7%. According to ISO/DIS 11138-1, a recovery of 50 to 300% of the manufacturer's inoculation specification is acceptable for bacterial count (14). Hence the rates found for the indicators used in the study all meet the required criteria.

Bacteriostasis

The only material which showed an inhibitory effect by the sample itself was EPDM (ID 12). For NBR (ID 40), polyamide (PA) hose (ID 41), and PU belt (ID 45), an inhibition was observed only for the decontaminated samples. All other materials showed no retardation of the outgrowth of the test organisms.

Artifact Study

Effects of the inoculation count on the estimated D-values were investigated in the artifact study. Four materials that showed unexpectedly high D-values during the main study were chosen: stainless steel 1.4404 e-polished, polyamide 12GF 30 (PA 12), FPM, and PVC-U (ID 3, 16, 23, and 24). This study was conducted two times with the same selected materials. As shown in Figure 5, a very good reproducibility was found between the first and second experiment. The results for the 10^4- and 10^6-inoculated carriers of every material were compared to each other. Only for PVC-U on the 5% level of significance differences were found between the samples (18). For PVC-U the difference between the D-values calculated for 10^4-

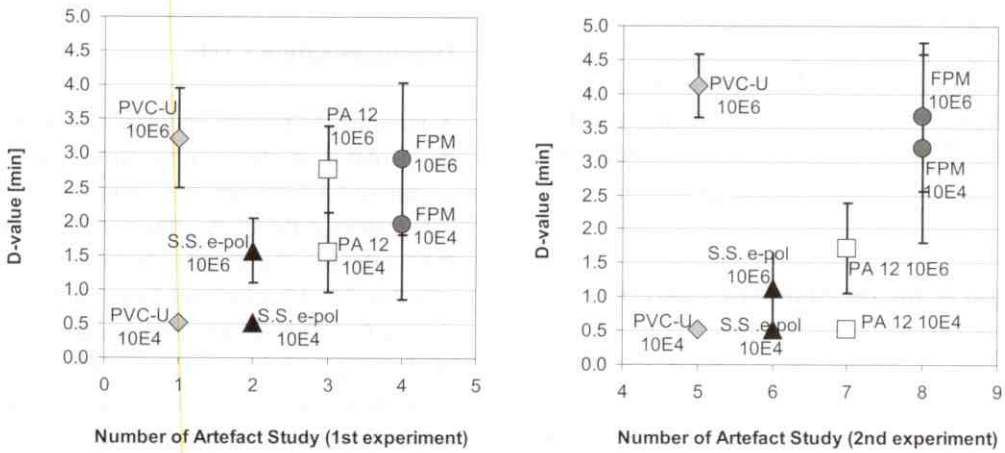
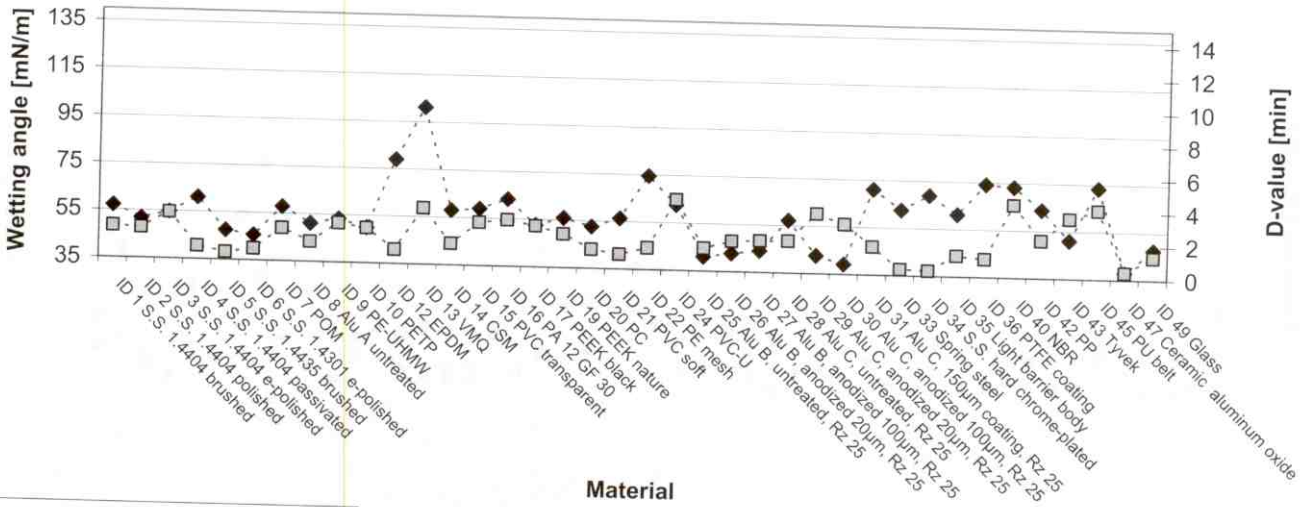


Figure 5

Results of first and second experiment of the artifact study: D-values for 10^4- and 10^6-inoculated materials. For some data no error bars could be calculated for 10^4-inoculated materials due to rapid kill and no sufficient fractional window.



$$\rho_{x,y} = \frac{Cov(X,Y)}{(\sigma_x \cdot \sigma_y)}$$

Figure 6

Wetting angle and average D-value of materials.

and 10^6-inoculated carriers is clearly higher than for the remaining samples, which show partial overlap of the 95% confidence interval of the D-values.

The test revealed that in some cases the inoculation could influence the D-value. The D-values for the carriers inoculated with 10^4 spores are all found to be at least 1 min lower than the carriers inoculated with 10^6 spores. Particularly for PVC-U, the inoculation seems to have an impact on the D-value. For this material, the estimated D-values for 10^4- and 10^6-inoculated carrier differ by more than 3 min. For the other tested materials, the difference in the D-value of the 10^4- and 10^6-inoculated material ranges only from 0.5 to 1.3 min. The 10^4 D-value for FPM is higher than the other 10^4-inoculated materials.

Contact Angle

Although in Figure 6 it looks like for some materials a correlation exists between the average D-value and the wettability, this is not confirmed by the correlation coefficient $\rho^1 = 0.18$ for the whole sample size. The contact angle of the test liquid on the different materials varies from 38 mN/m for aluminum oxide ceramic (ID 47) to 101.2 mN/m for VMQ (ID 13). The plastic-based materials tend to be more wettable with the test liquid than the metal based materials. For some materials, a determination of the contact angle was hardly possible mainly due to the geometry of the samples, which made reading difficult.

$$\rho^1 x,y = \frac{\mathrm{Cov}(X, Y)}{(\sigma_x \cdot \sigma_y)}$$

Roughness

Roughness parameters Ra and Rz were measured for the different materials. Ra is plotted together with the average D-value for every material in Figure 7. Again the correlation coefficient of the whole sample $\rho = 0.07$ shows no direct connection between the two parameters. Nevertheless in the xy-plot of the data in Figure 8 a dependency of the D-value on the roughness could be assumed for some of the materials.

Discussion

Analysis of the Study Results

Variation in D-values and Decontamination Cycle

The D-values of the standard biological indicators for all cycles ranged between 1.05 min maximum and 0.15 min minimum with an average of 0.5 min (see Table IV). The control system shows a basic variation of around 37%, which has to be considered when evaluating the material D-values.

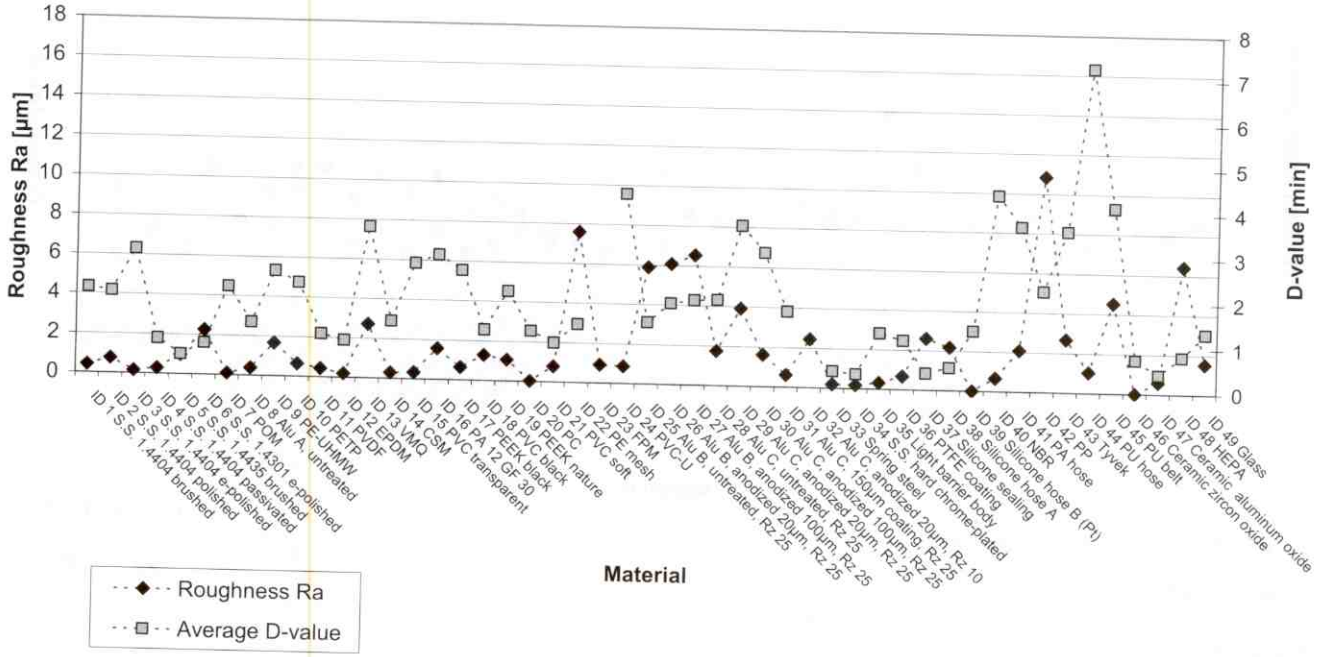


Figure 7

Roughness and average D-value of materials.

Few decontamination cycles deviated slightly from the optimum course demonstrated in Figure 4. For these runs, the concentration of hydrogen peroxide at the end of the conditioning phase was found to vary by approximately 75 ppm according to NIR reading. These cycles were examined separately with respect to the derived material and standard control D-values. No measurable effect on the D-values was observed, and it was shown that slight concentration deviations in the decontamination cycle have no detectable effect on the D-values. A subtraction of the standard, control D-values from the corresponding material D-values derived from the same cycle did not provide any additional information. The ratio between the different

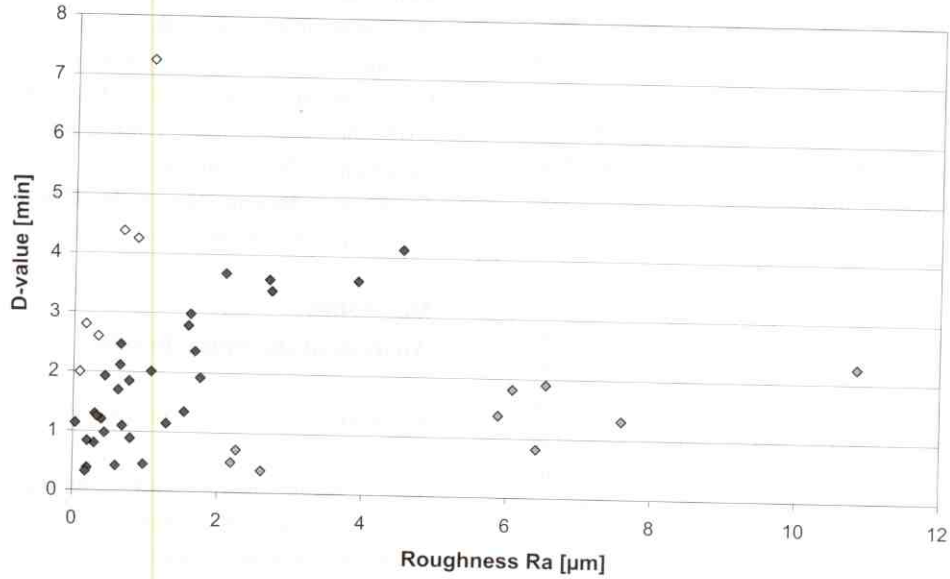


Figure 8

XY-plot of roughness and D-value.

material D-values remained consistent. It can be assumed that variations in the decontamination cycle during the study had no relevant influence.

This was also shown in the experiment where three D-values were determined during the same decontamination run with identical sampling windows. Although any cycle effect was eliminated, still the range in the controls reached from 0.39 to 0.63 min D-value. This variation of 24% could be assumed to result mainly from the natural inhomogenities in the biological test system. For the less standardized material carriers, an additional element of uncertainty might be presumed for the inoculation procedure. This and the D-value estimation with minimized Limited Spearman Karber Method suggest to some extent a qualitative approach to the results.

Best- and Worst-case Materials

The presented data demonstrates that the tested materials show a highly variable behavior towards the inactivation of spores on their surface with gaseous hydrogen peroxide. The findings are valid for the tested combination of materials with the applied decontamination system but a similar behavior can be assumed for other configurations with comparable cycle parameters (9). The experiments have resulted in the identification of a number of worst-case materials having an estimated average D-value exceeding 3 min. FPM (ID 23), Alu C, anodized 20 µm, with Rz 10 (ID 32), and VMQ (ID 13) are not recommended as construction materials in aseptic processing areas, or extra measuring during cycle development has to be taken into regard. Also, PVC-U (ID 24), NBR (ID 40), PA hose (ID 41), PU hose and belt (ID 44, 45) as well as Alu C, anodized 20 µm, with Rz 25 (ID 29) are classified as critical. These materials need a careful review and risk analysis before application in isolation systems sterilized with gaseous hydrogen peroxide. The PU conveyor belt (ID 45) may cause particular problems due to its large surface area and its potential vicinity to the product. The results obtained for NBR, PA hose, and PU belt (ID 40, 41, and 45) have to be treated with special care because these materials showed inhibitory effects during the bacteriostasis study. It can be assumed that the D-values might be underestimated due to growth retardation by traces of hydrogen peroxide absorbed into the samples. Therefore the time needed to decontaminate the named materials without occurrence of growth inhibition could be even higher than the estimated D-values. However, most of the materials tested show good to satisfactory results and were found suitable for the use in aseptic processing systems. Among the materials with the lowest D-values are stainless steel materials with different surface finishes, ceramic materials used for pumps, and some silicone-based materials.

Material Groups

Aluminum

Most of the results regarding the suitability of different materials for decontamination with gaseous hydrogen peroxide are in line with the literature. However, some results deviate from previously published data. Aluminum, which showed critical qualities before, was examined in detail. The study shows that the manufacturing process and the surface finish applied have an impact on the decontamination success for aluminum-based materials. Figure 9 illustrates that anodized aluminum shows higher D-values than untreated aluminum. The data also indicates increasing D-values for higher anodic layer thickness. This correlation may be related to the growing porosity and the increasing depth of cavities on the material surface during the anodizing process. Also, the high variation in the D-values for aluminum anodized 100 µm (ID 27) can be explained with increasing irregularities that arise during the treatment. As shown in the SEM picture (Figure 10) of the inoculated surface of aluminum anodized 100 µm, Rz 25 (ID 30), spores and comparable contaminants can hide inside the pores, and their exposure to the sporicidal agent is reduced.

A polytetrafluoroethylene (PTFE) coating provided similar results to the untreated sample. Surprisingly, the Alu C, anodized 20 µm, with Rz 10 µm (ID 32) showed by far the worst outcome of all aluminum-based materials. Complete inactivation was not achieved for this material in any of the cycles. Microscopy reveals a highly porous and irregular surface of the 20-µm anodized surface, which has more irregularities than the Rz 25 subjected to the same anodizing process (ID 29). Obviously, the Rz 10 sample produces a different surface topography during the treatment, which leads to a different inactivation behavior. The contact angles for the two materials were equal in the range of measurement accuracy. This finding reveals the importance of the decontamination studies.

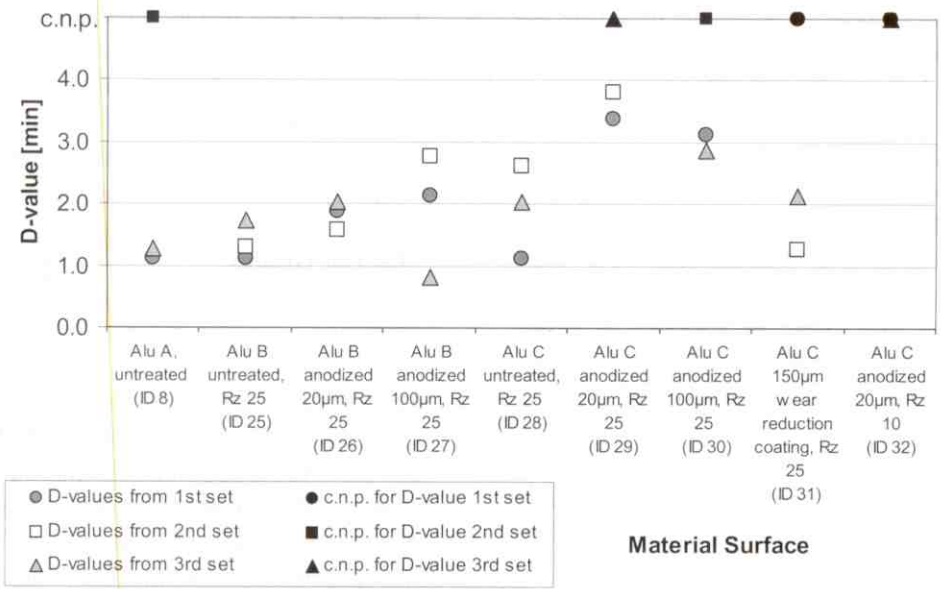


Figure 9

D-values of different aluminum qualities. c.n.p. = calculation of D-value not possible.

The inactivation kinetic of each material is the result of a multitude of effects interfering with one other, which makes it difficult to predict the behavior of any material. A comparison of the D-values estimated for Aluminum B and C reveals an impact of the aluminum grade on the achieved inactivation of spores: The Aluminum B samples were manufactured using a forged alloy technique whereas the Aluminum C sam-

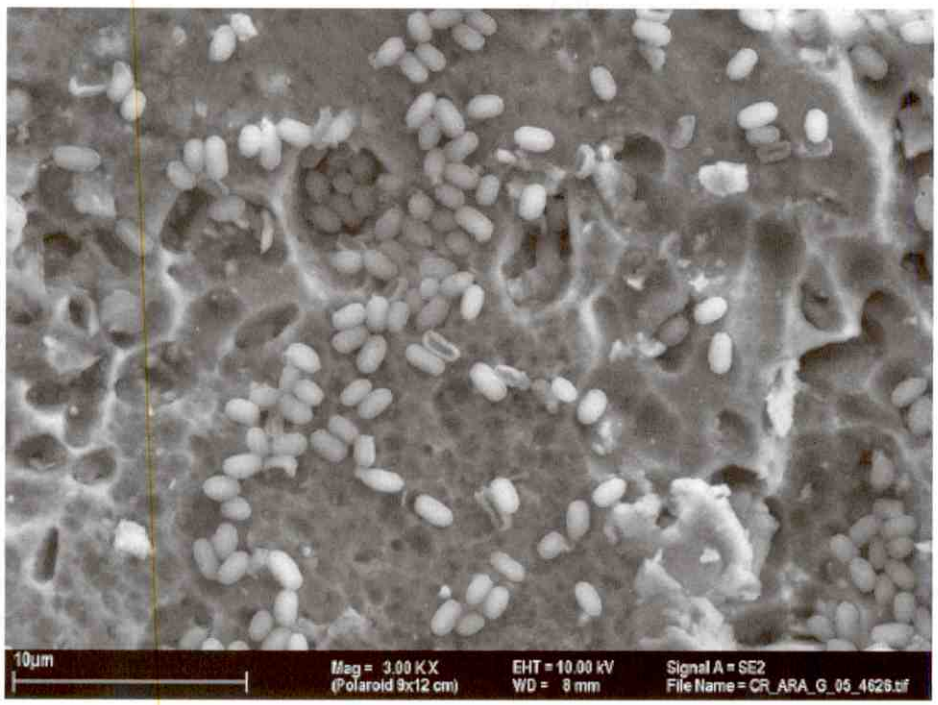


Figure 10

SEM picture of 10^6-inoculated surface of aluminum anodized 100 µm with Rz 25 (ID 30) with magnification 3000x.

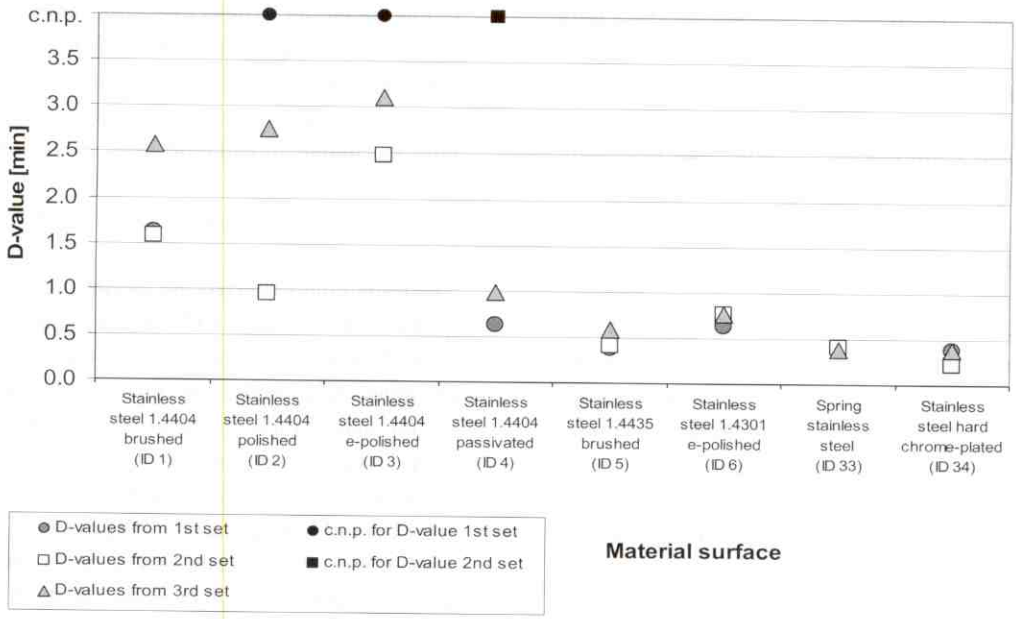


Figure 11

D-values of different stainless steel qualities. c.n.p. = calculation of D-value not possible.

ples were cast-alloy fabricated. Every detail in the manufacturing process and surface treatment of the materials seems to have an impact on decontamination success. This creates the need for individual case studies regarding the behavior of every single construction material surface.

Stainless Steel

In Figure 11 the D-values for the stainless steel materials tested in the study are summarized. All stainless steel qualities were found suitable for decontamination with vaporized hydrogen peroxide, although clear variance between the different grades was apparent. This is an important finding because stainless steel grades are used as main construction material in the inside and outside of the aseptic processing system and represent the highest surface area. The three D-values that could not be calculated were caused by undersized sampling windows. The stainless steel grade 1.4404 was found to have higher D-values than the grades 1.4435 and 1.4301. During the standard microscopic analysis of the materials, it was noted that the electropolished stainless steel samples (ID 3) partially showed deep scratches on the surface where single spores were concealed from full peroxide exposure. Consequently, the comparatively high D-value of the electropolished material can be explained as being an artifact generated during sample preparation.

The fact that the scratches were detected in the study is an indicator for the sensitivity of the D-value test method. The electropolished stainless steel 1.4404 (ID 3) was further studied in the artifact study. Here special care was taken not to scratch the surface of the carriers. As expected in this experiment, the resulting D-values for 10^6 inoculation were clearly lower. With 1.1 and 1.6 min at the 10% level of significance, a difference to the values from the main study (see Figure 5) can be determined (18). The result for 10^4 in the artifact study was 0.5 min in both cycles and differed comparably little from the higher inoculated samples. It can be concluded that no significant inoculation effects occurred for this material.

Silicone

The strong dependency of the inactivation kinetic on the material processing is again demonstrated for silicone qualities. The tested silicone sealing (ID 37) showed non-critical D-values, with an average of 0.37 min. However, silicone sealing has other disadvantages regarding bio-decontamination with gaseous hydrogen peroxide. It is known to be permeable for hydrogen peroxide. Silicone can absorb high quantities of peroxide. Later on, the peroxide gases out and can prolong the aeration phase. Additionally, silicone exhibits distinctive deterioration after exposure to hy-

drogen peroxide. Yet no data is available dealing with inactivation kinetic of aged silicone. The silicone tubes (ID 38 and 39) also showed good results regarding the inactivation time of the inoculated spores. The D-values ranged from 0.4 to 1.6 min. The platinum-cured hose showed a slightly higher D-value. An explanation for this could be increased peroxide decomposition caused by the platinum.

In contrast to these results, the VMQ (ID 13) was found to have a distinctly higher D-value of 3.4 min (see Table III). For this material, several factors seem to have negative influence on the inactivation kinetic. The material shows the highest contact angle of all tested surfaces (see Figure 6). This has an impact on the spreading of the spores during drying process of the inoculum and encourages the formation of multilayers. Besides that, VMQ has a sponge-like texture with a rough and porous surface. Although VMQ showed acceptable model behaviour during the first set, in the two other decontamination runs no complete kill was achieved despite long exposure times (2nd set: 55 min; 3rd set: 60 min). This indicates that the variability of the material has to be taken into consideration when developing a decontamination cycle for systems where VMQ is included.

PVC

Although no correlation between contact angle and average D-value was found for the whole sample size, for the different PVC qualities such a relationship can not be ruled out (see Figure 6 and Table III). The correlation coefficient between average D-value and contact angle of the three PVC materials (ID 15, 21, 24) takes a value of 0.996. Due to the shape of the samples, no determination of the roughness was possible with the applied method. For PVC-U, extremely long decontamination periods were needed to reach complete kill. The D-values varied from 3.9 up to 4.7 min. During the artifact study, an inoculation effect exceeding the one for other tested materials was observed. The SEM picture revealed that the spores dried partially clustered, with increased likelihood for multilayer formation on the 10^6 -inoculated PVC-U parts. 10^4-inoculated samples of PVC-U showed no noticeable irregularities, neither in the inactivation behavior nor in the spore distribution on the SEM pictures. Due to the large space between the single spores at 10^4 inoculation, the hydrogen peroxide can probably interact well with each biological entity. The inactivation takes place very quickly, so that differences between the materials are harder to detect because of the limited removal intervals of samples from the isolator. This finding justifies the conclusion that certain differences in the inactivation characteristics of materials can only be detected with the higher inoculation level of 10^6 spores.

Other Thermoplastic and Elastomer Materials

The remaining materials are thermoplastic and elastomer qualities. Elastomeric EPDM (ID 12) and chlorosulfonepolyethylene (Hypalon) (CSM) (ID 14) revealed low D-values. Nevertheless, no final conclusion can be drawn for the inactivation characteristics of EPDM. The calculated D-values were all very low but might be underestimated due to EPDM's inhibitory material properties, which could have prevented outgrowth of the test organisms. CSM (ID 14) came out to be the better choice for glove material from a D-value point of view than the alternative PVC transparent (ID 15), which showed clearly higher D-values. For the elastomeric material FPM (ID 23), no D-value could be calculated during the main study. In the course of the artifact study, two D-values with 2.9 and 3.7 min were estimated for the 10^6-inoculated FPM. Similarly, NBR (ID 40) was shown to have extremely high D-values with an average of 4.3 min. A closer inspection of the two hard-to-decontaminate materials showed they both had a microscopically grooved surface that seemed to inhibit the inactivation process. Furthermore, the examination of FPM in the artifact study showed that no distinctive difference exists between the 10^4- and 10^6 inoculated carriers.

For only some of the the thermoplastic materials, a distinct coherence between roughness, contact angle, and D-value is demonstrated, as illustrated in Figure 12. The higher the measured roughness was, the higher the contact angle and D-value of the particular material. However, the data are not conclusive and the interrelationship between these variables is not obvious for all materials tested. Further work and extended sampling is required to deepen the understanding of the coherence. For plastic materials it would be particularly interesting to gain further knowledge concerning the inactivation kinetic of different roughness grades of the same material. No clear relationship between contact angle and spreading of the spores on the carriers was observed in light optical and scanning electron microscopical investigations. Due to limitations in time and number of samples, a simplified method was used for the contact angle measurements in this study. Instead of using surface energy determina-

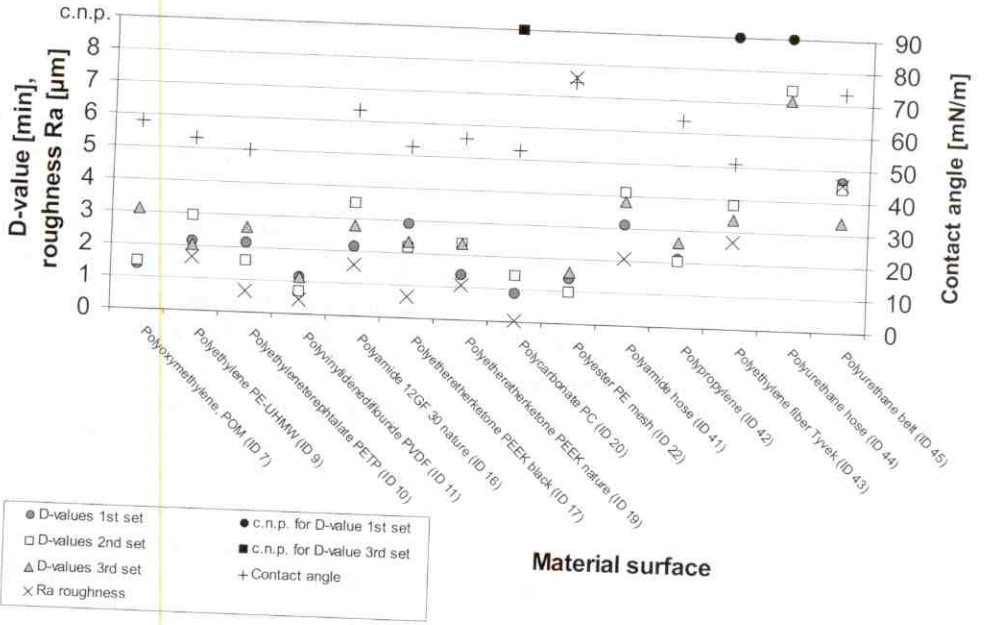


Figure 12

D-values of thermoplastic materials. c.n.p. = calculation of D-value not possible.

tion, which demands testing of the materials with at least four liquids with different polarity, only wettability with 40% ethanol solution was chosen for the measurements. This procedure reflects the inoculation conditions of the material biological indicators used in the study. In future investigations it might be valuable to further study the influence of variable compositions of the inoculation suspensions on the resulting D-value.

The types of PU belt and hose (ID 44 and 45) that were tested in this study were found to have very high D-values exceeding 4 min. PU belt also had the highest roughness and contact angle among the thermoplastic materials. Due to the sample geometry, neither roughness nor contact angle could be determined for PU hose. PA hose and Tyvek (ID 41, 43) also rank among thermoplastics, which require a long exposure for complete inactivation of the spores. For Tyvek, the absorption of the spore suspension and the fibrous structure makes the decontamination difficult. PA hose and PA 12GF (ID 41, 16), as well as polyetheretherketone (PEEK) black and PEEK nature (ID 17 and 19), produce very similar results concerning D-value and roughness.

Particularly interesting is the result for polyoxymethylene (POM) (ID 7), which provided the highest D-values among a range of tested materials in a previous publication (9). In the study presented here, POM showed good results. The D-values determined with the applied decontamination system ranged between 1.4 and 3.1 min with a comparably high variance between the estimated values. Nevertheless, spores could be inactivated within a reasonable time on POM surfaces during all the three decontamination cycles. Further investigations including SEM, microscopy, roughness, and contact angle analysis did not show any irregularities, which would indicate that the previous behavior has to be questioned. The remaining thermoplastic materials tested during the study, polyethylene ultra high molecular weight (PE-UHMW) (ID 9), polyethyleneterephthalate (PETP) (ID 10), polyvinylidenedifluoride (PVDF) (ID 11), polycarbonate (PC) (ID 20), polyester mesh (PE mesh) (ID 22), and polypropylene (PP) (ID 42), all provided good results with D-values between 0.7 min minimum for PVDF (ID 11) and 2.9 min maximum for PE-UHMW (ID 9). PC (ID 20) exhibited a rather low roughness; all other parameters for the materials did not show any distinctive features.

Ceramic Materials

Both tested ceramic qualities (ID 46 and 47) showed remarkably low and well reproducible D-values (see

Table III). The compact texture with low surface porosity has a positive impact on the inactivation. Under the tested conditions these materials can also be classified as suitable for construction of components in aseptic processing areas. The same conclusion applies for the glass-based materials (ID 48, 49). Although the high contact angle of the inoculation fluid on the HEPA filter resulted in a compact spore layer on the glass fibers, the filter material showed short inactivation periods. The variation in the three calculated D-values probably results from the deviation between the samples caused by the fibrous texture. The good result for glass (ID 49) is important due to the large surface area of the window panes in isolation systems. Coated materials (ID 31, 34, 35, 36) tend to have low roughness combined with high contact angle. This seems to have a positive impact on the inactivation rate. Robust and durable coatings with PTFE or chrome plating can be an option to enhance the decontamination properties of certain materials.

Summary

In this paper, different materials of construction for aseptic processing areas were examined for their suitability to be decontaminated with gaseous hydrogen peroxide. D-values were determined repeatedly for every material to investigate individual inactivation patterns. Throughout the study, this approach was shown to be sensitive to detect differences between the materials. The objective of the study was to identify worst-case and best-case materials concerning the inactivation properties and to learn more about the origin of the different behaviors. The stainless steel- and ceramic-based materials tested were all found suitable for decontamination with gaseous hydrogen peroxide. The results for aluminum depended strongly on the surface treatment of the particular sample. For the applied sporicidal cycle, some types of aluminum showed acceptable D-values while others were classified as not recommendable for the use in aseptic processing systems.

High variations in the D-values and a correlation of the inactivation times with roughness and wettability were observed for plastic-based materials. All in all, smooth materials with a low roughness tended to show good results, and a well applied coating can promote inactivation quality. Furthermore, the porosity of the material surface seemed to have an impact on the efficiency of the kill. Spores can be hidden in cavities of porous exteriors, and this decreases exposure to the sporicidal agent (9).

In conclusion, it can be stated that the performance of materials concerning the inactivation pattern of spores can not be accurately predicted without inactivation study results. Different effects complexly overlay each other in varying intensities for each material. Inoculation phenomena, material conditions like roughness, wettability, and absorption, and catalytic characteristics towards the gaseous hydrogen peroxide can play a role for decontamination success. For this reason it is suggested to include a simplified version of the material investigation applied in this study into the construction process of new isolation and filling systems. When conducting material analysis it is particularly important to test original material parts that are identical to materials with their entire surface characteristics to be built in later. This point is important because the data derived from the study indicate that variations in grade, manufacturing, and treatment process of the parts could affect the inactivation kinetic. The tested materials have to be certified, and availability at a constant quality level has to be guaranteed.

Common stainless steel carriers, which are available standardized and in high quality, are representative for the largest material surface area inside hard wall isolators. They are a valuable tool and should be used for cycle development and validation purposes. Aside from other fundamental criteria, such as chemical resistance to hydrogen peroxide, the inactivation pattern of materials should become a basic rationale for the decision regarding which materials are incorporated into a machine. The authors have the opinion that worst-case material surfaces as determined from this type of study should be tested during the validation runs in order to prove the full inactivation capability of the applied bio-decontamination process. If necessary, the parameters of the cycle can be adapted to the most challenging construction material. Wherever possible, materials that are tested and found to be critical should be replaced by substitutes with proper inactivation kinetics.

References

1. Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S). PI 014-2: 2004 "Isolators used for Aseptic Processing and Sterility Testing." Geneva, 2004.

2. International Organization for Standardization/ Draft International Standard (ISO/DIS) 13408: Draft: Aseptic processing of health care products—Part 6: Isolator systems. Geneva, 2004.

3. General Information, Chapter 1035 Biological Indicators for Sterilization. *United States Pharmacopoeia (USP) 27,* United States Pharmacopoeial; 2003; pp 2413–2416.

4. General Information, Chapter 1211 Sterilization and Sterility Assurance of Compendial Articles *USP 27,* United States Pharmacopoeial; 2003; pp 2616–2621.

5. Chapter 5.1.2. Biological Indicators for Inspection of Sterilization Methods. *European Pharmacopoeia,* 3rd ed., Deutscher Apotheker Verlag Stuttgart; Stuttgart, 1997; pp 381-382.

6. General Information, Chapter 1208 Sterility Testing—Validation of Isolator Systems. *USP 27,* United States Pharmacopoeial; 2003; pp 2613–2616.

7. Lysfjord, J. "2006 Barrier Isolator History and Trends." Oral Presentation and Conference Handbook, International Society for Pharmaceutical Engineering, Barrier Isolation Technology Forum, Vienna, Austria, September 18–22, 2006.

8. Gail, L.; Sirch, E. Chapter 8. Development of Isolator Technology. *Reinraumtechnik,* 2nd ed.; H.-P. Hortig: Berlin, Germany, 2004; p 174.

9. Sigwarth, V.; Stärk, A. Effect of carrier materials on the resistance of spores of *Bacillus stearothermophilus* to gaseous hydrogen peroxide. *PDA J. Pharm. Sci. Technol.* **2003,** *57* (1), 3–11.

10. Dalmasso, J. "What to Consider when Making or Buying Biological Indicators." Oral Presentation and Conference Handbook, International Society for Pharmaceutical Engineering, Barrier Isolation Technology Forum, Prague, Czechoslovakia, September 19–23, 2005.

11. FDA Guidance for Industry: "Sterile Drug Products Produced by Aseptic Processing–Current Good Manufacturing Practice." 2004.

12. International Standard, Deutsches Institut für Normung Europäische Norm International Organization for Standardization (DIN EN ISO) 4287: "Geometrical Product Specifications (GSP); Surface texture: Profile method; Terms, definitions and surface texture parameters." Deutsches Institut für Normung; Berlin, 1997.

13. Microbiological Tests, Chapter Sterility Tests. *USP 27,* United States Pharmacopoeial; 2003; pp 2157–2162.

14. International Organization for Standardization/ Draft International Standard ISO/DIS 11138-1: Draft: Sterilization of health care products—Biological indicator systems—Part 1: General requirements. 2004.

15. Sigwarth, V.; Moirandat, C. Development and quantification of H_2O_2 decontamination cycles. *PDA J. Pharm. Sci. Technol.* **2000,** ***54*** (4), 286–304.

16. Microbiological Tests, Chapter 55 Biological Indicators—Resistance Performance Tests. *USP 27,* pp 2150–2152.

17. Pearson, E. S.; Stephens, M. A. The ratio of range to standard deviation in the same normal sample. *Biometrika* **1964,** *51,* 484–487.

18. Sachs, L.; Hedderich, J. Chapter 7. Hypothesis Test. *Applied Statistics,* 12th ed.; Springer: Berlin, 2006; pp 367–369, 377–381.