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TNO report

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Disinfection using hydrogen peroxide

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Summary

On the instructions of Infection Control B.V., TNO has conducted a study of the efficiency and reliability of the disinfection effects of hydrogen peroxide vaporised with the IC-4™ ultra-mist system.
Initial tests in the TNO laboratories established a relationship between the material to be treated (construction and interior) and the (local) concentration of hydrogen peroxide. On the basis of this relationship, the disinfection capability of hydrogen peroxide mist on materials inoculated with the vegetative cells *MRSA* and *Staphylococcus aureus* and the spores *Bacillus subtilis* and *Clostridium difficile* (*027*) was determined.

Based on the study, it can be concluded that there is a strong relationship between the construction and interior materials to be disinfected (concrete, wood, aluminium, steel, glass, ceiling boards, wallpaper, floor coverings, etc.) and the efficiency of the disinfection process. Highly (water) adsorbent materials in particular, such as plaster, untreated wood, ceiling boards and carpet, resulted in a much lower concentration (< 3 ppm) of airborne hydrogen peroxide measured close to the surface of the material, which contrasted with vapour-tight materials such as linoleum, steel, laminated wood and glass. These materials, often used in hospitals, retained the concentration of hydrogen peroxide in the ambient air at the desired value (25-50 ppm). The concentration of hydrogen peroxide measured close to the surface of the material was also relatively high (>10 ppm).

The study was conducted in TNO laboratories and validated in Tergooi Hospitals in Blaricum, where a log reduction of 6 was realised for the vegetative organisms *Staphylococcus aureus* and *MRSA* and for the spores *Bacillus subtilis* and *Clostridium difficile.* The vegetative organisms were effectively eliminated within 90 minutes and the spores within 150 minutes on stainless steel, wallpaper, linoleum and laminate. Mineral wool ceiling boards were not disinfected.

It can be concluded that hydrogen peroxide mist from the IC-4™ (5% H_2O_2, 0.01 % Ag+) is an effective, reliable and quick method to use as a surface disinfectant for the above materials, which are frequently used in hospitals.

On completion of the work, the room can be most quickly ventilated by opening the windows, and re-entered after approximately 30 minutes.

Exposure to 50 ppm of hydrogen peroxide for 1-2 minutes is not likely to produce any harmful effects in humans.

It is, of course, recommended to take normal precautionary measures such as wearing safety goggles while in the room. It is also a good idea to limit inhalation of air containing hydrogen peroxide as much as possible. A second person must ensure that the first person actually vacates the room concerned shortly after entry.

Document structure

This report is divided into six chapters.

Chapter 1 begins with a brief introduction, after which chapter 2 addresses the objectives of the study. Chapter 3 describes the equipment used and the analyses performed.

Chapter 4 describes the experimental set-up of the study in individual paragraphs, with the results being described, where possible, in comparable paragraphs in chapter 5. This means that certain paragraphs in chapter 4 refer to results in chapter 5.

Chapter 6 contains the conclusions.

Contents

1 Introduction

According to previous studies, hydrogen peroxide (5%), marketed under trade name Sanosil S010Ag, has an eliminating effect with a log reduction of at least 5. To achieve this log reduction, the bacteria, viruses and spores must be in contact with diluted hydrogen peroxide liquid. Infection Control B.V. has test results that demonstrate this. Infection Control B.V. wishes to study whether vaporisation of hydrogen peroxide using the IC-4 ^{TM} delivery system has a similarly effective eradicating effect on bacteria, viruses and spores.

On the instruction of Infection Control B.V., TNO studied the eliminating effect of hydrogen peroxide mist on selected bacteria and spores. The prerequisites for effective and reproducible use were also studied.
The study focused on the effectiveness of elimination in relation to the concentrations of hydrogen peroxide in the ambient air and close to the surfaces of the materials to be disinfected, as well as on the dependency of elimination and the nature of the materials.

2 Objective

The objective of the study was to determine the effectiveness of hydrogen peroxide as a disinfectant when vaporised with the IC-4^{TM} delivery system.

The study comprised various phases. During the initial study phases, test facilities (pilot room) at TNO were used to determine the relationship between materials, hydrogen peroxide concentration and elimination of micro-organisms.
In the final phase of the study, the operation of the IC-4^{TM} was validated in a practical situation (Tergooi Hospitals).

3 Equipment and analyses

3.1 Basic principle of the IC-4™

The IC-4™ is a delivery system that vaporises hydrogen peroxide using ultrasonic vibrations. The hydrogen peroxide is a stabilised aqueous solution with a concentration of 5%, in which silver crystals have been dissolved (5% H_2O_2 / 0.01% Ag+). This solution is marketed under the trade name Sanosil S010 Ag and its specifications are described in the Material Safety Data Sheet in accordance with 91/155 EC's most recent version dated 28-05-2007. The intended use of the delivery system is the disinfection of material surfaces.
The operation of the IC-4™ delivery system is as follows.
The delivery system used to disinfect a room uses a 5% hydrogen peroxide solution vaporised in fine liquid particles with an average diameter of 2 µm. The delivery system is linked to measurement of the relative humidity (RH) in a room. Delivery is active until the pre-set offset of + 25-40% RH is reached. This situation is then stabilised for a period of 90 to 150 minutes. The room in question is air sealed, which means that there is no mechanical air supply or discharge during the disinfection process.

3.2 Measuring the hydrogen peroxide concentration

Two measuring principles were used to determine the hydrogen peroxide concentration. Firstly, the concentration was measured instantly. During the study, it became clear there was a need for on-line measurement of the hydrogen peroxide concentration as well.

3.2.1 Instant determination using a reagent

This method works as follows. A liquid containing titanium oxysulfate ($TiOSO_4$) is introduced into an impinger. The air in the pilot room is directed through the liquid using active suction. The colourless $TiOSO_4$ liquid reacts with hydrogen peroxide, turning into a yellow solution.
Colorimetric analysis based on the degree of discoloration (yellowing) establishes the average concentration of the gaseous hydrogen peroxide during the active sampling period.
On the instructions and under the supervision of TNO, these measurements and analyses were performed by RPS Analyse B.V. in accordance with OSHA IMIS 1470.

3.2.2 Continuous hydrogen peroxide measurement

During the second part of the project, the Dräger Polytron 700 measurement system with DrägerSensor® H_2O_2 LC was used. This is an electrochemical diffusion sensor for the continuous monitoring of the hydrogen peroxide concentration in the ambient air.

3.3 Determining relative humidity and temperature

The relative humidity was determined using capacitative sensors, the temperature using thermistors. The data was stored on external Squirrels and read out later.

3.4 Determining the disinfective effect on micro-organisms

Various protocols can be used to determine the disinfective effect of hydrogen peroxide. Modified protocols, e.g. customised to practical materials, can be developed and applied during the study.

Protocol 1
Various spore strips were applied aseptically to a previously sterilised/autoclaved (121°C for 30 minutes) inert surface (e.g. stainless steel 316L with a surface roughness of 0.5 µm Ra). These spore strips contained *Bacillus subtilis* spores in a concentration of 10^2, 10^4 and 10^6 CFU spores per strip, respectively. In the TNO pilot room, the stainless steel plates (with strips) were exposed to 5% H_2O_2 in the air for 90 minutes. After exposure, the spore strips were removed aseptically and placed in sterilised 50 ml bottles/plastic bags containing a specific growth medium. For the Bacillus subtilis, Staphylococcus aureus and the MRSA, this was BPW (Buffered Peptone Water); for Clostridium difficile this was SAB (Sabaroud Dextrose Broth)

Photograph 1 below shows the test materials in the growth medium as used in the practical situation in Tergooi Hospitals.

Photograph 1. *Test materials in growth medium.*

These plastic bags were placed in an incubator at 30°C and incubated for two periods of 24 hours. After incubation, the bottles/medium were checked for possible growth of the test organism. This way, the log reduction of the test organism under the conditions specified was determined.

Protocol 2 Quantitative test method / agar plates
A certain concentration of test organism was applied to sterile agar plates (9 cm plates and 14 cm plates) using the spatula method. The test organisms were:
- Bacillus subtilis spores
- ATCC S. aureus 29213
- Clostridium difficile 027
- MRSA
- Acinetobacter
- VRE

Optionally:
i - ATCC *S. aureus* 25923
ii - Norovirus
iii - Asperillus fumigatus

Test organism concentrations were: 0, 10, 10^2, 10^3, 10^4, 10^5 and 10^6 CFU test organisms per agar plate.

After exposure, the test plates and the control plates were placed in the incubator at 30°C and incubated for two periods of 24 hours.
After incubation, the plates were analysed by counting the (remaining) culturable test organisms to get an idea of the test organism's log reduction under the conditions specified.

Protocol 2 can also be conducted semi-quantitatively on stainless steel plates.

4 Experimental

The study was conducted at laboratory scale (TNO pilot room) and validated in the field (Tergooi Hospitals, Blaricum).

4.1 Laboratory analysis of the operation of the IC-4^{TM}

To gain a good understanding of the relationship between materials, hydrogen peroxide concentration and elimination of micro-organisms, the tests in the first study phase were carried out in a pilot room at TNO.

4.1.1 Description of the pilot room

Figure 1 below is a schematic representation of the pilot room, including measurement positions. The dimensions of the pilot room were 4.83 x 3.41 x 2.48 m. Three of the four walls were made of wood (primer only), one was made of glass, the floor was covered with carpet and the ceiling with ceiling boards (plaster, mineral wool).
See Appendix D for impressions of the pilot room.

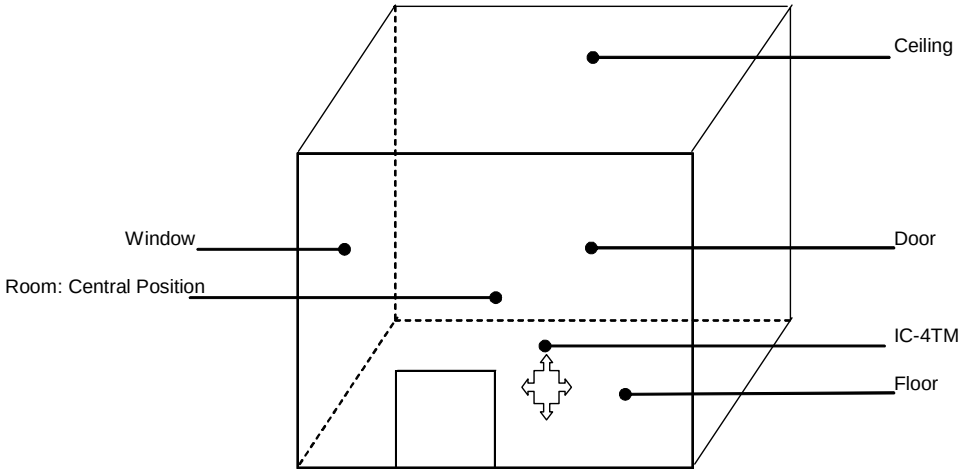


Figure 1. *Schematic representation of the pilot room, including measurement positions.*

4.1.2 Effects of ventilation on the distribution of relative humidity and concentration of hydrogen peroxide in the pilot room

In order to achieve, where possible, a better distribution and increased concentration of hydrogen peroxide, the effect of ventilation in the pilot room on the relative humidity and hydrogen peroxide concentration in the pilot room was studied. The settings of the IC-4^{TM} delivery system and the prevailing conditions during these tests are included in Table 1.

Table 1. *Prevailing conditions and settings of the IC-4$^{TM.}$*

Conditions and settings	**Value**
Temperature at start of test (^{o}C)	45-50
Relative humidity at start of test (%)	18-20
ΔRH IC-4^{TM} set (%)	30
Treatment time set (hour)	1.5

4.1.3 Disinfective effect of the IC-4^{TM} on spores inoculated on strips

Various spore strips were applied aseptically to a previously disinfected/ sterilised/autoclaved inert surface.
These spore strips contained:

- Bacillus subtilis spores in concentrations of 10^1, 10^2, 10^3, 10^4, 10^5 and 10^6 CFU spores, respectively, per strip
- Geobacillus stearothermophilus spores in concentrations of 10^1, 10^2, 10^3, 10^4, 10^5 and 10^6 CFU spores, respectively, per strip

In the TNO pilot room, the test strips were exposed to 5% H_2O_2 solution (5% H_2O_2 / 0.01%Ag+) vaporised in the air for 90 minutes.
After exposure, the spore strips were removed aseptically and put into 50 ml bottles containing a previously sterilised growth medium.
These bottles were placed in an incubator at 30°C and incubated for two periods of 24 hours. After incubation, the bottles/medium were checked for possible growth of the test organism.
This way, the log reduction of the test organism under the conditions specified was determined.

Table 2 below presents the settings of the IC-4^{TM} delivery system and the prevailing conditions during these tests.

Table 2. *Prevailing conditions and settings of the IC-4^{TM}.*

Conditions and settings	**Value**
Temperature at start of test (^oC)	21 (19)
Relative humidity at start of test (%)	25 (35)
ΔRH IC-4^{TM} set (%)	40
Treatment time set (hour)	1.5

4.1.4 Effects of waterproof and vapour-tight packaging and construction materials on relative humidity, the basic concentration of hydrogen peroxide and the local concentration of hydrogen peroxide

Given the results in *5.1.3* and following consultation with the commissioning party, it was decided to study the relation between materials and (local) concentration of H_2O_2 in more detail. To properly chart the effects of highly absorbent materials compared to water-repellent materials on the H_2O_2 concentration, the tests were repeated in the pilot room, which was completely sealed off with transparent, vapour-tight agricultural tarpaulin. In this phase of the project, it was important to measure the H_2O_2 concentration continuously, which was why an H_2O_2 detector was purchased (see 3.2.2.)

The surface concentration of various construction materials was determined, with the sensor being placed 1 mm and 8 mm, respectively, above the materials:

- ceiling board (plaster)
- carpet
- wood (plywood)
- metal (aluminium)
- standard wallpaper

The surface concentrations were determined in both the sealed and the unsealed pilot room. 0.1-0.3 m^2 of construction material was placed in the sealed pilot room.

The settings of the IC-4^{TM} delivery system and the prevailing conditions during these tests are presented in Table 3 below.

Table 3. *Prevailing conditions and settings of the IC-4^{TM}.*

Conditions and settings	Value
Temperature at start of test (^{o}C)	20-21
Relative humidity at start of test (%)	25-50
ΔRH IC-4^{TM} set (%)	40
Treatment time set (hour)	1.5

4.2 Practical validation of IC-4^{TM}: Tergooi Hospitals

The laboratory study showed that elimination depends strongly on the types of materials used in a room. It is important to find out what construction materials are used in hospitals. To that end, an overall inventory of the construction materials/furniture in two hospitals in the Netherlands (Groene Hart Hospital in Gouda and Tergooi Hospitals in Blaricum) was made.

The concentration in a practical room (Tergooi Hospitals) can be determined and, based on the knowledge obtained from the inventory, the practical values measured are compared with the values measured in the pilot room at TNO (sealed and unsealed).

4.2.1 Inventory of construction materials in hospitals

During the study (see 5.1.4), it appeared that highly absorbent materials have a detrimental effect on the hydrogen peroxide concentration and, therefore, on the eliminating effect of hydrogen peroxide. As a result, it was desirable to inventory what construction materials are generally used in hospital rooms before carrying out the practical tests. If these construction materials are water-repellent, it is likely that the hydrogen peroxide mist from the IC-4^{TM} has an eliminating effect on bacteria and spores.
Tables 4a and 4b below contain overviews of the construction materials used in Tergooi Hospitals in Blaricum and Groene Hart Hospital in Gouda.

Table 4a. *Inventory of materials in Tergooi Hospitals (room A16 K04), 6 beds, 6 TVs.*

Materials	Description
Door/window frames	Painted (blue) aluminium
Walls	Wallpaper
Ceiling	Ceiling boards, glass wool/ painted white
Floor	Linoleum
Cupboards	Veneer/laminate/wood
Beds	Plastic/metal
Bathroom	Tiles
Headboards	Softboard

See Appendix D for impressions of the materials present in the test rooms of Tergooi Hospitals in Blaricum.

Table 4b. *Inventory of materials in Groene Hart Hospital in Gouda.*

Materials	**Description**
Door/window frames	Steel/aluminium
Walls	Concrete (painted), wallpaper
Ceiling	Concrete, modular ceiling (mineral wool), woodwool cement
Floor	Linoleum, vinyl
Doors	Wood
Cupboards	Veneer, laminate
Beds	Steel
Bathroom	Tiles
Miscellaneous	Plastic, glass

Photograph 2 shows the different materials, such as linoleum, ceiling boards, laminated/veneered cupboards, concrete, woodwool cement boards, etc.

Photograph 2. *Construction materials and interior of Groene Hart Hospital, Gouda.*

4.2.2 Description of the practical pilot rooms in Tergooi Hospitals in Blaricum

The practical study was validated in Tergooi Hospitals in Blaricum, where two hospital rooms served as test locations. For room A16 K04, the distribution and level of the hydrogen peroxide concentration was determined (see 4.2.3.). In room A16 K11, the disinfective effect of IC-4^{TM} on bacteria and spores applied to practical materials was determined. The dimensions of room A16 K04 are approx. 5.5 x 6 x 2.8 m, those of room A16 K11 approx. 3.5 x 5 x 2.8 m.
See also 4.2.1 for a description of the construction materials used.
See Appendix D for impressions of the practical test rooms in Tergooi Hospitals in Blaricum.

4.2.3 Distribution and level of the concentration of hydrogen peroxide and relative humidity in hospital room A16 K04

For room A16 K04, the distribution and level of the basic concentration of hydrogen peroxide with use of the IC-4^{TM} was determined. Wall effects and the effects of internal ventilation were also studied.
The settings of the IC-4^{TM} delivery system and the prevailing conditions during these tests are shown in Table 5 below.

Table 5. *Prevailing conditions and settings of the IC-4^{TM}.*

Conditions and settings	**Value**
Temperature at start of test (^{o}C)	23
Relative humidity at start of test (%)	43
ΔRH IC-4^{TM} set (%)	40
Treatment time set (hour)	2

4.2.4 Disinfective effect of IC-4^{TM} on bacteria and spores applied to construction materials in hospital room A16 K11

The practical study showed that construction materials have significant impact on the local concentration of hydrogen peroxide and, therefore, the degree of elimination of bacteria and spores.
In the light of this information, follow-up practical studies were performed by applying spores and vegetative cells onto practical samples. These practical samples consist of small pieces (5x5 cm) of construction material generally found in hospitals (linoleum, laminated wood, ceiling board, wallpaper). A reference material was also added to the materials matrix (stainless steel 316L; 0.4 µm Ra).

Two spores, *Bacillus subtilis*, *Clostridium difficile* (*027*), are applied to the practical samples. In addition, samples are prepared on which the vegetative cells *Staphylococcus aureus* and *MRSA* are applied.
All samples are packaged aseptically and irradiated using gamma radiation. Following this treatment, the selected micro-organisms are applied to the practical samples. Next, the samples are subjected to three H_2O_2 exposure times, after which their viability is determined.
To obtain a certain concentration of test organism on a small, sterile surface (5x5 cm), the test organism is applied aseptically onto the surface as a liquid particle. This minuscule droplet is then reduced through evaporation (under aseptical conditions; laminar flow cabinet) and applied unlayered to the material sample. Photograph 3 below shows the practical samples (5x5 cm) of linoleum, laminated wood, ceiling board and wallpaper as placed in the room at Tergooi Hospitals.

Photograph 3. *Overview of placement of test samples in the hospital room.*

The concentrations of test organisms were 10^2, 10^4, 10^6 CFU (spores or bacteria) per test surface, respectively.
For each material and each test organism, two tests are carried out.
The load /exposure (H_2O_2; 5 / 0.01% Ag+) is carried out at different exposure times.

Test cycle 1: 30 / 60 and 120 minutes
Test cycle 2: 30 / 60 / 90 minutes

Table 6. *Prevailing conditions and settings of the IC-4™ day 1 (26 June 2007).*

Conditions and settings	Value
Temperature at start of test (°C)	24
Relative humidity at start of test (%)	51
ΔRH IC-4™ set (%)	40
Treatment time set (hour)	2

Table 7. *Prevailing conditions and settings of the IC-4™ day 2 (27 June).*

Conditions and settings	Value
Temperature at start of test (°C)	25
Relative humidity at start of test (%)	38
ΔRH IC-4™ set (%)	40
Treatment time set (hour)	2

5 Results and Discussion

5.1 Laboratory analysis of the operation of the IC-4™

5.1.1 Effects of ventilation on the distribution of relative humidity

Figures 2a,b,c and 3a,b,c below present the results of the development of relative humidity in the pilot room at TNO with and without the use of internal ventilation. The delivery system was filled with the stabilised hydrogen peroxide solution.

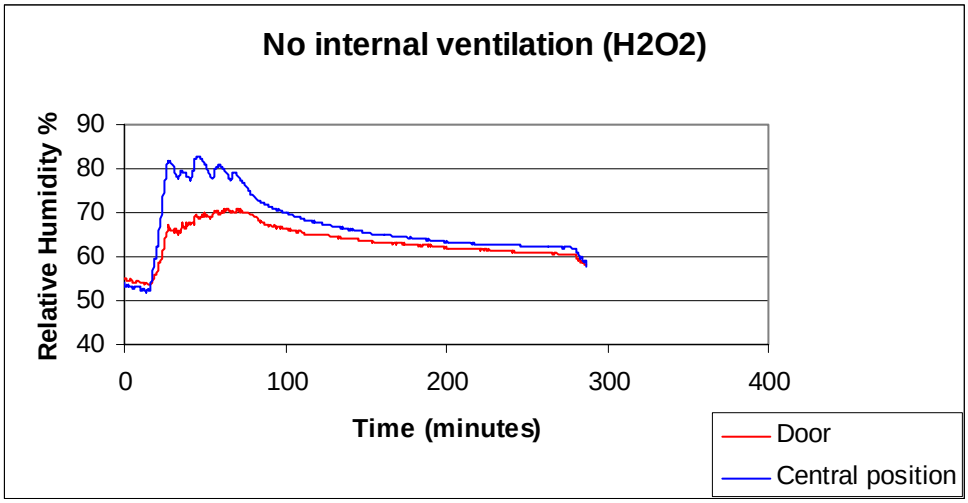


Figure 2a. *Development of relative humidity at measurement positions at the door and the central position, no internal ventilation, delivery system filled with H_2O_2.*

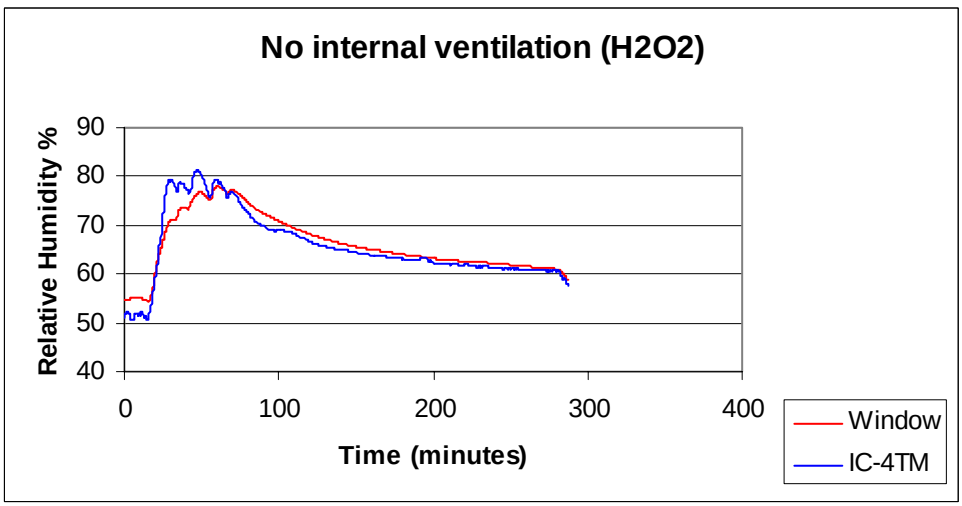


Figure 2b. *Development of relative humidity at measurement positions at the window and the IC-4™, no internal ventilation, delivery system filled with H_2O_2.*

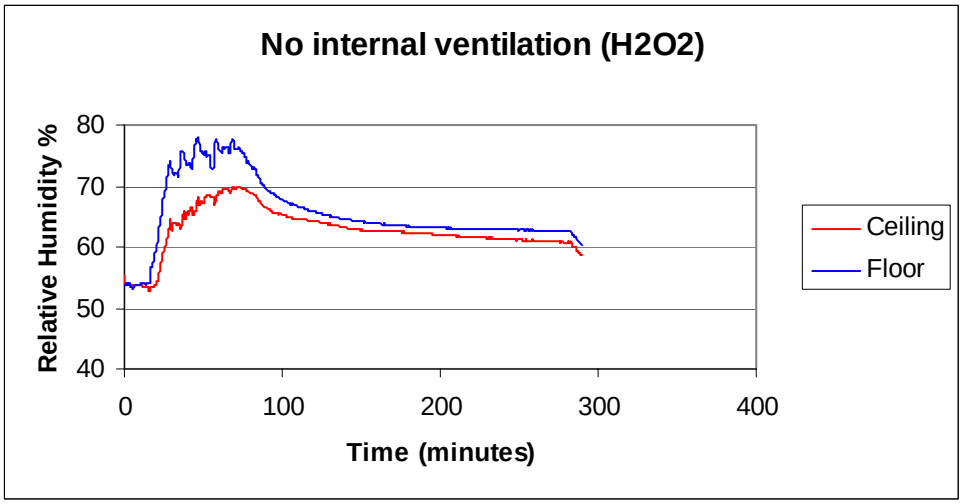


Figure 2c. *Development of relative humidity at measurement positions on the ceiling and the floor, no internal ventilation, delivery system filled with H_2O_2.*

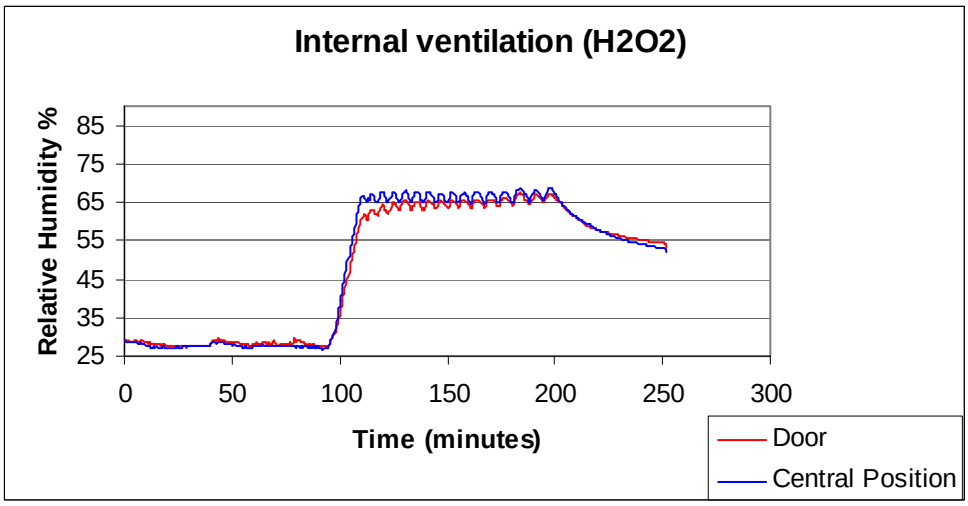


Figure 3a. *Development of relative humidity at measurement positions at the door and the central position, with internal ventilation, delivery system filled with H_2O_2.*

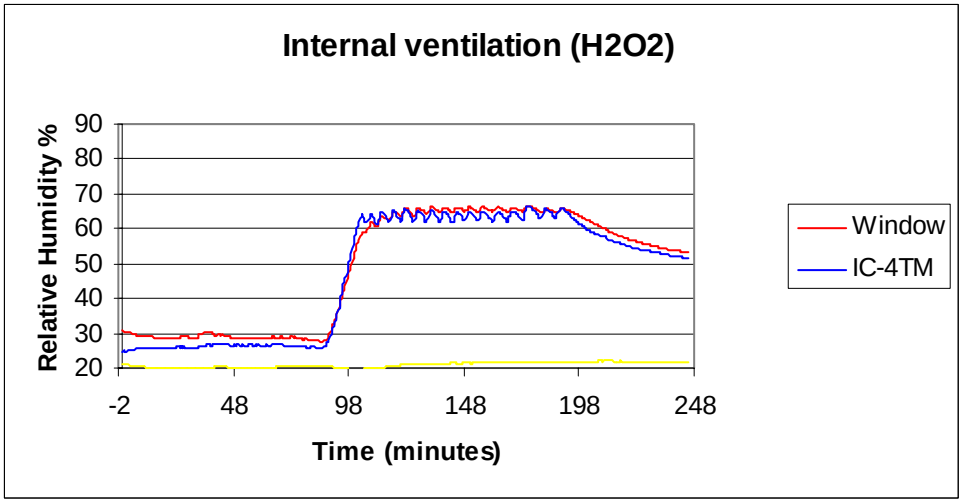


Figure 3b. *Development of relative humidity at measurement positions at the window and the IC-4^{TM}, with internal ventilation, delivery system filled with H_2O_2.*

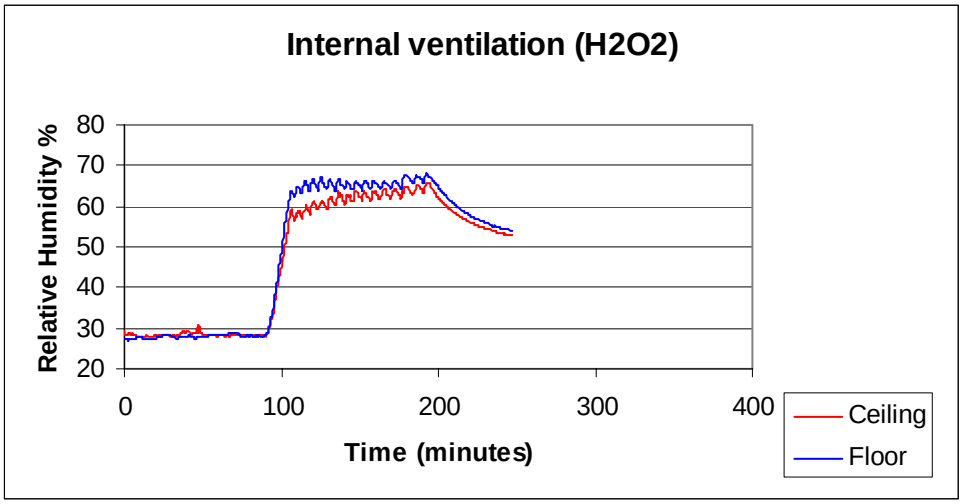


Figure 3c. *Development of relative humidity at measurement positions on the ceiling and the floor, with internal ventilation, delivery system filled with H_2O_2.*

The above figures show that ventilation has a positive effect on the distribution of relative humidity in the pilot room.

Similar trends in the development of relative humidity are observed if the IC-4^{TM} is filled with tap water instead of hydrogen peroxide. See Appendix A for a graphic representation of the development of relative humidity in the pilot room, when the IC-4^{TM} is filled with tap water.

5.1.2 *Instant determination of H_2O_2 concentration and effect of waterproof and vapour-tight packaging*

The figures below show the results of the concentration of hydrogen peroxide for the sealed and the unsealed pilot room.

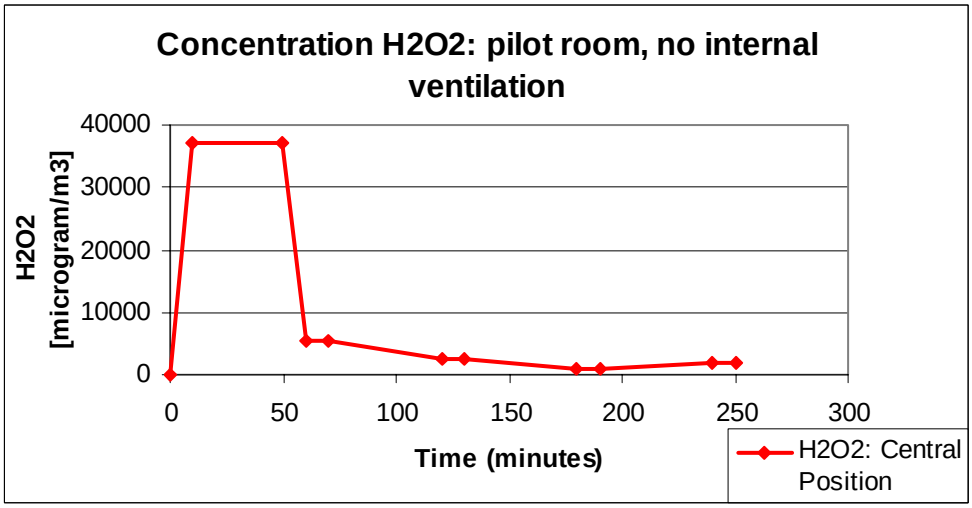


Figure 4. *Average concentration of H_2O_2 (OSHA IMIS 1470) per time interval in the unsealed pilot room, no internal ventilation.*

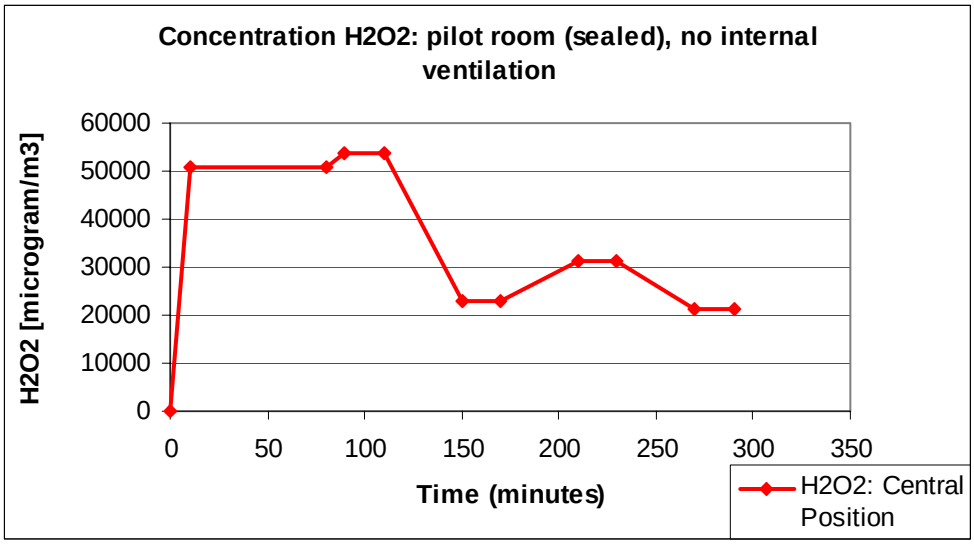


Figure 5. *Average concentration of H_2O_2 (OSHA IMIS 1470) per time interval in the sealed pilot room, no internal ventilation.*

Figures 4 and 5 above show that a vapour-tight packaging results in both a much higher concentration and longer exposure to hydrogen peroxide in the pilot room. The decade development is much more rapid in the unsealed situation than in the sealed pilot room.

Figures 6 and 7 below present the results for the relative humidity and the concentration of hydrogen peroxide of both the sealed and the unsealed pilot room.

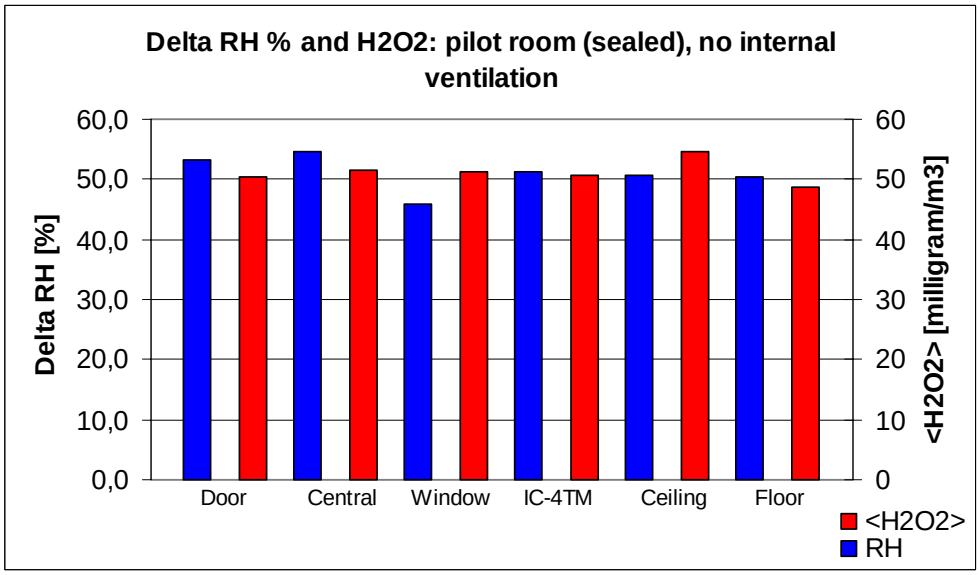


Figure 6. *Average concentration of H_2O_2 (OSHA IMIS 1470) and ΔRH per measurement position per time interval in the sealed pilot room, no internal ventilation.*

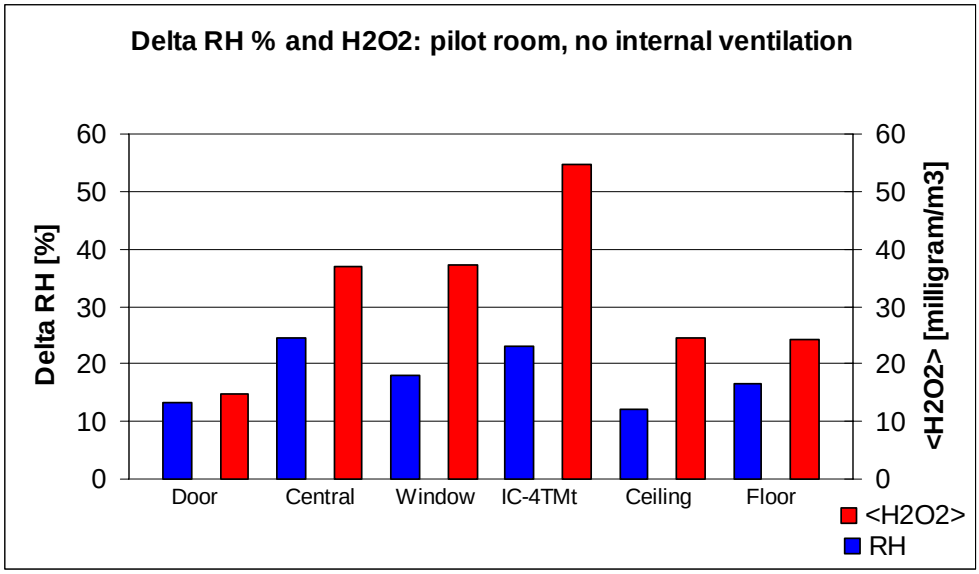


Figure 7. *Average concentration of H_2O_2 (OSHA IMIS 1470) and ΔRH per measurement position per time interval in the unsealed pilot room, no internal ventilation.*

Figures 6 and 7 above show that a vapour-tight packaging results in both a much higher concentration of hydrogen peroxide and a better distributed and higher RH value for the different measurement positions in the pilot room.
Consumption of hydrogen peroxide in the sealed pilot room is much lower than in the unsealed pilot room. Consumption of hydrogen peroxide increases when the tests are carried out with internal ventilation. For the exact values of the different test series, see Appendix A.

5.1.3 *Disinfective effect of the IC-4™ on spores inoculated onto strips*

Tables 8a,b and 9a,b present the test results for the two measurement days.

Table 8a. *Results for B. subtilis, unsealed pilot room, ventilation on, test day 1; 24 January 2007.*

B.subtilis ATCC 5230	Position 1	Position 2	Position 3	Position 4	Control
10^1	+	+	+	+	+
10^2	+	+	+	+	+
10^3	+	+	+	+	+
10^4	+	+	+	+	+
10^5	+	+	+	+	+
10^6	+	+	+	+	+

Table 8b. *Results for G. stearothermophilus, unsealed pilot room, ventilation on, test day 1; 24 January 2007.*

G. stearothermophilus ATCC 7953	Position 1	Position 2	Position 3	Position 4	Control
10^1	+	+	+	+	+
10^2	+	+	+	+	+
10^3	+	+	+	+	+
10^4	+	+	+	+	+
10^5	+	+	+	+	+
10^6	+	+	+	+	+

+ = growth
- = no growth

For the relative humidity at the different measurement positions, see Appendix A. The concentration of hydrogen peroxide was not determined during this test.

Position 1 : On a stand / shelf to the right of the door at a height of approx. 2m (surface: wood)
Position 2 : On a unit at a height of approx. 1 m (surface: stainless steel)
Position 3 : On the floor (surface: petri dish)
Position 4 : On steps at a height of approx. 1.6 m (surface: corrugated stainless steel)

Table 9a. *Results for B. subtilis, sealed pilot room, ventilation off, test day 2; 31 January 2007.*

B.subtilis ATCC 5230	Position 1	Position 2	Position 3	Position 4	Position 5	Control
10^1	-	-	-	-	n.d.	+
10^2	+	-	-	-	-	+
10^3	+	-	-	-	n.d.	+
10^4	+	-	-	-	-	+
10^5	+	-	-	-	n.d.	+
10^6	+	-	-	-	-	+

Table 9b. *Results for G. stearothermophilus, sealed pilot room, ventilation off, test day 2; 31 January 2007.*

G. stearothermophilus ATCC 7953	Position 1	Position 2	Position 3	Position 4	Position 5	Control
10^1	-	-	-	-	n.d.	+
10^2	-	-	-	-	-	+
10^3	-	-	-	-	n.d.	+
10^4	-	-	-	-	-	+
10^5	+	-	-	-	n.d.	+
10^6	+	-	+*	+*	-	+

+ = growth
- = no growth
n.d. = not determined
* = questionable result

For the relative humidity and the concentration of hydrogen peroxide at the different measurement positions, see figure 6 in section 5.1.2.

Position 1 : On a stand / shelf to the right of the door at a height of approx. 2 m (surface: untreated wood surface)
Position 2 : On a unit at a height of approx. 1 m (surface: stainless steel surface)
Position 3 : On the floor (surface: vapour-tight sheet)
Position 4 : On steps at a height of approx. 1.6 m (surface: corrugated stainless steel surface)
Position 5 : Threaded on iron wire directly above the discharge opening of the unit at a height of approx. 1.6 m

The results of test day 1 with *Bacillus subtilis* and *Geobacillus stearothermophilus* spores indicate that there was no elimination.

The results of test day 2 with *Bacillus subtilis* and *Geobacillus stearothermophilus* spores indicate that there was elimination.
Depending on the measurement position, a log reduction of 1 to 6 was found for *Bacillus subtilis*. Log reduction for *Geobacillus stearothermophilus* was 5.

On measurement day 1, the required H_2O_2 concentration in the air was not achieved due to the highly absorbent capacity of the materials in the pilot room.

On measurement day 2, the required H_2O_2 concentration in the air was achieved in the room, while elimination was achieved at almost all positions, with the exception of the wood surface (position 1). It is assumed that, due to its highly absorbent capacity, a local, low concentration of H_2O_2 emerged on the interface of the wood surface, see also *4.1.4* and *5.1.4*. As a result, the strips inoculated with spores were not exposed to a sufficiently high concentration of H_2O_2.
It can be deduced from tables 9a,b and 10a,b that there is a relationship between elimination and the (water/ H_2O_2) absorbing capacity of the materials in the pilot room. A highly absorbent capacity has a negative effect on elimination. Materials like plaster, paper and untreated wood apparently have a detrimental effect on the effectiveness of this method. This aspect is studied in more detail in *4.1.4* and *5.1.4*.

5.1.4 Effects of construction materials and vapour-tight sealing on the basic concentration of hydrogen peroxide and on the local concentration of hydrogen peroxide

Figures 8a,b,c,d below present the effect of vapour-tight sealing and fans on the basic and local concentration of hydrogen peroxide.

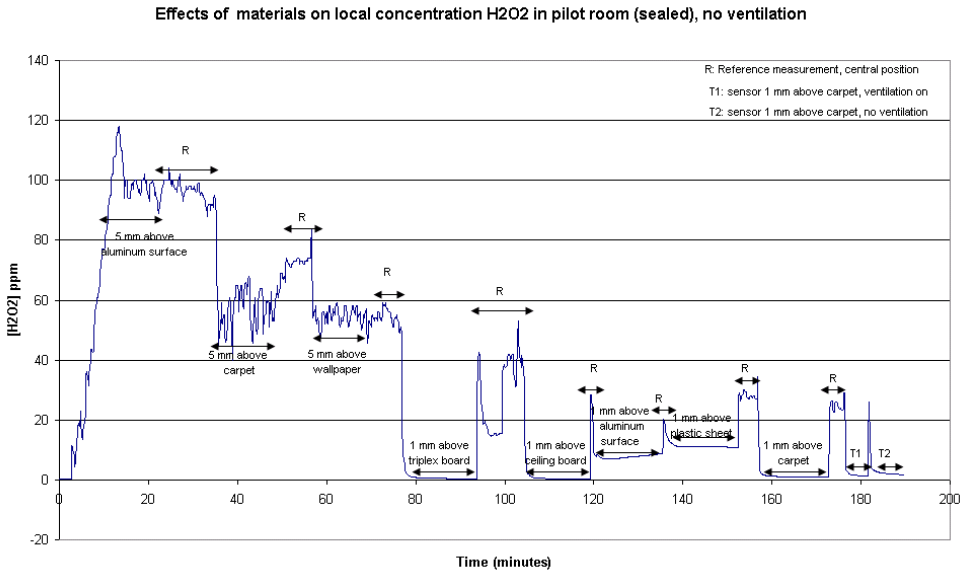


Figure 8a. *Development of local concentration of H_2O_2 on materials in the pilot room (sealed).*

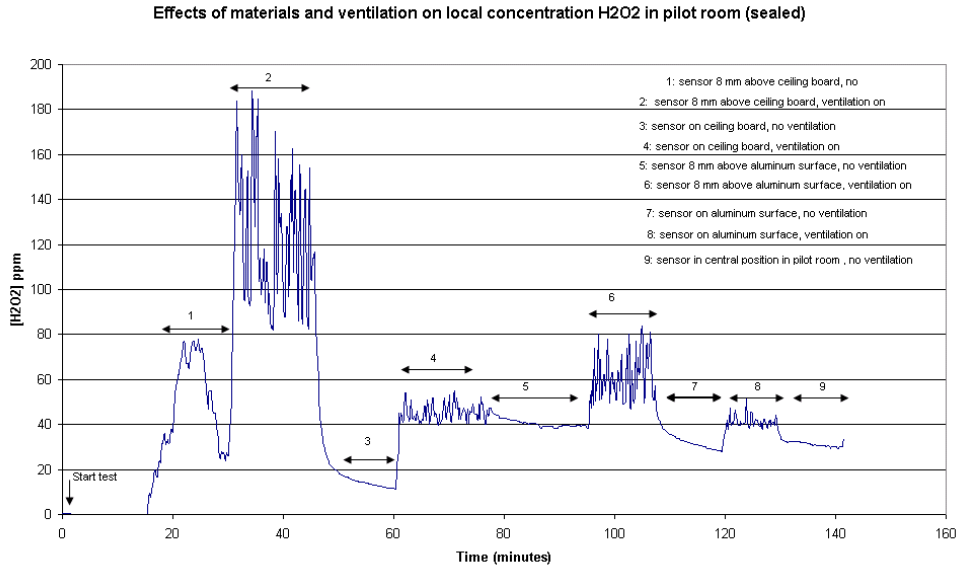


Figure 8b. *Effects of materials and ventilation on the local concentration of H_2O_2 in the pilot room (sealed).*

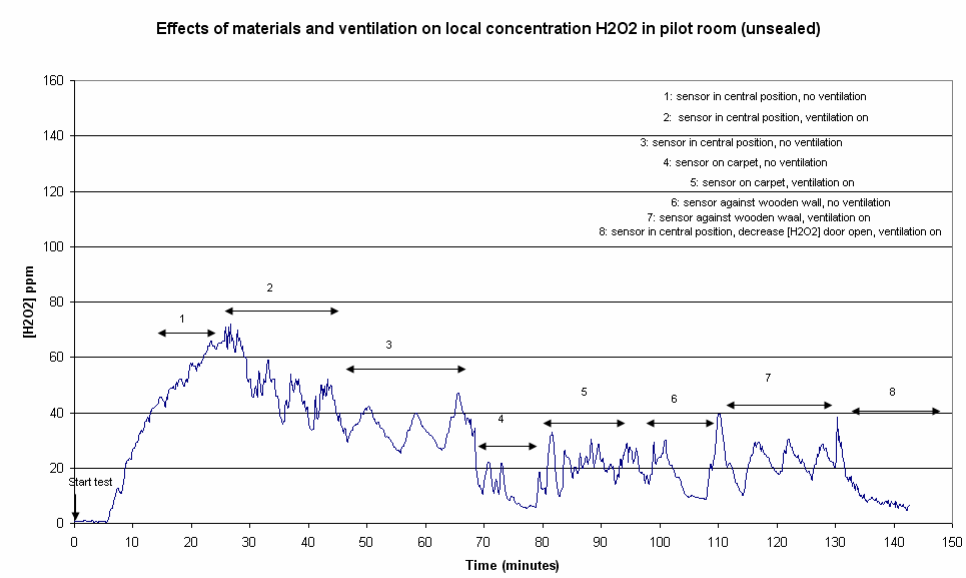


Figure 8c. *Development of local concentration of H_2O_2 and wall effects in the unsealed pilot room.*

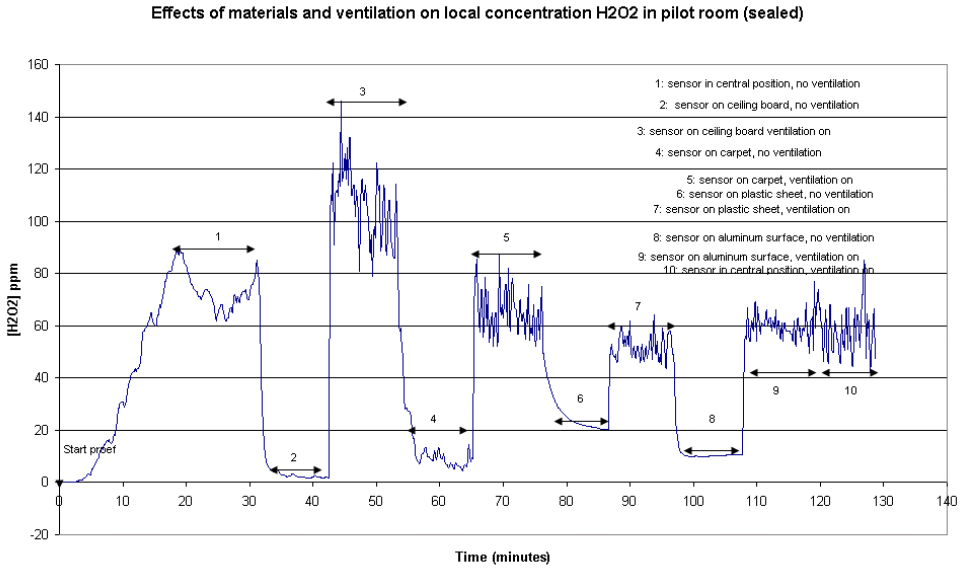


Figure 8d. *Effects of ventilation on the local concentration of H_2O_2 and wall effects in the sealed pilot room.*

It can be deduced from figures 8a,b,c,d above that a large presence of water-absorbent materials in a room results in a low basic concentration in the room (compare figures 8a and 8c). Even in a vapour-tight room, small surfaces (0.3 m^2) of absorbing materials result in a very low concentration of hydrogen peroxide at the material surface (interface concentration). Water-absorbent materials generally produce a surface concentration of hydrogen peroxide lower than 3 ppm. For water-repellent materials, the surface concentration is generally higher than 10 ppm.
Use of a ventilator results in a slightly higher concentration of hydrogen peroxide at the material surface, but the drawback is that ventilation causes the basic concentration of hydrogen peroxide to be lower and the concentration to fluctuate significantly. Although it was shown in 5.1.1 that ventilation produces a better distribution and, consequently, a higher value of the relative humidity, this relationship does not apply, or not as much, to the concentration of hydrogen peroxide.

For the development of relative humidity in the situation in Figure 8d, see Appendix A.

5.2 Practical validation of IC-4^{TM}: Tergooi Hospitals in Blaricum

5.2.1 Distribution and level of the concentration of hydrogen peroxide and relative humidity in hospital room A16 K04

Figure 9 below shows the distribution and level of the (local) concentration of hydrogen peroxide, as well as the wall effects and the effects of internal ventilation.

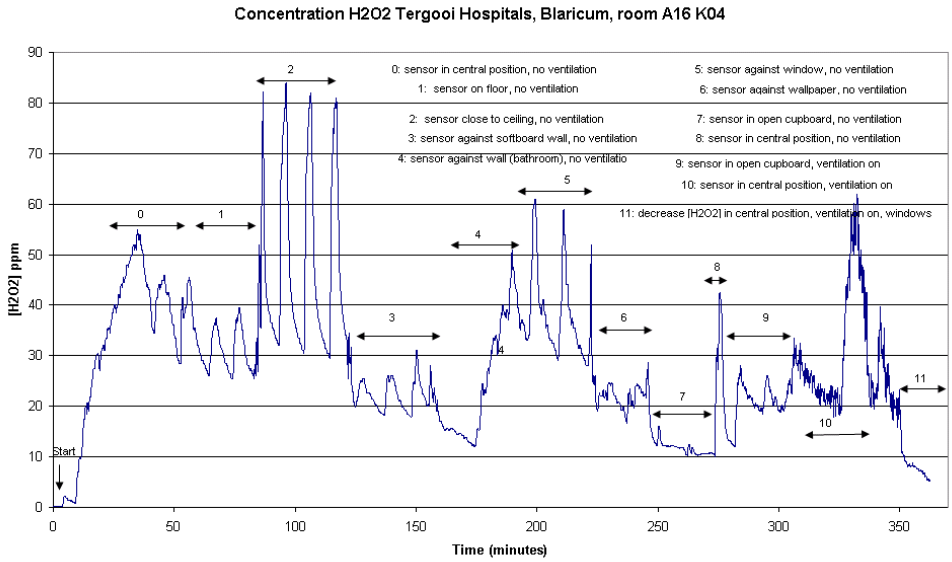


Figure 9. *Distribution of the hydrogen peroxide concentration in hospital room A16 K04, Tergooi Hospitals, Blaricum.*

It can be deduced from Figure 9 above that the concentration in the room (situations 0, 1) during treatment varies between 25-50 ppm. Ventilation results in lower basic concentrations in the room and larger fluctuations.
For the development of relative humidity during this test, see Appendix B.

5.2.2 *Disinfective effect of IC-4™ on bacteria and spores applied to construction material in hospital room A16*

Figures 10 a,b below present the concentration of hydrogen peroxide during the practical tests. Some of the data for the start of the test on 26 June is missing.

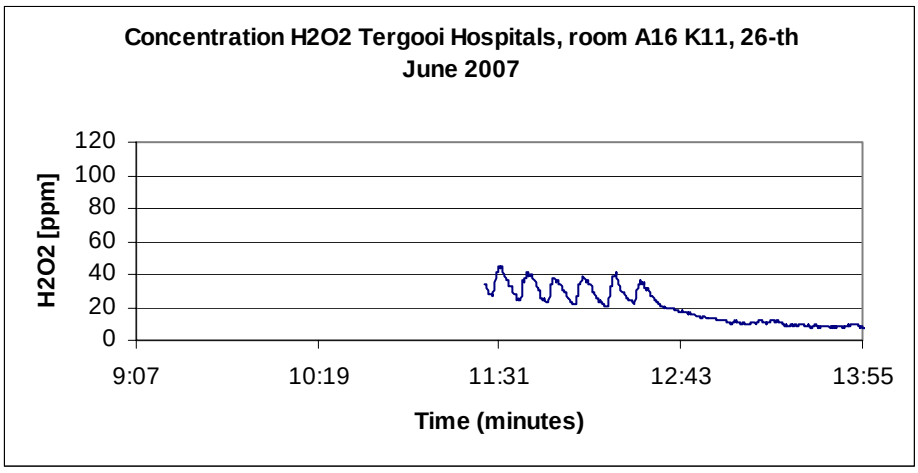


Figure 10a. *Hydrogen peroxide concentration on day 1 (26 June), room A16 K11.*

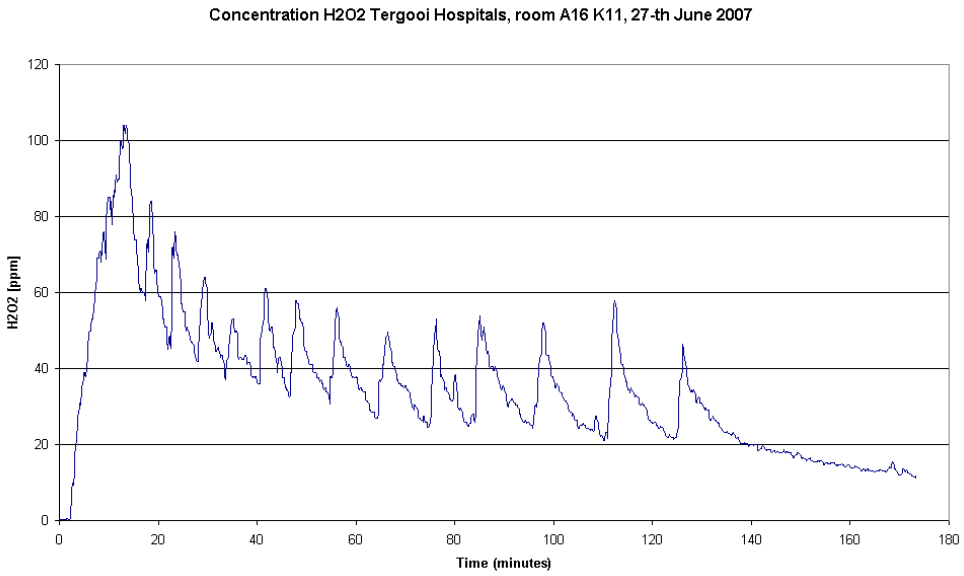


Figure 10b. *Hydrogen peroxide concentration on day 2 (27 June), room A16 K11.*

As the figures above show, the basic concentration of hydrogen peroxide varied between 25-80 ppm during the practical tests.
For the relative humidity during the practical tests, see Appendix B.

Table 10 below summarises the key results of the practical tests in Tergooi Hospitals in Blaricum.
For each material, each treatment time and each micro-organism inoculation level, two samples were tested. This means that over 500 tests have been performed for this practical validation.

Table 10. *Summary of results of the eradicating effect of hydrogen peroxide from the IC-4™ delivery system on practical materials, room A16 K11, Tergooi Hospitals in Blaricum, 26 and 27 June 2007.*

Micro-organism inoculation level: 10^2	Delivery system on: 150 minutes		Delivery system on: 90 minutes	
Materials	Clostridium difficile spores	Bacillus subtilis spores	Staphylococcus aureus vegetative	MRSA vegetative
Stainless steel	-	-	-	n.d.
Wallpaper	-	-	-	-
Linoleum	-	-	-	-
Laminate	-	-	-	-
Ceiling	+	+	+	+

-: 100% elimination
+: growth
n.d.: not determined

Micro-organism inoculation level: 10^4	Delivery system on: 150 minutes		Delivery system on: 90 minutes	
Materials	Clostridium difficile spores	Bacillus subtilis spores	Staphylococcus aureus vegetative	MRSA vegetative
Stainless steel	-	-	n.d.	n.d.
Wallpaper	-	-	-	-
Linoleum	-	-	-	-
Laminate	-	-	-	-
Ceiling	+	+	+	+

-: 100% elimination
+: growth
n.d.: not determined

Micro-organism inoculation level: 10^6	Delivery system on: 150 minutes		Delivery system on: 90 minutes	
Materials	Clostridium difficile spores	Bacillus subtilis spores	Staphylococcus aureus vegetative	MRSA vegetative
Stainless steel	-	-	n.d.	n.d.
Wallpaper	-	-	-	-
Linoleum	-	-	-	-
Laminate	-	-	-	-
Ceiling	+	+	+	+

-: 100% elimination
+: growth
n.d.: not determined

It can be deduced from the practical tests performed in Tergooi Hospitals that hydrogen peroxide mist from the IC-4^{TM} delivery system is an effective method to use as a surface disinfectant for hard, smooth materials such as floors, metal and laminate.

With highly water-absorbent materials such as ceiling boards (glass wool), the micro-organism drop that is applied does not dry on the surface, but penetrates the material. The reactivity of the hydrogen peroxide mist from the IC-4^{TM} is limited to the (wall) surfaces and does not, therefore, elicit a response in the materials themselves.

For a complete overview of the elimination results, see Appendix C.

5.3 Development of the concentration of hydrogen peroxide over time after termination of vaporisation in the room.

Based on the hydrogen peroxide measurements in two hospital rooms in Tergooi Hospitals and the TNO calculation model VentControl, the development of the hydrogen peroxide concentration over time after turning off the IC-4^{TM} hydrogen peroxide delivery system was calculated. Three situations were distinguished:

- room with closed doors/windows and no ventilation;
- room with closed windows/doors and ventilation;
- room with open windows (1 m^2) and closed door and no ventilation.

The following data was used for the calculation:

- hospital room with dimensions (l*w*h) of 5 * 3.5 * 2.8 m.; a floor area of 17.5 m^2 and a volume of 50 m^3;
- ventilation of 80 m^3/hour in accordance with the standards of the Buildings Decree for an occupancy rate of two bed-ridden patients;
- a final hydrogen peroxide concentration of 50 ppm immediately after the delivery system was turned off.

Figure 11 below presents the development of the hydrogen peroxide concentration over time.

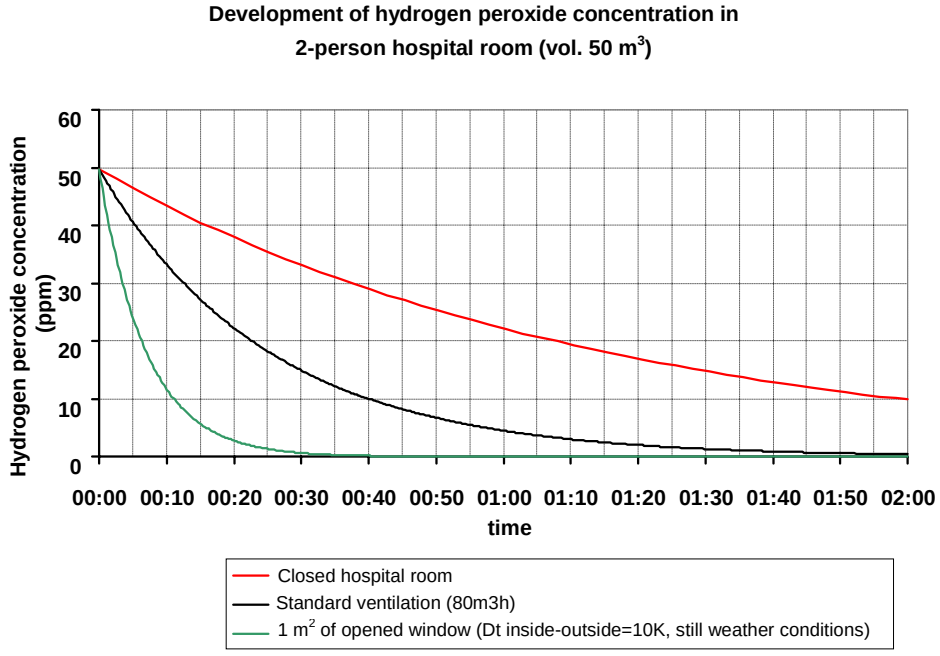


Figure 11. *Development of the hydrogen peroxide concentration over time.*

In a closed hospital room, the concentration of hydrogen peroxide decreased due to natural ventilation and degradation of hydrogen peroxide. After 90 minutes, the H_2O_2 concentration is still 15 ppm, which is high compared to an 8-hour MAC TWA value of 1 ppm for H_2O_2 as stipulated by the Dutch government for exposure in a work environment. By using mechanical ventilation with a standard ventilation of 80 m^3/hour in the case selected, the concentration of hydrogen peroxide is reduced much more quickly.

When the windows are opened (surface 1 m^2), the concentration of hydrogen peroxide is less than 1 ppm after 30 minutes. The reduction is even quicker when more window surface is opened and there is a slight draught. After 30 minutes, it is still safe to enter the room.
It is recommended to give the person who renders the ventilation system suitable for use or opens the window some form of protection upon entering the room immediately after delivery has stopped. At that time, the concentration is approx. 50 ppm. A wet cloth in front of nose and mouth and, preferably, refraining from inhaling for 30 seconds (when opening the windows) are acceptable. The IC-4^{TM} delivery system comes equipped with a zero-potential contact switch, so that turning off the delivery system can be linked to the reactivation of ventilation in the hospital room without anyone having to enter the room.

5.4 Corrosive properties of hydrogen peroxide

Hydrogen peroxide is a chemical compound that comprises the peroxide ion O_2^{2-}. The hydrogen peroxide molecule has one oxygen molecule more than the much more stable water molecule. The bond between the two oxygen atoms – the peroxide bond – gives way quite easily due to the formation of oxygen radicals. The contaminations are degraded by free oxygen radicals, which have both an oxidising and a disinfecting effect.
The corrosiveness of hydrogen peroxide in liquids is a well-known phenomenon, although this only applies to hydrogen peroxide solutions up to 90% . The contact with materials (plastics, metal, wood etc.) is a contact with a hydrogen peroxide liquid, not with a hydrogen peroxide vapour.

This study concerns the effect of hydrogen peroxide in a concentration of 50 ppm in ambient air and a short exposure time of no more than 4 hours. Based on information in the Corrosion Guide, hydrogen peroxide liquid is corrosive for materials such as copper and iron.
In concentrations of 50 ppm in the air, problems caused by corrosion are not likely (telephone information from TNO expert Gabriele Ferrari, who works for Materials Technology at TNO Science and Industry). The very short contact time between materials and hydrogen peroxide is another important parameter.
Moreover, the vulnerable materials in equipment (circuit boards, contacts) are protected by a housing, reducing the contact time even further.
Under the conditions specified and based on the information given here, TNO experts do not consider corrosion a problem. It may be desirable to further substantiate this using experimental study.

5.5 Estimate of the risk to humans after exposure to hydrogen peroxide

Based on the European Union Risk Assessment Report hydrogen peroxide (CAS No: 7722-84-1, EINECS No: 231-765-0 of the European Commission (2003), TNO expert Dr. E.D. Kroese (Quality and Safety of TNO Quality of Life) concluded that no harmful effects are expected for humans following exposure to 50 ppm hydrogen peroxide for 1-2 minutes.
It is, of course, recommended to take normal precautionary measures such as wearing safety goggles while in the room. It is also a good idea to limit inhalation of air containing hydrogen peroxide as much as possible. A second person must ensure that the first person actually vacates the room concerned shortly after entry.

6 Conclusions and recommendations

One of the most remarkable findings of this study is the strong dependency of the hydrogen peroxide concentration in the ambient air in relation to the building materials and the interior of a specific room . In a room with adsorbent materials (carpet, paper, textile, mineral wool, etc.), the concentrations of hydrogen peroxide and the relative humidity are considerably lower than in a room with materials like steel, glass, laminate, linoleum, plastic, etc.
The hydrogen peroxide concentration very close to the surface of the materials is strongly related to the nature of the material. It can be concluded on the basis of this study that water-adsorbent materials like plaster, mineral wool, untreated wood and carpet cause a reduction in both the surface concentration (<3 ppm) and the basic concentration of hydrogen peroxide in the room in question. With water-repellent and vapour-tight materials such as linoleum, (washable) wallpaper, laminated wood and glass, on the other hand, which are often used in hospital rooms, the basic concentration (25-50 ppm) and the surface concentration (>10 ppm) of hydrogen peroxide remain relatively high.

In laboratory tests and the practical study in Tergooi Hospitals in Blaricum, a log reduction of 6 was realised for the spores *Clostridium difficile* and *Bacillus subtilis* and the vegetative organisms *Staphylococcus aureus* and *MRSA*. In all, over 1,000 test samples were analysed.
The conclusion is that hydrogen peroxide vaporised using the IC-4^{TM} delivery system (5% H_2O_2, 0.01 % Ag^+) is an effective method to use as a surface disinfectant for water-repellent materials.

On completion of the work, the room can be most quickly ventilated by opening the windows, and re-entered after approximately 30 minutes.

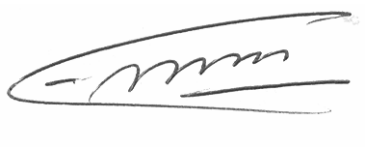
Exposure to 50 ppm of hydrogen peroxide for 1-2 minutes is not likely to produce any harmful effects in humans.

The (initial) results are promising. It is recommended to extend the study to include other micro-organisms. The following bacteria and spores are eligible:
- Acinetobacter baumanii
- VRE (vancomycin-resistant enterococci)
- Clostridium spores (2 other varieties of type 27 and other epidemic type)

It is, of course, recommended to take normal precautionary measures such as wearing safety goggles while in the room. It is also a good idea to limit inhalation of air containing hydrogen peroxide as much as possible. A second person must ensure that the first person actually vacates the room concerned shortly after entry.

7 Responsibility

Delft, 20 September 2007



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Author

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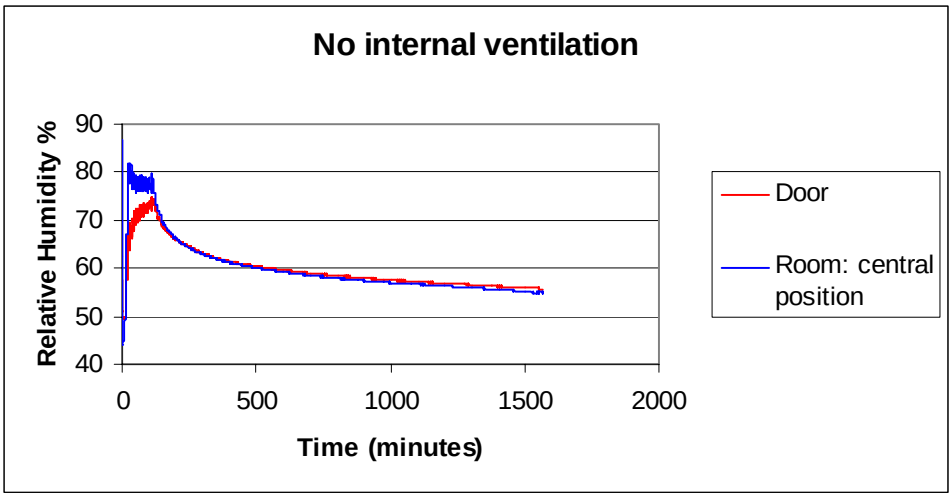
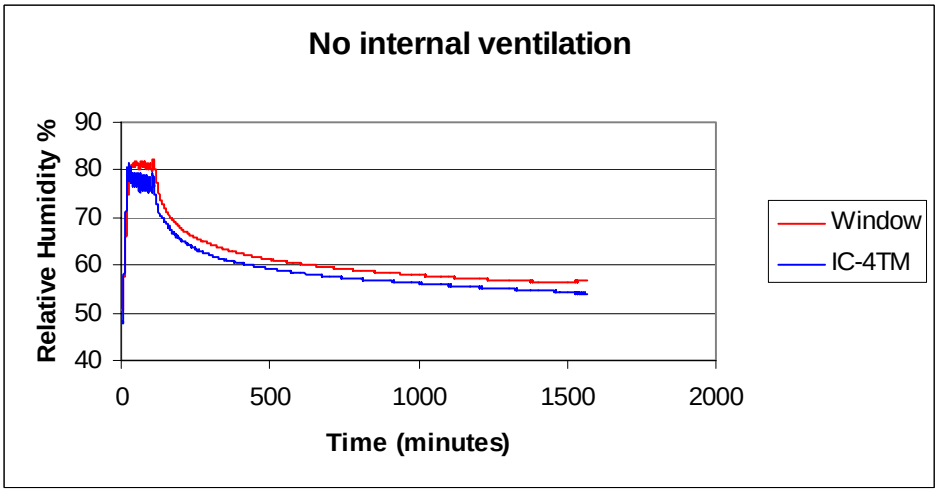
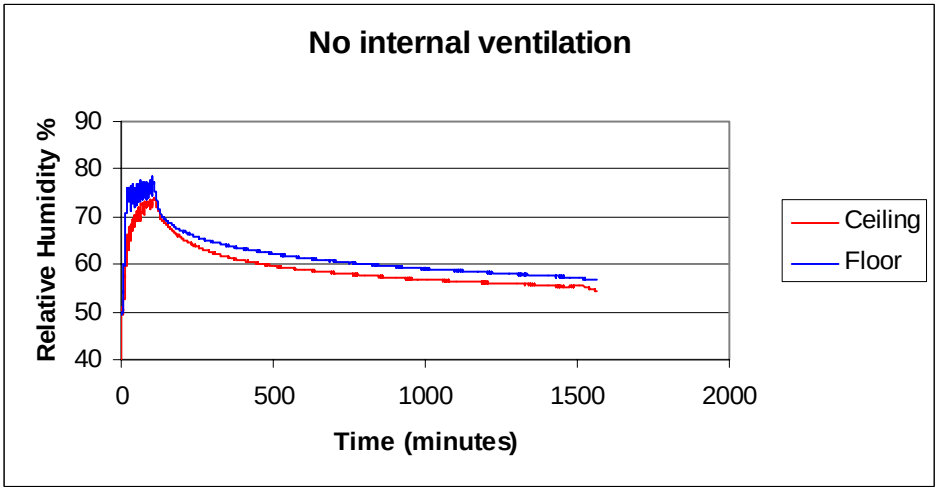
Head of the department
Interior Environment and Health

Appendix A Relative humidity tests in TNO pilot room, IC-4TM

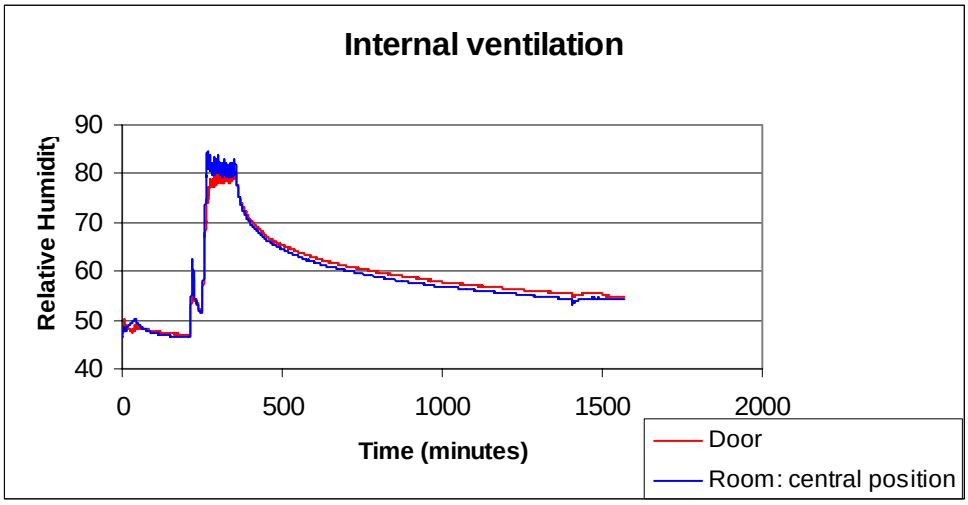
The three figures below present the development of relative humidity for the measurement positions in the pilot room at TNO, without ventilation and when the IC-4^{TM} is filled with tap water.

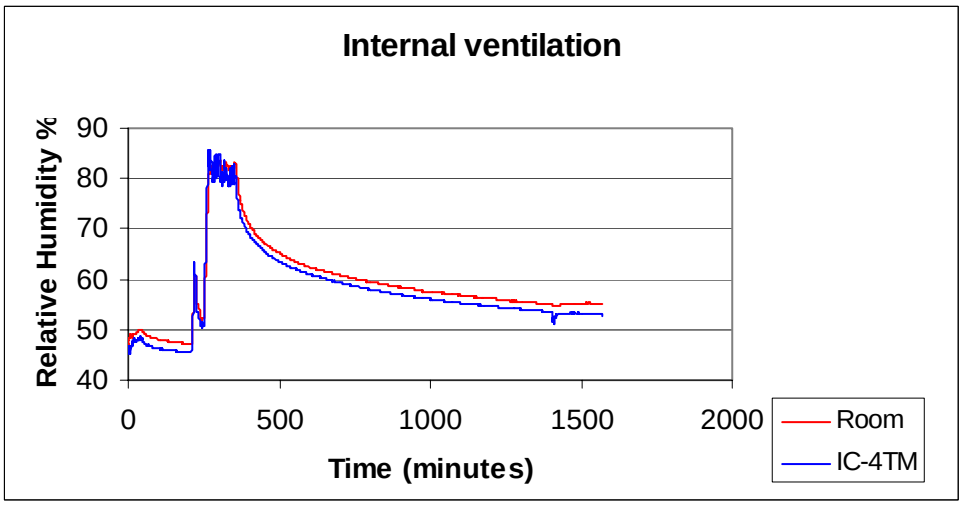


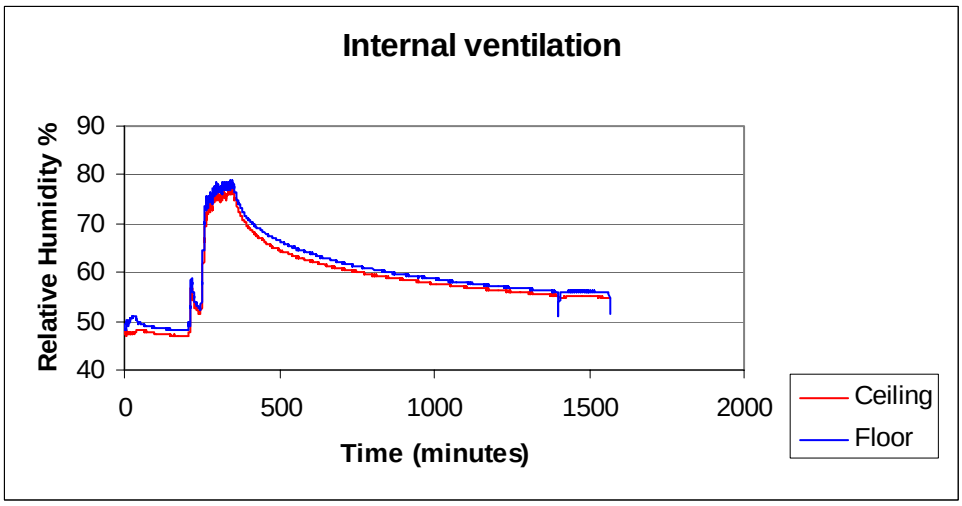




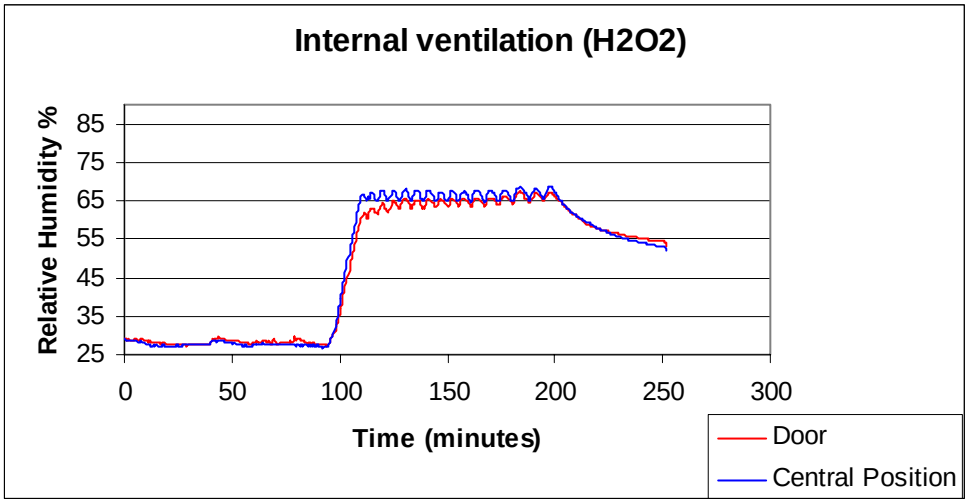
The three figures below present the development of relative humidity for the measurement positions in the pilot room at TNO, with ventilation on (level 3) and when the IC-4^{TM} filled with tap water.

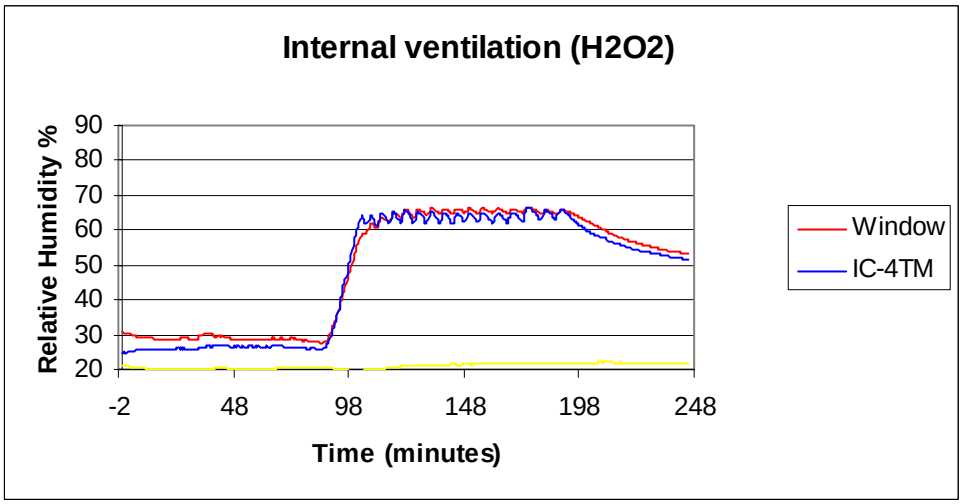


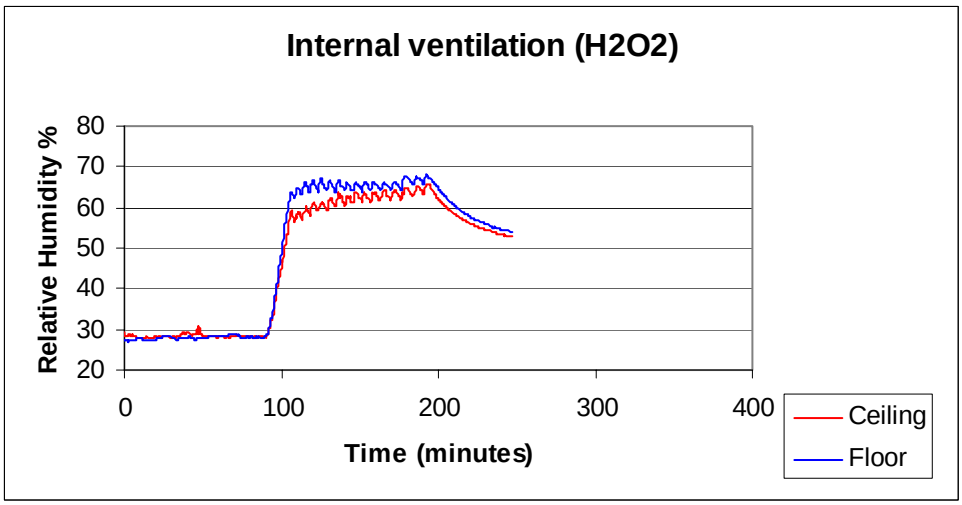




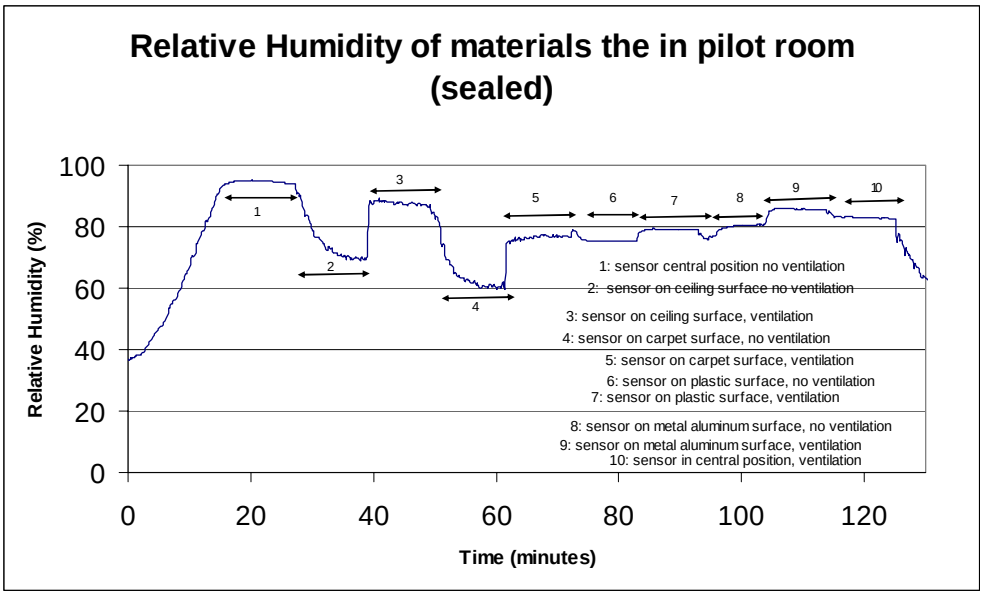
The three figures below present the development of relative humidity during the test in the unsealed pilot room on 24-01-2007, which was conducted to determine the eliminating effect on the spores *B. subtilis* en *G. stearothermophilus*, with internal ventilation.







The figure below presents the development of relative humidity for the situation shown in figure 8d.



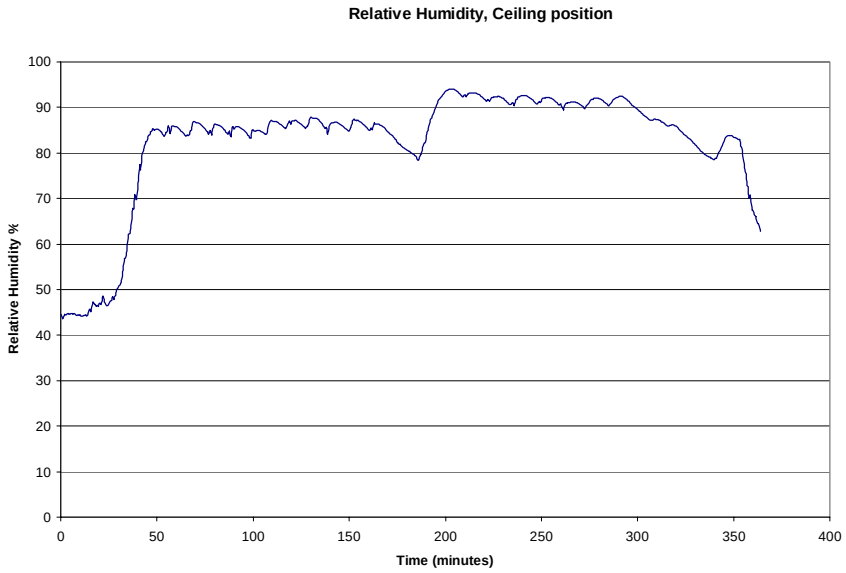
The table below presents the consumption of hydrogen peroxide during several test series in the pilot room

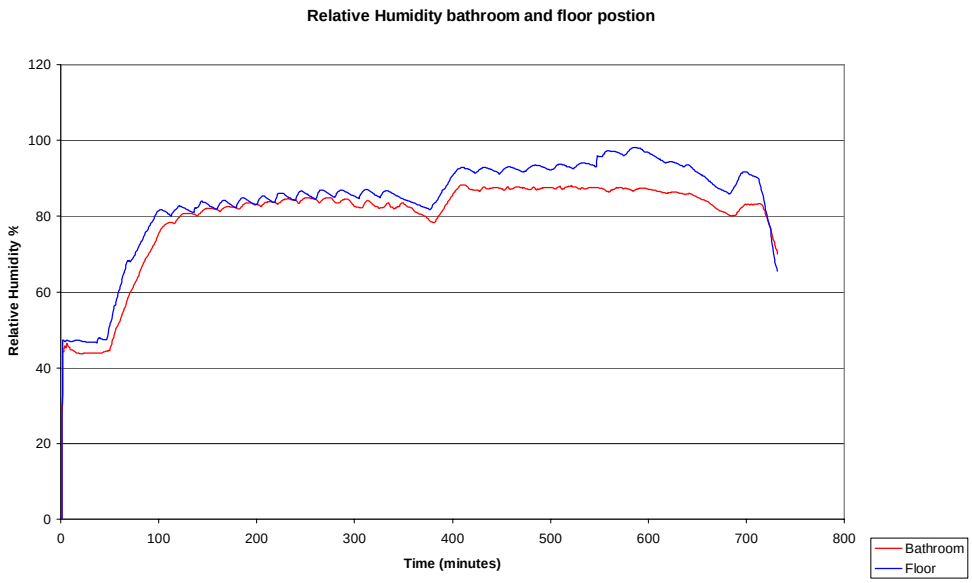
Test date	Pilot room	Ventilation	H_2O_2 consumption	ΔRH (%)	Time (hr)
6-12-2006	unsealed	on	1.8 (kg)	30	1.5
11-12-2006	unsealed	off	0.8 (kg)	30	1.5
24-01-2007	unsealed	on	1.1 (kg)	40	1.5
31-01-2007	sealed	off	0.4 (kg)	40	1.5

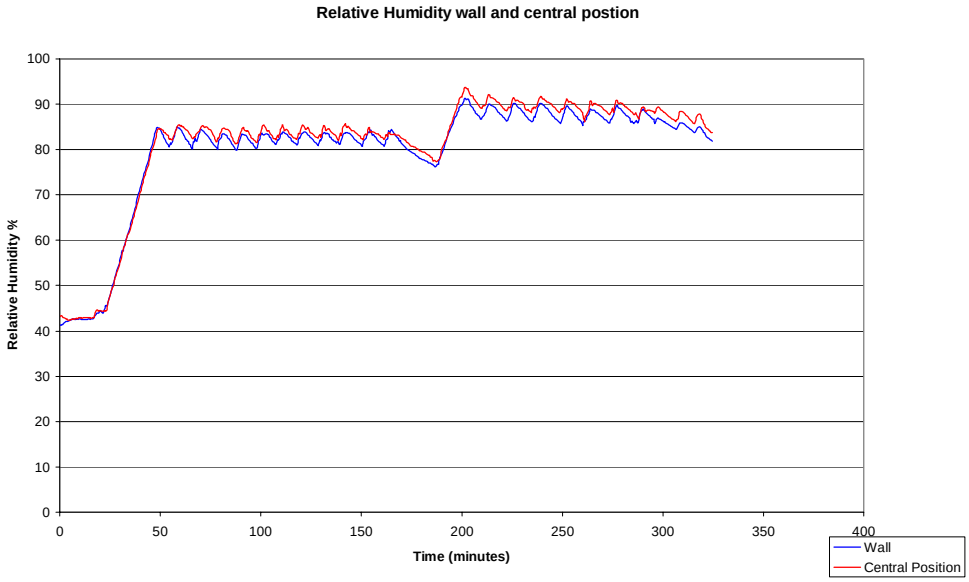
Appendix B Practical relative humidity tests in rooms A16 K04 and A16 K11

B.1 Measurements taken on 13 April 2007, Tergooi Hospitals room A16K04

The three figures below present the development of relative humidity during determination of the distribution and level of the hydrogen peroxide concentration in room A16 K04, 13-04-07, Tergooi Hospitals in Blaricum

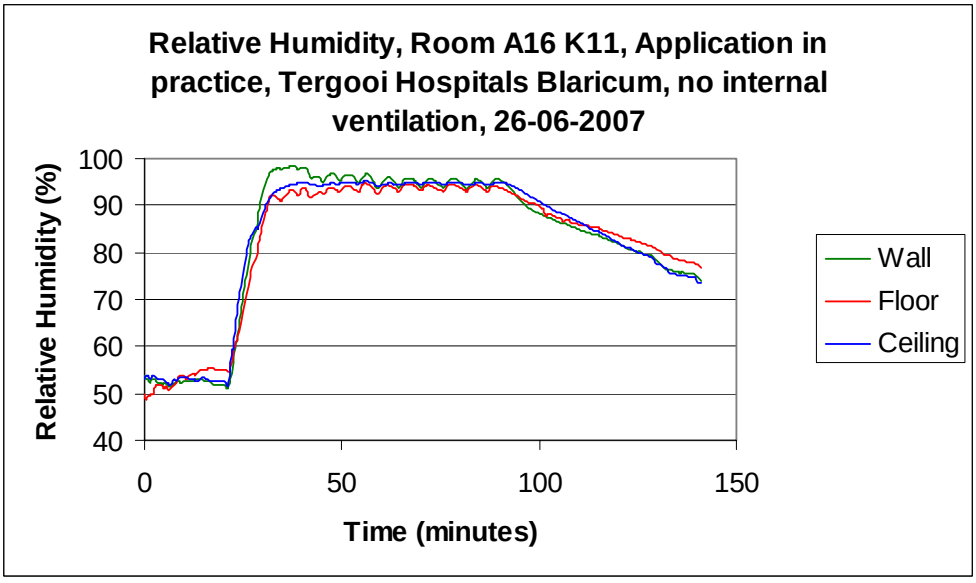




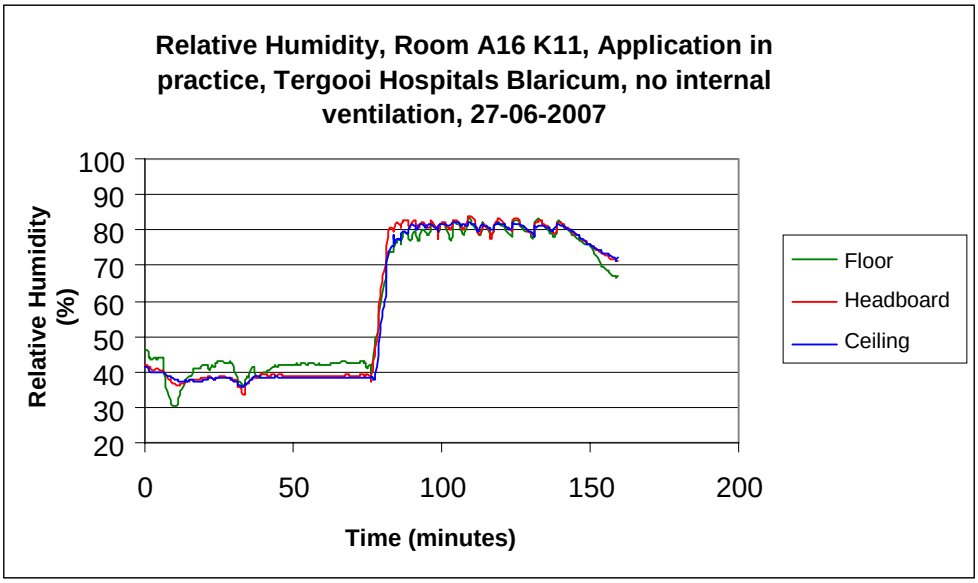


B.2 Measurements taken 26 June 2007, Tergooi Hospitals room A16 K11

The figure below presents the development of relative humidity during the validation tests in room A16 K11 on 26-06-2007, which were conducted to determine the eliminating effect on the spores *Bacillus subtilis* and *Clostridium difficile.*



The figure below presents the development of relative humidity during the validation tests in room A16 K11 on 27-06-2007, which were conducted to determine the eliminating effect on the vegetative cells *MRSA* and *Staphylococcus aureus.*



Appendix C Detailed overview of results of practical test in Tergooi Hospitals

Clostridium difficile 27A spores

Material	Inoculation level	Treatment time in minutes Delivery system on: 150 minutes							
		0		30		60		220	
Stainless steel	**10^2**	+	+	-	-	-	-	-	-
	10^4	+	+	-	-	-	-	-	-
	10^6	+	+	-	-	-	-	-	-
Wallpaper	**10^2**	-	+	-	-	-	-	-	-
	10^4	+	-	-	-	-	-	-	-
	10^6	+	+	-	-	-	-	-	-
Linoleum	**10^2**	+	+	-	-	-	-	-	-
	10^4	+	+	-	-	-	-	-	-
	10^6	+	+	-	-	-	-	-	-
Laminate	**10^2**	-	+	-	-	-	-	-	-
	10^4	+	+	-	-	-	-	-	-
	10^6	+	+	-	+	-	-	-	-
Ceiling	**10^2**	+	+	+	+	+	+	+	+
	10^4	+	+	+	+	+	+	+	+
	10^6	+	+	+	+	+	+	+	+

Bacillus subtilis spores ATCC 35021

Material	**Inoculation level**	**Treatment time in minutes** **Delivery system on: 150 minutes**							
		0		**30**		**60**		**220**	
Stainless steel	**10^2**	+	+	-	-	-	-	-	-
	10^4	+	+	-	-	-	-	-	-
	10^6	+	+	-	-	-	-	-	-
Wallpaper	**10^2**	+	+	+	+	-	-	-	-
	10^4	+	+	+	+	+	+	-	-
	10^6	+	+	+	+	+	+	-	-
Linoleum	**10^2**	+	+	-	-	-	-	-	-
	10^4	+	+	-	-	-	-	-	-
	10^6	+	+	-	-	-	-	-	-
Laminate	**10^2**	+	+	-	-	-	-	-	-
	10^4	+	+	-	-	-	-	-	-
	10^6	+	+	+	-	-	-	-	-
Ceiling	**10^2**	+	+	+	+	+	+	+	+
	10^4	+	+	+	+	+	+	+	+
	10^6	+	+	+	+	+	+	+	+

Staphylococcus aureus ATCC 6538

Material	**Inoculation level**	**Treatment time in minutes** **Delivery system on: 90 minutes**								
		0	**30**		**0**	**60**		**0**	**90**	
Wallpaper	**10^2**	+	+	-	+	-	-	+	-	-
	10^4	+	+	-	+	-	-	+	-	-
	10^6	+	+	-	+	-	-	+	-	-
Linoleum	**10^2**	+	-	-	+	-	-	+	-	-
	10^4	+	-	-	+	-	-	+	-	-
	10^6	+	+	-	+	-	-	+	-	-
Laminate	**10^2**	+	-	-	+	-	-	+	-	-
	10^4	+	-	-	+	-	-	+	-	-
	10^6	+	+	-	+	+	-	+	-	-
Ceiling	**10^2**	+	+	+	+	+	+	+	+	+
	10^4	+	+	+	+	+	+	+	+	+
	10^6	+	+	+	+	+	+	+	+	+

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Material	**Inoculation level**	**Treatment time in minutes** **Delivery system on: 90 minutes**								
		0	**30**		**0**	**60**		**0**	**90**	
Wallpaper	**10^2**	+	-	-	+	-	-	+	-	-
	10^4	+	-	-	+	-	-	+	-	-
	10^6	+	+	-	+	-	-	+	-	-
Linoleum	**10^2**	+	-	-	+	-	-	+	-	-
	10^4	+	+	-	+	-	-	+	-	-
	10^6	+	+	+	+	-	-	+	-	-
Laminate	**10^2**	+	+	-	+	-	-	+	-	-
	10^4	+	+	-	+	-	-	+	-	-
	10^6	+	-	-	+	+*	+*	+	+*	-
Ceiling	**10^2**	+	+	+	+	+	+	+	+	+
	10^4	+	+	+	+	+	+	+	+	+
	10^6	+	+	+	+	+	+	+	+	+

*** = questionable result**

Appendix D Recordings and photographs of the tests

The DVD contains recordings and photographs of the tests as conducted at TNO (pilot room) and during practical validation (Tergooi Hospitals).

Photographs of TNO pilot room, see directory:
- Recordings of test 13-12-06 (RH distribution, instant determination of H_2O_2 concentration)
- Recordings of test 24-01-07 (1^{st} test with strips: Bacillus subtilis)
- Recordings of test 31-01-2007 (2^{nd} test with strips: Bacillus subtilis)
- Recordings of test 15_16-03-07 (surface concentration of H_2O_2: continuous measurement)

Photographs of practical validation (Tergooi Hospitals)
- Recordings of test 13-04-07 (surface and basic concentration of H_2O_2: continuous measurement)
- Recordings of test 26_27-06-07 (elimination of bacteria and spores on practical materials)