DISINFECTION WITH VAPORIZED HYDROGEN PEROXIDE FLUID IN DIFFERENT ENVIRONMENTS

and its applications and advantages in crisis management

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Abstract

During a global pandemic, mitigating the impact of the disease and coordinating efforts to manage not only the medical but also the logistical and administrative aspects of such an all-encompassing phenomenon are of paramount importance. An extremely important but less publicised issue in this context is laboratory management and safety in analytical laboratories. In times of high capacity utilisation, as is the case during a pandemic or endemic outbreak of disease, other routine processes have to be abbreviated or are cancelled altogether due to lack of planning owing to the rapid emergence of the outbreak. In order to achieve high level of cleanliness in laboratories of all shapes and sizes and with different requirements, a universal solution seems unimaginable. Our experiments show a promising, automated approach of disinfection of various spaces. Within a short timeframe of 1 h – 3 h it is possible to disinfect any desired room to achieve a laboratory grade hygiene status. This was proven by employing biological indicators validated for this procedure. The tested technology reduced the indicator germs by a concentration of the mathematical log 6 reduction. Achieving this high level of cleanliness is possible by assigning a single person to the task for the set-up at the scene. Steering and monitoring of the process can be done remotely. While the machine used in our experiments is not a completely new concept, our experiments in a real-life setting such as laboratories and clinics alike, show that the applied hydrogen peroxide vapour distributed by a specialized fogger, disinfects even hard to reach spots within closed-off spaces. This program can be performed while automated (PCR) machines are running and highly trained personnel can apply their expertise elsewhere. Moreover, while the program is running real-time data is available and the process can be remotely monitored and steered digitally. It is of major concern to ensure maintainability of infrastructure e.g. COVID labs, ambulances, laboratories or veterinary practitioners to ensure treatment of directly and indirectly related health issues within a crisis. We concentrated on evaluating the usability of the disinfection technology presented in real-life settings.

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1. Introduction to the methods

The fogger used in these experiments was provided by Braincon GmBH & Co KG. It was developed to disinfect surfaces and airborne contaminants in clinics and hospitals, producing dry vapour hydrogen peroxide droplets of ~ 0,3 µm in size without heating the fluid (Braincon GmbH). We used commercially available, validated biological indicators, containing different concentrations of viable of the standard microbe, as well as validated chemical indicators used for monitoring disinfection with H_2O_2 as controls for the process. These indicators were placed mainly on hard to reach spots or highly frequented areas within the rooms where the disinfection was performed. With the biological indicators we tested different concentrations, namely 10^4, 10^5 and 10^6 viable, colony forming units or CFU, of *G. stearothermophilus* to see how powerful the disinfection we performed was. After the distribution of the indicators the doors and windows were sealed with duct tape and ventilation was turned off to prevent the vapour from escaping the assessed area. The disinfection program was adapted to the size of the room and the unique requirements i.e. relative air humidity (RAH) at the start. As a disinfectant 7.5 % hydrogen peroxide solution DCXF, was nebulized to achieve and maintain the desired RAH during the disinfection program of 3 h. In some cases with 50 ppm silver ions as an additive to the solution. After the disinfection protocol, the air ventilation was turned on and, if feasible (i.e. in the ambulance), doors and windows were opened to ensure air exchange and sufficient diffusion of hydrogen peroxide out of the disinfected room. To ensure safety the highly sensitive and portable Dräger X-am 5100 device was used to verify that the peroxide concentration had fallen under the maximum workplace level of 1.0 ppm before the respective areas were deemed safe and declared safe.

2. Validation

2.1. Empty Room

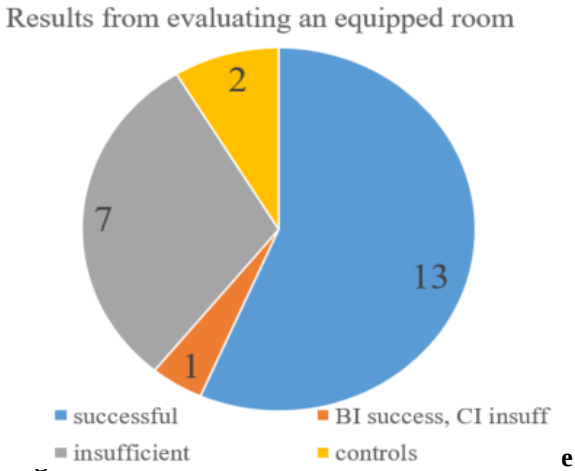
As a first step, the disinfection program was performed three times within a sealed cellar of approx. 38 m^3 to validate the fogger under comparable conditions. For this experiment various biological and chemical indicators where distributed on four different places within the room at the AGES Mödling. The positions of the indicators were chosen to achieve a distance of approx. 2 m from the vaporizer while all seven different indicators could be placed at the same position at once. The following locations were selected to evaluate the peroxide distribution within the room: in a plastic tube sealed by 2 copper sponges, on the opposite side of the room and on the top of the ventilation shaft (10 cm below the ceiling, this was approx. 3 m from the vaporizer, for further information see **4. Materials and Supplementary**). The fourth position, on the device itself, was added after the first experiment, because it was suspected that 90 % RAH could lead to condensation. In this case, the condensation would be most severe and most noticeable near the outlet of the vaporizer. The indicators inside the plastic tubes served as a negative control, since hydrogen peroxide is an oxidizing agent. Consequently, it reacts strongly with the copper ions in the sponges, ensuring that the vapour cannot reach the indicators inside.

Based on the practical experience of Braincon, 90 % of RAH was set as target value and the room was disinfected with DCXF solution over a span of 360 min. The DCXpro recorded RAH and temperature for further two hours after the nebulisation to measure the development of the humidity in the room and to compare the internal readout with the Porta Sense II device installed in the room. This was done to prove that the humidity curves measured by the DCX internal probe correlates with the ppm measurements of the Porta Sens II device. The humidity and the ppm measurements did match. The ppm-values peaked after fluid vaporization was finalized. This may be due to fluid

dynamics within the room. 90 % RAH is feasible to perform a log 6 disinfection in a 38 m^3 sealed cellar with no additional air ventilation. All tested biological indicators are applicable and can be used interchangeably.

2.2. Equipped room

After the room evaluation, the experiment was extended by assessing 23 positions of the biological and chemical indicators within and around office staples and miscellaneous objects of common use (i.e. drawer of a cupboard, in a folder between sheets of paper, HEPA filter, rubber boots, aluminium ventilation tube, etc.). The goal was to determine the accessibility of hydrogen peroxide fog to various locations in an equipped room. For the examination, a RAH of 80 %, 7.5 % hydrogen peroxide and an active disinfection time of 360 min was chosen. In this case, only one biological indicator and one chemical indicator were used to verify the reduction, and all indicators were placed approx. two meters from the vaporizer.



e differentially equipped room showed a clear success, though some equipment was hard to decontaminate; see 4. Materials and Supplementary for more detailed results, BI= Biological indicator (Spore Strip G. *stearothermophilus* ChemVapor), CI= C)

The gaseous hydrogen peroxide achieved a successful bacterial reduction within a two meter radius and was able to reach laterally open horizontal cavities such as sewer pipes or underneath the laptop. The disinfection in vertical cavities open at the top, however, was insufficient. Furthermore, the fog was able to access a coat pocket but not into the pile of clothes or narrow spaces such as filters and straw bales. The disinfection of closed drawers and behind cabinet doors was not successful. As described in the initial room disinfection experiment the indicators inside the plastic tube were used as negative control.

3. Real Environment Experiments

3.1. Dentist practice

Another proof of concept was the disinfection of a dentist practice in Linz, with the DCXplus in “boost mode” (continuous steam injection) for 120 min, then continued measuring of humidity and temperature for 60 min, before the biological indicators were taken out. In this experiment 50 ppm silver ions were added to the DCXF solution. In total four positions were monitored with biological indicators, to determine the disinfection power of the DCXplus.

Table 1 Overview of Apex Discs positions and results at the dentist practice, blue/(-): successful disinfection, orange/(+): insufficient disinfection/growth

Position / CFU	10^4	10^5	10^6		10^4	10^5	10^6
Water fountain patient chair	-	-	-	Bench	-	-	+
utensil tableau	-	-	-	Suction device and tools	-	+	-

The insufficient disinfection of the bench and suction device could be due to the shorter vaporization time. Another issue could have been that that the temperature in the room was much higher (~28°C)

than the range of effective temperature for the DCXF fluid and dry vapour which lies between 15 and 22°C. In order to obtain more information swab tests at real locations and more real-life scenarios need to be assessed to see if temperature is really such a hindering factor.

3.2. Veterinary practice

The effectiveness of disinfection in real-life situations with the DCX technology was tested in an operational veterinarian practice. For the evaluation of the disinfection, biological indicators and swab tests were used to see if additionally optimization is required. The treatment room as well as the operation room were disinfected with DCXplus placed in the centre of the rooms and using 7.5 % hydrogen peroxide containing 50 ppm silver ions. The disinfection was performed with respect to the target humidity using the "boost mode" for 90 min. The samples were taken before (swab) and after completion (swab/biological indicators) of the disinfection process. The biological indicators were distributed across four different locations and evaluated after exposure time in two laboratories. The operating room (OR) with an approx. volume of 37 m^3 and the treatment room (TR) with a volume of approx. 46.8 m^3 were both equipped with two biological indicators and the operating room was swabbed for microorganisms as well. Biological indicators (Apex Discs) showed a decimation of log 5, and in the TR a CFU reduction of log 6 could be reached on both positions. In the OR, however, a log 6 reduction could not be achieved. The bacterial analysis of the swabs displayed successful disinfection at all places.

Table 2 Results of the swab tests in the veterinary practice before and after disinfection with the DCXplus; blue: no microorganisms were found

Location of Swabbing	Time	Detected Microorganism	Pathogenicity
Handles overhead cupboard OR	Before	-	-
	After	-	-
Anaesthetic device	before	*Staphylococcus haemolyticus*	nosocomial pathogen, carrier of resistance genes (Takeuchi et al. 2005; Froggatt et al. 1989; Barros et al. 2012)
	after	-	-
Handles cupboard OR	before	*Acinetobacter lwoffii*	opportunistic pathogen, can cause nosocomial infections , carrier of resistance genes (Regalado, Martin, and Antony 2009)
	after	-	-
Keyboard x-ray PC	before	*Acinetobacter lwoffii*	opportunistic pathogen (see above)
	after	-	-

3.3. PCR Lab

Next a container laboratory with a total area of approximately 111 m^2 and a volume of approx. 265 m^3 as well as its storage container with a size of 6.66 m^2 and a volume of 15.9 m^3 was disinfected. Two DCXplus (located at opposite ends of the room) and one DCXpro (located at the centre) were positioned in the container lab. Only one DCXplus device was placed in the storage container. The

PCR lab was disinfected with the same setting as the veterinary practice and the biological indicators were distributed on five positions within the laboratory. Additionally, two positions were chosen within the storage container. The disinfection program could be fully performed while automated (PCR) machines were running.

The results show a high efficiency of the DCX technology, indicating a promising application for PCR laboratory containers. A bacterial decimation of log 6 could be achieved, except for one indicator in the storage container, with which a log 5 reduction was attained.

3.4. Ambulance cleaning protocol comparison

Finally, two different approaches in cleaning protocols were compared in two almost identical ambulances. One was cleaned by hand by well trained staff via the established cleaning protocol while the other was submitted to the fumigation protocol of the DCXplus with 7.5 % hydrogen peroxide with 50 ppm silver ions in "boost mode". A RAH of 88 % was achieved after 60 min of vaporization, although the first charge of biological indicators were taken out after 30 min and the main door was opened for approx. one min. The usual cleaning protocol took the two trained persons approx. 45 min each, meaning 90 min personnel hours in total. The used biological indicators were placed at five different locations in duplicates and removed after 30 and 60 min. In order to receive more data on existing contamination within the ambulances and the actual disinfection, the five locations where the biological indicators were placed and five additional locations in both ambulances were swabbed before and after the disinfection process. All selected places for the swab tests are areas of contact for paramedics and patients which could be hazardous for both parties. The analysis of the indicators and swabs was again performed in the same laboratories as indicated in 3.2. Veterinary practice.

Table 3 Summary of findings of the swab analysis before and after disinfection by hand, * biological indicator at the same location, orange: remaining contamination after disinfection treatment; grey: non-pathogenic microorganisms, remaining after disinfection

Location of Sampling	Time	Detected Microorganism	Pathogenicity
Emergency bags*	before	*Micrococcus luteus*	Non-pathogenic, part of skin flora (Boldock et al. 2018)
	before	*Bacillus pumilus*	Food spoilage organism, rare cases of food poisoning (Drobniewski 1993)
	after	*Staphylococcus hominis*	Opportunistic pathogen, nosocomial infections, part of skin flora (Kloos and Schleifer 1975), antibiotic resistance (Mendoza-Olazaran et al. 2015)
Handle recliner	before	*Staphylococcus epidermidis*	Opportunistic pathogen, nosocomial infections, part of skin and mucus membrane flora (A report from the NNIS System 2004)
	after	-	
Harness stretcher	before	*Staphylococcus epidermidis*	Opportunistic pathogen, see above

	after	-	
Headrest stretcher*	before	*Bacillus cereus*	Foodborne pathogen (Bottone 2010)
	after	*Bacillus mycoides*	Foodborne pathogen, belongs to B. cereus group (Drobniewski 1993)
Rear patient chair seat*	before	*Micrococcus luteus*	Non-pathogenic (see above)
	after	-	
Seat belt (rear patient seat)	before	*Staphylococcus epidermidis*	Opportunistic pathogen, see above
	after	*Staphylococcus hominis*	Opportunistic pathogen, see above
Tray/ledge entrance*	before	*Staphylococcus warneri*	Opportunistic pathogen, nosocomial infections, part of normal skin and mucus membrane flora (Becker, Heilmann, and Peters 2014)
	after	*Bacillus subtilis*	Non-pathogenic (Earl Ashlee M. 2008)

Microorganisms could still be found in the swabs of the assessed areas after the conventional disinfection protocol. These were often even different species than before the disinfection which could be due to the large sampled areas, or perhaps due to the fact that through hand disinfection a smearing of microorganisms can occur. In one occasion the same can be seen after the DCXpro disinfection as described in Table 5. Since *Micrococcus luteus* is commonly found on human skin (Boldock et al. 2018), it is suspected that it was brought in when the biological indicators were taken out of the vehicle which was done before the swab testing. In this experiment the disinfection program achieved an up to log 5 disinfection at all locations after 30 min.

Table 4 Summary of findings of the swab analysis before and after disinfection with automated DCXpro program, * biological indicator at the same location, grey: non-pathogenic microorganisms, remaining after disinfection

Location of sampling	**Time**	**Detected microorganism**	**Pathogenicity**
emergency bags*	before	*Bacillus cereus*	Foodborne pathogen, see above
		Bacillus megaterium	Non-pathogenic, rare infection cases (Bocchi et al. 2020)
	after	-	
Handle recliner	before	*Bacillus cereus*	Foodborne pathogen, see above
	after	-	
harness stretcher	before	*Micrococcus luteus*	Non-pathogenic, see above
	after	-	
rear patient chair seat*	before	*Bacillus mycoides*	Foodborne pathogen, see above
		Bacillus megaterium	Non-pathogenic, see above

		Staphylococcucs haemo-lyticus	Opportunistic pathogen, part of skin flora, nosocomial pathogen, carrier of resistance genes (Takeuchi et al. 2005) (Barros et al. 2012)
	after	-	
Tray/ledge entrance*	before	*Bacillus niacini*	Soil bacterium (Nagel and Andreesen 1991)
	before	*Corynebacterium glucuronolyticum*	Opportunistic pathogen, causes urogenital tract infections (Gherardi et al. 2015)
	after	*Micrococcus luteus*	Non-pathogenic, see above

4. Materials and Supplementary

Figure 2 QR-Code linking to a PDF with supplementary information of all experiments for interested parties

5. Discussion

Our experiments show that disinfection protocols based on the DCXpro technology can keep valuable parts of infrastructure safe for both staff and patients. While more testing and fine tuning is needed we are confident that digitalisation and automation of disinfection protocols in critical infrastructure is a crucial part of effective crisis management.

As part of our investigation we could show that the intended reduction of log 6 was well achieved in most of the experiments performed under real environment conditions, though not in all. Although biological indicators are very good, validated measures of log reduction, we wanted to show the effect on true contamination in the tested areas and how the disinfection handles real life conditions. The effect of H_2O_2 on various species of bacteria is also necessary to be evaluated. Hence, we defined several locations at the different evaluated settings and swabbed areas of interest before and after the treatment in addition to assessing the biological indicators. This helped to determine the bacteria occurring in these environments and also to define potential sources of human and animal infections. We are aware that these results are not as standardized as the biological indicators and their interpretation has to be done with caution. Nevertheless, these data are of great interest and represent important hints for further studies. Although in some cases the biological indicators showed a reduction lower than expected after the treatment in some spots (equipped room, dentist practice, and veterinary practice OR) the trend to a sufficient reduction (of at least log 5) is obvious. This becomes even more evident, when looking at the swab tests in the veterinary practice and the ambulance before and after treatment with H_2O_2. The comparison of the hand cleaning in the ambulances with the DCX technology showed that the disinfection is more time efficient and more thorough. Furthermore, from the five bacteria detected before disinfection only one was found after the disinfection program and the microbe in question is non-pathogenic. The disinfection by hand was also successful but only in 3 of the 7 found bacteria. While in the ambulance cleaned by hand, the swab test showed a greater distribution of bacteria species, the vehicle treated by the DCXplus featured more species of bacteria.

The disinfection procedure with DCX technology is not only faster and more efficient than conventional cleaning procedures, as proven by the real life examples of the veterinary clinic and the ambulance cleaning protocol comparison, but also less manual labour intensive. Furthermore the DCXpro is a validated and reproducible technology that can be easily monitored and controlled online. In the PCR lab it was shown that with little disruption during shift work schedule and high capacity workload, digital process management and control as well as online data viewing are tremendously advantageous in this regard.

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