Hydrogen Peroxide and Dental Environment

Subjects: Others

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Hydrogen peroxide is an effective biocide in its gaseous (vaporized and aerosolized) form against viruses, spores, fungi, and bacteria. The vaporized solution of hydrogen peroxide, which is based on water, is activated by plasma and acts as an oxidizing and disinfecting agent when it settles and contacts the surfaces of all objects in the room.

hydrogen peroxide | vaporized hydrogen peroxide | bio-decontamination | enumeration

1. Introduction

Infection control has always been a core objective in dentistry; however, it has risen to greater importance given the SARS-CoV-2 pandemic. Aerosolized viruses and bacteria, such as Tuberculosis, Candida auris, and Staphylococcus aureus, once inhaled by either patient or healthcare worker, can result in far-reaching health consequences [1][2][3].

Infected droplets can be spread by dental instruments from the mouth of the dental patient, such as high-speed rotating handpieces and ultrasonic devices. The contaminated aerosol settle on exposed surfaces, resulting in environmental contamination. Despite surface disinfection protocols, many of the inanimate objects are not routinely disinfected and together with the hands of staff become vectors for transmission of healthcare-associated infections [3][4]. Studies determined that established disinfection methods showed their inability to eliminate environmental contamination of certain pathogens that are associated with direct transmission [5]. In addition, an increase in the bacterial load after the use of a neutral detergent was reported. A neutral detergent is composed of surfactants, fillers, and chelating agents. They are typically not used in pathogen eradication [6]. Enhanced cleaning that deviates from traditional surface cleaning (traditional surface cleaning or terminal cleaning aims to reduce the number of pathogens on surfaces to reduce transmission) not only reduced the bacterial load in the environment but also reduced the number of organisms on the hands of staff. Disinfection procedures that involve physical contact with the surfaces (spray, wipe, and spray techniques) are widely used but are usually labor-intensive and not always effective, as it is impossible to reach all hidden surfaces. For this reason, it is imperative to investigate the efficacy and then adopt other infection control approaches to decontaminate the dental environment between patients and minimize the risk of transmission of diseases [5][7].

VHP generators are no-touch decontamination and therefore circumvent problems associated with operators during manual disinfection such as incorrect application and use of cleaning agents.

2. Vaporized Hydrogen Peroxide as a Non-Contact Decontamination System for Pathogens Associated with the Dental Environment

The characteristics of VHP decontamination are presented in Table 1 and Table 2. Bioquell was the manufacturer of 13 of the 19 machines assessed in the selected studies [8][9][10][11][12][13][14][15][16][17][18][19][20]; however, irrespective of the VHP unit used, all the studies reported favorable outcomes (towards the VHP generators rather than the aerosolized hydrogen peroxide (aHP) generators) for log reduction of the assessed pathogens. Methicillin-resistant Staphylococcus aureus (MRSA) was the most used bacterial pathogen in five studies and with the viruses Feline calicivirus, Human norovirus, and Murine norovirus featured in three studies.

Most of the VHP generators are equipped with monitoring systems, but part per million (ppm) monitoring is essential to ensure the desired concentration of hydrogen peroxide is reached for the desired dwell time. Additionally, the use of standardized validated Geobacillus stearothermophilus biological indicators [9][10][11][12][15][16][17][18][21], are important to set the benchmark for the efficacy of the VHP with the manufacturer's instructions. The surface that received the pathogen was predominantly stainless steel in the form of discs, coupons, or tape with the exceptions of cell culture well plates [8][11][12] and cryogenic tube caps [14]. Two authors used stainless steel as well as some additional materials [22][20]. The log kill was sufficient for all the authors to conclude that VHP generation was effective for the assessed pathogens. The studies that assessed aHP found a greater log kill with VHP generators [9][10].

Table 1. The characteristics of the included studies for VHP decontamination (*n* = 17).

Characteristics	*n* or *n* (%)
Publication Year	*n* = 17
2010	1 (0.05)
2011	3 (0.17)
2012	3 (0.17)
2014	1 (0.05)
2015	1 (0.05)
2016	2 (0.11)
2017	3 (0.17)
2019	2 (0.11)
2020	1 (0.05)

Characteristics	*n* or *n* (%)
Location	*n* = 17
United Kingdom	8 (0.47)
Sweden	3 (0.17)
USA	2 (0.11)
Brazil	1 (0.05)
France	1 (0.05)
Germany	1 (0.05)
The Netherlands	1 (0.05)
Hydrogen Peroxide Vapourizing machine	(*n* = 19) or *n*% of total machines
Aeroclave	1 (0.05)
Bioquell	1 (0.05)
Bioquell BQ-50	1 (0.05)
Bioquell Clarus C	2 (0.10)
Bioquell Clarus L	1 (0.05)
Bioquell Clarus R	3 (0.15)
Bioquell Clarus S	1 (0.05)
Bioquell Q10	4 (0.21)
Liquid Verne Veiling equipment	1 (0.05)
Sterinis aHP	1 (0.05)
Steris La Calhene VHP	1 (0.05)
Steris VHP	1 (0.05)
Sterinis system SR2	1 (0.05)
Assessed Pathogen:	*n* or *n*% of total pathogens
Candida	(*n* = 34)
Various *Candida species*	34 (100)

Characteristics	*n* or *n* (%)
Bacteria	(*n* = 27)
Acholeplasma laidlawii	1 (0.03)
Acinetobacter baumannii	1 (0.03)
Bacillus anthracis (Ames) spores	1 (0.03)
Brucella abortus	1 (0.03)
Burkholderia pseudomallei	1 (0.03)
Clostridium difficile	1 (0.03)
Escherichia coli	1 (0.03)
Geobacillus stearothermophilus biological indicators	9 (0.33)
MDR *Acinetobacter baumannii*	1 (0.03)
Methicillin-resistant *Staphylococcus aureus* (MRSA)	5 (0.18)
Mycoplasma pneumoniae	1 (0.03)
Mycoplasma gallisepticum	1 (0.03)
Mycobacterium tuberculosis	1 (0.03)
Vancomycin- resistant Enterococcus (VRE)	1 (0.03)
Yersinia pestis	1 (0.03)
Virus	*n* = 21
Adenovirus	2 (0.09)
Avian influenza virus (AIV)	1 (0.04)
Escherichia virus MS2	1 (0.04)
Feline Calicivirus	3 (0.14)
Foot and mouth disease (FMDV)	1 (0.04)
Human adenovirus type 1	1 (0.04)
Human norovirus	3 (0.14)
Influenza A virus (H1N1)	1 (0.04)

Characteristics	*n* or *n* (%)
Murine norovirus (MNV)	3 (0.14)
Pseudomonas virus phi6	1 (0.04)
Poliovirus	1 (0.04)
Rotavirus	1 (0.04)
Swine influenza virus (SwIV)	1 (0.04)
Transmissible *Gastroenteritis coronavirus* of pigs (TGEV)	1 (0.04)
Characteristics:	
Method of inoculation	*n* = 29, *n*% of total surfaces
Sabouraud's dextrose agar and fabric	2 (0.06)
Stainless steel 10 mm-diameter discs/coupon	5 (0.17)
Stainless steel 3 mm-diameter discs/coupon	1 (0.03)
Stainless steel 2.2 cm × 2.5 cm disc	1 (0.03)
Tyvek-pouched stainless steel disc/coupon	5 (0.17)
Plastic plates	2 (0.06)
Steel embossing tape 2.5 cm × 5 cm	1 (0.03)
Roller bottle	1 (0.03)
Unspecified stainless steel/coupon	4 (0.14)
Gauze	1 (0.03)
Glass	1 (0.03)
Painted joint tape	1 (0.03)
Wood	2 (0.06)
Ceramic tile	1 (0.03)
N95 Filter medium	1 (0.03)
Efficacy: Log kill	
>8 log	1

Characteristics	*n* or *n* (%)
>6 log	4
>4 log	4
>3 log	2
<2 log	2
1.3–3.5 log reduction	1
Log reduction not specified	4

Table 2. Summary of items regarded in the risk assessment of the chosen article.

Author	Country of Study	Aim/Objective	Pathogen Used	Methodology:- Hydrogen Peroxide Concentration	Blinding and Controls	Sample Hanling and Contamination Prevention	Failed Experiments and Data/Results Not Presented	Pathogens Placed on Material	Outcome (Level of Bio-Decontamination)
A Abdolrasouli et al. 2017	United Kingdom	In vitro evaluation of the efficacy of VHP on standard and outbreak *C. auris.*	34 different yeast isolates: 4 strains (*Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis*) 28 outbreak isolates of *C. auris*	Bioquell machine, No H_2O_2 liquid concentration, performed following manufacture instructions. 8 g of H_2O_2/m^3.	No blinding. One *C. aurus* control plate with no exposure to VHP. Six yeast-free control wells. No BI used. Done in triplicate. Wells of pathogen grown in a 96-well plate and desiccated, sealed, and kept at 4 °C until exposure to VHP. Viability was then assessed on SDA with *C. auris* control plate.	Did not state how long after fogging well plates were closed to prevent contamination.	One Indian *C. auris* and a specific Indian strain not named. Non-exposed *C. auris. Candida species* and VHP exposed C. aurus survive in a desiccated state. Data not shown.	Well plates.	Data provided evidence that *C. auris* (and other *Candida species*) are effectively killed with a 96.6–100% by H_2O_2 vaporization.
E Berrie et al. 2011	United Kingdom	In vitro efficacy of inactivation of	Dried recombinant *adenovirus*	Bioquell Clarus S machine, 60 mL	Exposed and non-exposed	Immediately after the	One to two logs of	Stainless steel 10-mm-	Data provided evidence

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		recombinant adenovirus by VHP.	(Ad5GFP)	of 30% H_2O_2 liquid concentration, performed dwell time 45 min. Whole VHP cycle 3 h	samples to VHP. BI (Biological Indicator) indicators used. The experiment is one disc per dilution and repeated in triplicate	experiment, the samples were transferred to a sterile microbiological safety cabinet.	pathogen lost due to drying or recovery method compared to wet reference samples compared to experiment two at the titer. Viability reduction data explained in the article.	diameter discs.	that Adenovirus are effectively killed with a 7.6 to 9.4 log kill by H_2O_2 vaporization.
M Eterpi et al. 2010	France & United Kingdom	In vitro evaluation of the efficacy of VHP and cold VHP sterilization against *Mycoplasma.*	*Mycoplasma gallisepticum, M. pneumoniae*, and *A. laidlawii*	VHP100 Steris machine. 30% H_2O_2 liquid concentration. Three cycles 1200 ppm/15 min; 400–500 ppm/60 min; 180–200 ppm/4 h.	No blinding. VHP unexposed samples kept under a laminar flow hood in sealed Petri dishes for the same time cycle and managed the same as exposed coupons. No BI used. Six treated samples with each method and repeated for times.	Samples were transferred to an SP4-glucose broth immediately after VHP exposure.	Less than one log of pathogen lost due to drying or recovery method as described by Nagatomo et al. 2001 with loss due to recovery ≤0.5 log. Neutralization an additional ≤0.5 log. Viability reduction data explained in the article.	Stainless steel coupons of 1 cm × 3 cm.	Data provided evidence that Mycoplasma is effective with a >4 log kill by H_2O_2 vaporization.
T. Y. Fu et al. 2011	United Kingdom	Compare the efficacy, efficiency of VHP and aHP.	Methicillin-resistant Staphylococcus aureus (MRSA), Clostridium difficile and Acinetobacter baumannii.	Bioquell Clarus R machine. 30% H_2O_2 liquid. SR2 Sterinis machine with a 5% H_2O_2 liquid and silver ion (50 ppm) and orthophosphoric	No blinding. Both exposed and non-exposed to VHP. BI used. Four cycles per machine with each	Did not state how long after fogging discs were transferred to prevent contamination, nor the	No pathogens lost or contaminated samples were described or considered in	Stainless steel discs with a diameter of 10 mm.	The VHP system achieved a greater level of biological inactivation between 4–6 log for most locations than the aHP

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				acid (<50 ppm), dose 6 mL/m^3 recommended by the manufacturer.	cycle consisting of three unexposed VHP/aHP and three dry VHP/aHP discs for water, 3% BSA (Bovine Serum Albumin) and 10% BSA. The control was cycled separately over four cycles.	overnight drying to prevent contamination.	the methodology. All data presented.		system 1–5 log depending on the pathogen.
Goyal et al. 2014	United Kingdom	Evaluate the in vitro efficacy of three volumes of VHP on selected viruses with surface contamination.	FCV as a surrogate of *human norovirus*, TGEV as a surrogate for the SARS virus, *human adenovirus* type 1, AIV (A/chicken/Maryland/2007[H9N9]) and SwIV (A/swine/Minnesota/2010 [H3N2]).	Bioquell Clarus L machine. 35% H_2O_2 liquid. Hydrogen peroxide at 2 mL/min for 1, 2, 5 min followed by 1.5 mL/min or 15 min equating to the following different volumes: 25, 27, and 33 mL with the treatment time between 2–3 h for the completed cycle.	No blinding. Non-VHP exposed inoculated discs at room temperature. Four BI were exposed to the VHP in corners of the environmental chamber. Positive BI control was not exposed to VHP. 8% Fetal Bovine Serum (FBS) served as soiling present in the culture medium. Each experiment had inoculated	Discs are left to dry in a biosafety cabinet to prevent contamination. After VHP the discs including the non-exposed control discs were transferred immediately to the environmental chamber for titration.	Data was determined concerning the control disc. This allows direct comparison to the test and control discs having the same log loss, making the comparison more accurate. But also leads to not knowing what the log loss of viral load is. Loss of virus log particles during the methodology of drying and	10 mm stainless steel discs.	VHP was virucidal for viruses assessed dried on surfaces, suggesting that VHP can be considered for the disinfection of virus-contaminated surfaces based on the 8% FBS surface contamination.

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					discs exposed to each vaporized volume of VHP and one disc not exposed to VHP.		recovery. Not calculated		
Holmdahl et al. 2011	Sweden	Comparison of VHP and aHP to BI in various locations.	BI with *G. stearothermophilus*	Steris VHP machine. 5% H_2O_2 liquid. 6 mL/m^3 with 100–150 ppm. Bioquell Q10 machine. 35% H_2O_2 liquid. 900 mL per test and results in 6.6 g/m^3. 338 ppm peak, 3 h.	No blinding. BI was used as control.	BI in Tyvek pouches	Direct comparison of two machines on BI. All results presented.	BI stainless steel disc placed in various locations in the room.	All results presented for the same areas assessed for the two machines. VHP showed a 100% negative result while aHP presented with multiple positive results, the inconsistency with the aHP was 10% kill (100 ppm) followed by two cycles of 79% kill, with the ppm in cycles 2 and 3 being 130 and >150 ppm respectively.
Holmdahl et al. 2016	Sweden	Evaluate the efficacy of VHP in six locations for two virus pathogens with surface contamination.	FCV, feline permissive cell line (FCWF). MNV and permissive murine cell line (RAW 264.7)	Bioquell Q10 machine. As per the manufacturer. No H_2O_2 liquid concentration. Gassing time 40–50 min, dwell time 15 min. VHP ppm range 474–505 ppm with a total cycle time of 3 h.	Virus prepared in triplicate in well plates. Two inoculated plates and BI not VHP exposed two areas of the control room. BI exposed at all the positions with	Well plates left to dry at room temperature under a hood for 2 h and stored.	Loss of virus log particles during the methodology of drying and recovery were calculated.	Well plates	VHP was virucidal for viruses assessed dried on surfaces, suggesting that VHP can be considered for the disinfection of virus-contaminated surfaces based on the 10% FBS surface contamination.

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					VHP next to virus inoculated plates. Each VHP exposure experiment was repeated in triplicate.				
Holmdahl et al. 2019	Sweden	Assess *norovirus* viability of cytopathic the effect after VHP.	Two *human norovirus* field strains, genogroup I and II. *Murine norovirus*.	Bioquell Q10 machine. No H_2O_2 liquid concentration. 860 ppm VHP for 33 min gassing and 55 min dwell. This resulted in 205 g of H_2O_2 used.	No blinding. BI and mock samples with no VHP exposure.	Virus samples dried in 35 mm diameter wells of six-well plates mock and VHP treated samples.	Data was determined concerning the lowest detection limit of $10^{-0.5}$. This allows direct comparison to the test and control discs having the same log loss, making the comparison more accurate. But also leads to not knowing what the log loss of viral load is.	Well pates	BI deactivated and *norovirus* log 5 kill.
Lemmen et al. 2015	Germany	Efficacy of VHP on five pathogens dried onto various hard surfaces.	MDR MRSA and MDR VRE, MDR *A baumannii*. BI as proxy for *D. difficile*	Bioquell Q10 machine. 30% H_2O_2 liquid. Three cycles were performed. The dose of 11.2 g/m^3 achieved after 50–52 min until hydrogen peroxide was 500–600 ppm.	No blinding. BI used. Four of each material inoculated with the pathogen and distributed in four locations exposed to VHP and the same number	Kept on a sterile basis until experiment and after VHP exposure transferred to a sterile glass tube with 1 mL distilled water.	Lost pathogens are known and presented in the article and mean log reduction is calculated.	Stainless steel discs, gauze	VHP inactivated all spore BI (>6 log_{10} reduction), and no MRSA, VRE, or MDR *A baumannii* were recovered from the stainless steel and cotton carriers (>4–5 log_{10} reduction, depending on the

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				20 min dwell time.	not exposed to VHP as controls. BI placed in 4 corners of the room and 3 challenge locations.				starting inoculum). VHP was equally effective at all carrier locations. No difference in efficacy
Montazeri et al. 2017	USA	Inactivation of *human norovirus* after VHP exposure.	FCV. Outbreak *human NoV* GI.6 and GII.4.	AeroClave System 3110. 7.5% H_2O_2 liquid. No air handling unit during vapor process, at end of cycle turned on for 20 min. 7.1–15.9 mL/m^3 was achieved, with 5 min dwell time following the manufacturer's recommendation. Then the air handling unit was switched on for 20 min.	No blinding. No BI used. 7 locations in BSL-3 laboratory assessed with VHP. Inoculated coupons not exposed to VHP were outside the laboratory for the duration of the experiment.	Air-dried in a biosafety hood. And used immediately for the experiment. After the experiment, the samples were transferred to PBS tubes.	Data was determined to the control disc. This allows direct comparison to the test and control discs having the same log loss, making the comparison more accurate. But also leads to not knowing what the log loss of viral load is.	Stainless steel embossing tape	No trend was observed for *human NoV* GI.6 reduction as a function of H_2O_2-based disinfectant formulation concentration. However, increasing the concentration from 7.1 to 12.4 mL/m^3 enhanced viral genomic copy number reduction for GII.4
Murdoch et al. 2016	United Kingdom	Assess the application of three different liquid concentrations for VHP.	MRSA and *Geobacillus stearothermophilus*	Bioquell BQ50 machine. 5, 10, and 35% H_2O_2. 640 g hydrogen peroxide over 40 min and 200 min dwell time.	No blinding labeled containers. BI used. Positive and negative controls. Every 10 min throughout the experiment a BI was exposed for 10 min.	All specimens were placed in labeled 30 mL containers.	No pathogens lost or contaminated samples were described or considered in the methodology. All data presented.	Stainless steel discs	35% hydrogen peroxide is ideal.
Otter et al. 2012	United Kingdom	Efficacy of VHP against	MRSA	Bioquell Clarus R machine. No	No blinding. No BI used.	Air-dried in the test room air,	No pathogens	Stainless steel discs	Relative susceptibility to

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		methicillin-resistant Staphylococcus aureus on various surfaces.		H_2O_2 liquid concentration. VHP mean concentration 134 ppm.	Control discs were not VHP exposed. The experiment ran in triplicate per period for each contaminant material.	then VHP exposure and immediately enumerated.	lost or contaminated samples were described or considered in the methodology. All data presented.		VHP was 10% BSA < TSB < 3% BSA = water. At a ppm achieved and >75 min exposure, no MRSA was recovered on the discs.
Petit et al. 2017	Brazil	Efficacy of VHP against *foot-and-mouth disease.*	Three serotypes of *Foot-and-mouth disease virus (FMDV)*	Bioquell Clarus R machine. 35% H_2O_2 liquid. 115 min. VHP injection time 75 min, 40 min dwell time.	No blinding. No validated BI manufactured by VHP producers. Positive controls of three serotypes. Three replicate cycles of 15 BI produced from FMDV for VHP exposure. Five samples for each viral serotype were produced. One plosive control per serotype for the duration of the experiment was stored in a refrigerator.	Dried in class 2 biological safety cabinet.	No pathogens lost or contaminated samples were described or considered in the methodology. All data presented.	Inside the cap of the polypropylene cryogenic tube.	Three *FMDV* serotypes showed full inactivation.
Pottage et al. 2012	United Kingdom	Comparison of log kill of BI vs MRSA after VHP exposure.	*G. stearothermophilus* and MRSA	A Steris VHP-1000ARD machine. 35% H_2O_2 liquid. 750	No blinding. Random removal of VHP exposed samples. 18	Inoculated stainless steel discs air-dried for 1 h.	No pathogens lost or contaminated samples	BI on stainless steel discs sealed in	BI greater log kill than MRSA for the same periods of exposure.

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				ppm maintained in chamber.	MRSA and 18 BI indicators placed in sterile Petri dishes and VHP exposed for pre-determined periods. Three unexposed stainless steel discs of each pathogen were used.		were described or considered in the methodology. All data presented.	Tyvek packages.	
Pottage et al. 2019	United Kingdom	Efficacy of VHP on dried bacteria.	*Bacillus anthracis* (Ames) spores, *Brucella abortus*, Burkholderia pseudomallei, Escherichia coli, Mycobacterium *tuberculosis* and *Yersinia pestis*.	Bioquell Clarus C machine. 35% H_2O_2 liquid. 90 min cycle.	No blinding. Three controls tied in double plastic bags to determine the loss of log pathogen. 3 control samples were used as the start pathogen load. 12 produced BI for each VHP run to allow triplicate exposure. Three control BI from a VHP manufacturer used per VHP cycle.	Dried in a biological cabinet for 1 h.	No pathogens lost or contaminated samples were described or considered in the methodology. All data presented.	Stainless steel coupons in Petri dishes	This study demonstrates that VHP can inactivate a range of HG3 agents at high concentrations with associated organic matter, but *M. tuberculosis* showed increased resistance to the process.
Tuladhar et al. 2012	The Netherlands	Virucidal efficacy of VHP against respiratory and	*Poliovirus, human norovirus* genogroup II.4 (GII.4), *murine norovirus* 1, *rotavirus, adenovirus*,	Boneco 7131 machine. 12% H_2O_2 liquid. 120–134 ppm at	No validated BI manufactured by VHP	Dried in a biohazard cabinet.	No pathogens lost or contaminated	Stainless steel, framing panel, and	VHP effective against pathogens assessed.

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		enteric viruses on various materials.	and *influenza A* (H1N1) *virus.*	a flow rate of 2.3 L/h.	producers. Triplicate samples per virus were performed twice. Control samples were not VHP exposed.		samples were described or considered in the methodology. All data presented.	gauze carriers.	
Wood et al. 2020	USA	Assess the decontamination efficacy of VHP on phages.	Bacteriophage viruses, MS2 and Phi6	Humidifier with 3 or 8% H_2O_2 liquid generated to 25 ppm. Bioquell Clarus C machine. 35% H_2O_2 liquid. 25 ppm and 400 ppm generated.	No validated BI manufactured by VHP producers. Inoculated samples, not VHP exposed, and inoculated samples VHP exposed. Two blank samples. Completed in triplicate.	Samples made and dried in a biosafety cabinet. After the experiment coupons were sealed and transferred to the biosafety cabinet.	No pathogens lost or contaminated samples were described or considered in the methodology. All data presented.	Stainless steel, glass, tile, N95 mask material, painted joint tape, wood.	Extrapolating from these results for both an enveloped and non-enveloped virus, we would expect LCHP would be a viable decontamination option for *EBOV* for relatively clean surfaces

3. Summary

The overarching conclusion is that H_2O_2 delivered as VHP was an effective method to achieve large levels of log kill on the assessed pathogens. All the articles have applications to dentistry bio-decontamination. They showed the efficacy of VHP in spaces and surfaces similar to a dental clinic. Further investigation of VHP in dental clinics is required with certain variables that must be known and standardized to assure the validity and reproducibility regarding the H_2O_2 concentration, dwell time, and a constant ppm or defined ppm range during the dwell time. The enumerated pathogens at every step of the methodology, from inoculation on the test surface to the enumeration of the exposed and unexposed samples, should be completed. This safeguard will ensure the correct determination of the log loss of pathogens. From the results of the reviewed articles, a statistically calculated sample size performed in triplicate should be standardized.

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