The use of Hypochlorous Acid (HOCL) on environmental surfaces in healthcare facilities.

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Aquaox, LLC. (Aquaox) manufactures, distributes, installs and services on-site generators that produce Electrochemically Activated Solutions (ECAS) used for cleaning, sanitizing and disinfecting. These ready-to-use solutions contain predominantly hypochlorous acid by electrolyzing a sodium chloride solution. Hypochlorous acid solutions clean surfaces and destroy microorganisms.

Hypochlorous acid is a weak, highly unstable acid which can only exist in a solution.[1] Aquaox produces hypochlorous acid by electrolysis of a dilute salt solution passing through an electrolytic cell. At an acidic to neutral pH, the predominant chemical species is hypochlorous acid (HOCL). [2] Hypochlorous acid has demonstrated antimicrobial activity against numerous bacterial, viral and fungal pathogens, including antibiotic-resistant strains.[3]

Hypochlorous acid has a history of safe use as a disinfectant in numerous applications including household, hospital, food preparation, industrial and pharmaceutical applications. Hypochlorous acid is approved by the FDA for direct patient tissue contact as a wound care agent. It has also been used in dental root canal therapy.[4]

Numerous studies have found ECAS to be highly efficacious, as both a novel environmental decontaminant and a topical treatment agent (with low accompanying toxicity). It is clear that ECAS is active against a broad spectrum of microorganisms (see table 1)[5].

The effect of ECAS on bacterial cells has been directly observed using transmission electron microscopy, atomic force microscopy and fluorescence microscopy providing evidence of the direct effects on the bacterial cell envelope. Moreover, pH-neutral ECAS was found to have a significant sporicidal activity, equivalent to that of 50,000ppm hypochlorite. The effective removal of mature biofilms from the surface and stainless steel after treatment with ECAS has been shown in vitro by light and electron microscopy. ECAS has been shown to effectively inactivate pathogenic eukaryotic species and is thought to damage cellular functional structures. PH neutral ECAS shows significant activity against *Cryptosporidium parvo* oocyst in contrast to little or no activity using a free chlorine solution. The ability of ECAS to inactivate fungal toxins has been

[1] National Center for Biotechnology Information—NCBI (2015) it Acid, PubChem 867 Compound Database; CID=24341, https://pubchem.ncbi.nlm.nih.gov/compound/24341 868 (accessed Aug. 9, 2015).
[2] Sansebastiano, G. et al. Page 262 in Food Safety: A Practical and Case Study Approach (Ed: R. J. Marshall) 2006, Springer Science & Business Media, Berlin.
[3] Wang TX, Kelly MD, Cooper JN, Beckwith RC, Margerum DW. Equilibrium, kinetic, and UV-spectral characteristics of aqueous bromine chloride, bromine, and chlorine species. *Inorg Chem.* 1994; 33:5872– 5878.
[4] European Union Risk Assessment Report, Sodium Hypochlorite, CAS No: 7681-52-9, EINECS No: 231-668-3, Final Report, November 2007.
[4] European Union Risk Assessment Report, Sodium Hypochlorite, CAS No: 7681-52-9, EINECS No: 231-668-3, Final Report, November 2007.
[5] European Journal of Clinical Microbiology & Infectious Diseases: Electrochemically activated solutions: evidence for antimicrobial efficacy and applications in healthcare environments. R.M.S. Thorn, S.W.H. Lee, G.M. Robinson, J. Greenman, D.M. Reynolds ISSN 0934-9723, DOI 10.1007/s10096-011-1369-9. July 2011.

investigated using the aflatoxin of *Aspergillus parasiticus* and a significant reduction in the mutagenic potential of this aflatoxin was measured using a conventional Ames test. Numerous studies have demonstrated the virucidal activity of ECAS against a broad range of targets. Fogged ECAS has been found to significantly reduce the surface levels of both human norovirus and surrogate viruses, and ECAS have shown significant activity against *Human immunodeficiency virus* (HIV), even when infectious particles are pre-adsorbed onto an animate surface[5].

Aquaox manufactures a number of devices that electrochemically generate hypochlorous acid products at concentrations up to 0.165% (1650ppm free available chlorine) as one-step cleaner & disinfectant for multiple uses.

Some clients purchase bottled solutions for use as one-step cleaner & disinfectant on hard, non-porous surfaces[6]. These products at concentrations of 0.0275% and 0.0525% can be applied using a mop/bucket, a trigger spray bottle, or a pressurized spray system. Some clients choose to have Aquaox install a device within their facility to produce on-site solution for the same purpose and effect. Some of these clients obtained FDA approval for bottling hypochlorous acid solutions used for topical applications. Some clients use bottled or on-site generated hypochlorous acid for water treatment or for use in food preparation.

This review was conducted to evaluate the efficacy of applying bottled hypochlorous acid solutions on environmental surfaces compared to applying on-site generated hypochlorous acid solutions on environmental surfaces in healthcare environments.

Based upon efficacy studies documented by Aquaox in two Technical Dossiers[7] titled: '*Aquaox On-site generated solutions – Technical Summary*' and ' *Aquaox EPA Certified solutions – Technical Summary*', I concluded that all three Aquaox-bottled hypochlorous acid solutions have met the requisite governmental regulatory requirements. I also confirm that all three hypochlorous products have achieved EPA registration through required rigorous testing at independent EPA-approved laboratories, assuring their safety and effectiveness as hospital disinfectants. Additionally, comparing Aquaox solutions with other EPA-certified disinfectants, I concluded that AX-1650 disinfectant is the highest concentration HOCL product registered by the EPA, and together with AX-525 disinfectant, is included in EPA's List N[8]: *'Disinfectants for Coronavirus (COVID-19)'* and the EPA List Q[9]:' *'Disinfectants for Emerging Viral Pathogens (EVPs)'*

Further, Aquaox has rigorously tested its AX-275 disinfectant solutions using USP51 Antimicrobial effectiveness studies and ASTM laboratory test methods, which were conducted in compliance with the ISO 10993 Standards. Testing included a wide range of bacteria, viruses, fungi and spores at various strengths and various contact times. The results of these studies confirmed that AX-275 disinfectant shows 99.99955% effectiveness against pathogens at concentrations as low as 250ppm within 30 seconds[10].

[6] https://www.aquaox.com/Aquaox On-site generated solutions – Technical Summary/
[7] https://www.aquaox.com/Aquaox EPA Certified solutions – Technical Summary/
[8] https://www.epa.gov/coronavirus/list-n-advanced-search-page-disinfectants-coronavirus-covid-19
[9] https://www.epa.gov/pesticide-registration/disinfectants-emerging-viral-pathogens-evps-list-q
[10] Table 1, Efficacy Test summary Aquaox Disinfectant with a contact time of 1 minute or less

AX-525 shows 99.99989% effectiveness against *Clostridioides Difficile* spore in 10 minutes in the presence of a three-part soil load[11]. AX-1650 (at 1305ppm) shows 99.9983% effectiveness against *Clostridioides difficile* spore in 5 minutes in the presence of a three-part soil load[12]. AX-1650 (at 1062ppm) shows 99.9946% effectiveness against Clo*stridioides difficile* spore in 10 minutes in the presence of a three-part soil load[13]. AX-1650 (at 1305ppm) shows 99.99975% effectiveness against *Clostridioides difficile* spore in 10 minutes in the presence of a three-part soil load[13]. Aquaox is pursuing laboratory confirmation that AX-1650 (at 1490ppm) is 99.9999% effectiveness against *Clostridioides difficile* spore in 5 minutes or less in the presence of a three-part soil load.

GLP efficacy tests for on-site generated hypochlorous acid solutions were conducted at various contact times at various strengths of active chlorine.

At 1490ppm, on-site generated HOCL shows 99.999 to 99.9999% effectiveness against bacteria, mycobacteria, enveloped viruses, yeast, and bloodborne pathogens at a contact time of 2 minutes or less. At 477ppm, on-site generated HOCL shows 99.999 to 99.9999% effectiveness against bacteria, mycobacteria, enveloped viruses, yeast and bloodborne pathogens at a contact time of 5 minutes or less. At 248ppm, on-site generated HOCL shows 99.999 to 99.9999% effectiveness against bacteria, enveloped viruses, and fungi at a contact time of 10 minutes or less.

On-site generated HOCL at 477ppm shows 99.9995 to 99.9999 effectiveness against *Clostridioides Difficile* spore in 10 minutes or less in the presence of a three-part soil load. HOCL, on-site generated at 1490ppm, shows 99.9995 to 99.9999 effectiveness against *Clostridioides difficile* spore in 5 minutes or less in the presence of a three-part soil load[14].

The results of these studies confirmed that less contact time is required to eradicate pathogens when the concentration of active chlorine is increased. This is because a higher concentration of active chlorine can more easily overcome the three-part soil load mimicking the biofilm and organic debris on environmental surfaces.

Further, it is widely observed that on-site generated HOCL is more efficacious against microorganisms than bottled HOCL products. Electrochemically activated solutions (ECAS) are transformed into an activated 'metastable' state, exhibiting elevated chemical reactivity resulting in the modification of molecular structures. The transformation into a 'metastable' state is not permanent. Upon the generation and recovery of ECAS, the chemical species present will shift spontaneously from this thermodynamically un-equilibrated condition to a stable non-active form, during what is known as the period of relaxation. In contrast to a reduction in residual free chlorine, studies have shown that the pH, ORP, conductivity and chloride ion concentration levels are all relatively stable. During short-time storage, indicating that the oxidizing potential of bottled

[11] Final Study Report Standard Quantative Disk Carrier Test method for Aquaox Disinfectant 460.Project#: A15758. ATS Labs, 1285 Corporate Center Drive, suite 110, Eagan, MN 55121. November 2013

[12] Final Report OECD Quantitative Method of AX-1650 against spores of *Clostridioides difficile* on Inanimate, Hard Non-Porous Surfaces. Project#: 1079-103. Microbac Laboratories Inc., 105 Carpenter Drive, Sterling, VA 20164, January 2023

[13] Final Report OECD Quantitative Method of AX-1650 against spores of *Clostridioides difficile* on Inanimate, hard No-Porous Syrfaces. Project#: 1079-103. Microbac Laboratories Inc., 105 Carpenter Drive, Sterling, VA 20164, April 2023

[14] Study Report against spores of *Clostridioides Difficile*. JDM Scientific Research Organisation Private Limited, Block No. 58, Village Lasundra-391775, Taluka Savli District-Vadodara, Gujarat (India)

HOCL is largely retained. Nevertheless, the antimicrobial efficacy of ECAS is thought to be superior because of 'non-specific', short-lived highly reactive oxygen-chlorine compounds present in on-site generated HOCL, but not necessarily any longer present in bottled HOCL.

The presence of organic loading has been shown to significantly reduce the antimicrobial potential of HOCL. Accurate cleaning methods for the removal of grease, dirt and organic debris from environmental surfaces play a major role in HOCL efficacy. Reviewing the Aquaox ICS manual[15], it is apparent that the use of microfiber is a prerequisite in Aquaox Infection Control System (ICS) cleaning protocols.

Siani et al. used multiple spore types to demonstrate that 10 seconds of contact time is not enough for a cleaning wipe soaked in a sporicidal agent to produce a 99.99% reduction on inoculated surfaces. Our data demonstrated that microfiber cloths could remove 99.4% reduction on spores from an inoculated ceramic surface when a cleaning agent (that has no sporicidal activity) was used to wet the surface and the contact wiping time was 5 seconds[16].

Microfiber cloths can remove significantly more *Clostridioides difficile* spores from surfaces compared with cotton cloths. In addition, the ability of microfiber cloths to retain spores provides convincing evidence that this cleaning approach could reduce transfer of microorganisms. Utilization of microfiber cleaning cloths in the health care environment may diminish the risk of spread of antibiotic-resistant organisms[17].

Wiping surfaces clean with a microfiber wetted with HOCL will remove this soil load effectively whereas the HOCL will kill all pathogens while the surface is allowed to air-dry. As such, due to wiping effect of the microfiber, the HOCL is not actively combatting an organic load and as such broad-spectrum antimicrobial activity is recorded whereby rapid disinfecting times are achieved. To illustrate this, efficacy studies with AX-275 disinfectant without organic load against spores shows that on-site generated HOCL provides 99.99955% effectiveness in 30 seconds[18].

Rapid re-contamination of environmental surfaces after manual cleaning/disinfection suggests that alternate mitigation strategies should be evaluated. Test whereas environmental service technicians conducted daily room cleaning and disinfection and then was followed by targeted cleaning of high-touch surfaces by research staff using hydrogen peroxide wipes, EPA-approved for C. auris showed that these surfaces located close to the patient were commonly re-contaminated with C auris by 4 hours after cleaning[19]. Environmental surfaces near C auris-colonized patients were

[15] Aquaox ICS Manual

[16] Siani H, Cooper C, Maillard J. Efficacy of "sporicidal" wipes against Clostridium difficile. Am J Infect Control 2011;39:212-8.

[17] Microfiber cloths reduce the transfer of Clostridium difficile spores to environmental surfaces compared with cotton cloths. Adriana N. Trajtman MS, Kanchana Manickam PhD, Michelle J. Alfa PhD, Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada, St. Boniface Research Centre, Winnipeg, MB, Canada, Diagnostic Services of Manitoba, Winnipeg, MB, Canada

[18] Final Study Report Time Kill Assay for Aquaox Disinfectant 250. Project#: A15628. TS Labs, 1285 Corporate Center Drive, suite 110, Eagan, MN 55121. November 2013.

[19] Candida auris Rapidly Recontaminates Surfaces Around Patients' Beds Despite Cleaning and Disinfection. Infection control Today, May 4, 2022, Tori Whitacre Martonicz

rapidly re-contaminated after cleaning/disinfection[20], told Infection Control Today®. "Rapid re-contamination of environmental surfaces after manual cleaning/disinfection suggests that alternate mitigation strategies should be evaluated."

HOCL is effective against Biofilm. Undiluted (500ppm) and mildly diluted (50ppm) HOCL removed the biofilm while the more diluted (5ppm) HOCL did not have any immediate effect on the biofilm. Re-growth of the biofilm occurred after 24 h of biofilm treatment with HOCL, showed by the increase in colony forming units. Re-growth of planktonic bacteria however, occurred only after 72 h of treatment [21]

Frequent cleaning/disinfection of surfaces around patients with (on-site generated) HOCL should be encouraged. Patients as well visitors could be handed wipes wetted with HOCL and be asked to wipe their immediate surroundings. Electrostatic spraying of HOCL after or in between cleaning of environmental surfaces with HOCL is a quick, not-labor intensive method to disinfect surfaces while encouraging wiping surfaces and hands with HOCL -wipes will prevent re-contamination and spread of C.auris.

Aquaox HOCL products are liquids which contain a maximum concentration of 0.1650%. As a liquid, inhalation exposure to applicators, patients and other potentially exposed individuals to hypochlorous acid would require exposure to an atmosphere which contains an aerosol of hypochlorous acid. Aquaox has determined that the recommended pressurized spraying system for product application generates an aerosol with a particle size diameter of approximately 30 to 50 µm. In the average adult, particles larger than 10 µm are deposited in the nasopharynx and cannot penetrate to tissue below the level of the larynx. Once deposited, particles are then subject to various particle clearance mechanisms.[22] Based on a particle size range of approximately 30 to 50 µm Aquaox HOCL products' potential inhalation exposure would be limited to the nose and oral pharynx. The bronchiolar and alveolar regions of the lung would not be exposed[23].

The preclinical testing described above has demonstrated that AQUAOX Disinfectant, and by extrapolation all AQUAOX Disinfectant products marketed at hypochlorous concentrations less than 1650 ppm are not irritants following inhalation, dermal or ocular exposure. Based on these findings AQUAOX Disinfectant products are not considered to represent a risk to the health of applicators, patients or other potentially exposed individuals by the inhalation route of exposure.

A study evaluating the effects of aerosolized HOCl cellular effects on lung tissue showed no signs of impaired cellular viability, cytotoxicity, oxidative stress, or inflammatory response (as indicated by 12 cytokine measures) were found.

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[20] Sarah Sansom, DO, MS, attending physician of infectious diseases, Rush University Medical Center, Chicago, Illinois, and coauthor of the study. Gabrielle Gussin, ASPPH, and the principal investigators Mary Hayden, MD, Rush University Medical Center; and Susan Huang, MD, MPH, University of California Irvine

[21] The effect of anolyte and a combination of anolyte and catholyte on biofilms,T E Cloete, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa website http://www.wrc.org.za ISSN 0378-4738 = Water SA Vol. 32 No. 2 April 2006

[22] Casarett & Doull's Toxicology The Basic Science of Poisons, Sixth Edition, McGraw-Hill, 2001, p523.

[23] Ira W. Daly, Ph.D., DABT, RAC, CBiol FRSB, ERT

That is, the 24-hour exposure of the pulmonary cell lines to 350-ppm and 550-ppm aerosolized solutions of HOCl, dispersed at a high rate, were comparable to exposing lung tissue to air[24].

After studying all materials referred to in the footnotes, I am confident that implementation of the Aquaox Infection Control System (ICS) in healthcare environments will improve cleanliness by safely reducing the overall soil load present on all surfaces (high touch surfaces, walls, ceilings, furniture, medical equipment, and curtains). I assert that following Aquaox ICS protocols will result in effective whole-room disinfection, and reduce labor and human error while applying safe, natural disinfectants with microfiber cloth wipes. Utilizing electrostatic sprayers, hypochlorous acid also gets applied to surfaces that cannot be touched or wiped, disinfecting these surfaces and (indirectly) sanitizing and improving air quality.

I therefore advise healthcare facilities to clean and disinfect using hypochlorous acid when treating and preventing infection from wounds or periodontal disease, and also to wipe and electrostatically spray (and allow to air-dry) medical equipment and all environmental surfaces. This will clean and disinfect these surfaces, and (indirectly) sanitize the air.

[24] Pre-Publication Draft Summary of ionogen IIVS Lung-Toxicology Study. Abstract of the Study Results Evaluating the Effects of Aerosolized HOCl On Lung Tissue Chuck Jones, PhD and Bergein F. Overholt, MD, MACP

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