

## BIOLOGICAL EFFICACY

EVIDENCE TO DEMONSTRATE THE DECONTAMINATION TECHNIQUE IS EFFECTIVE AT INACTIVATING COMMON HOSPITAL PATHOGENS AND AN ACCEPTABLE SURROGATE TO SARS-COV-2 UNDER THE RECOMMENDED OPERATING CONDITIONS.

Human Coronavirus Strain 229E- “AX250 demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log<sub>10</sub>” (1)

“The recommendation of 0.1% (1000 ppm) in the context of COVID-19 is a conservative concentration that will inactivate the vast majority of other pathogens that may be present in the health-care setting.” (2)

### SUMMARY OF RESULTS

Test Substance:	AX250 Batch # AX-13196-0210
Dilution Tested:	Ready to use A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.
Virus:	Human Coronavirus, strain 229E, ATCC VR-740
Exposure Time:	30 seconds
Exposure Temperature:	Room temperature (20.0°C)
Organic Soil Load:	1% fetal bovine serum
Efficacy Result:	Under these test conditions, AX250 (Batch # AX-13196-0210) demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log <sub>10</sub>

### STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, AX250 (Batch # AX-13196-0210), ready to use, demonstrated a 99.994% reduction in viral titer following a 30 second exposure time to Human Coronavirus as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25LOG<sub>10</sub>.**

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“...hypochlorous acid and hypochlorite ions promote amino acid degradation and hydration, what corroborates with the mechanism of action performed by WHO (2016), when affirming that chlorine compounds lead to inhibit actions enzymes, protein denaturation and inactivation of nucleic acids and, therefore, it is recommended as a disinfectant agent for the prevention and control of pandemics related to respiratory infections, such as COVID-19.” (3)

“Sterilox HG or 10% household bleach resulted in a 5 log<sub>10</sub> or greater CFU reduction of *C. difficile* spores, VRE, and MRSA in 10 minutes” (4)

“We conclude that HOCl solution as a liquid or fog is likely to be effective in disinfecting common settings to reduce NV exposures and thereby control virus spread via fomites.” (5)

“Within the framework of the results of our study, we believe that electrolyzed water can be regarded as a cost-effective disinfectant that is successful in controlling hospital infections, as it had an effect on bacteria showing various resistance patterns.” (6)

Sodium dichloroisocyanurate (NaDCC tablet): Hep A, Hep B, Porcine Epidemic Diarrhea virus (PEDs), Influenza Virus H1N1, Norovirus (7)

“NaDCC is an alternative source of FAC (HOCl). Accordingly, the significant body of evidence on the antimicrobial action of chlorine is as relevant to NaDCC as it is to NaOCl and other sources of chlorine... Susceptibility to hypochlorous acid has been established with respect to a wide variety of bacteria, including *Escherichia coli*, *Salmonella dysenteriae*, *Shigella sonnei*, *Campylobacter jejuni*, *Yersinia enterocolitica*; viruses, including hepatitis A, poliovirus (type 1), rotavirus, adenovirus and calicivirus; helminthes; and protozoa, including cysts of *Entamoeba histolytica* and *Giardia lamblia*.” (8)

## VIRUSES

Effectiveness of hypochlorous acid (free available chlorine) against a range of water borne						
ORGANISM	PH	TEMP °C	EXPOSURE TIME	AVAILABLE CHLORINE mgs/litre	BIOCIDAL RESULT	REF
Adenovirus (type 3)	7.8	22	5 mins	0.5	>99.9%	10
Enteroviruses:						
Poliovirus (type 1)	7.8	22	5 mins	0.5	>99.9%	10
Coxsackievirus (type A9)	7.8	22	5 mins	0.5	>99.9%	10
Coxsackievirus (type B5)	6.0	5	13.2 mins	0.5	>99.9%	27
Coliphages MS2	6.0	5	1.2 mins	0.5	>99.9%	27
Coliphages OX174	6.0	5	0.5 mins	0.5	>99.9%	27
Echovirus (type 7)	7.8	22	5 mins	0.5	>99.9%	10
Reovirus (type 3)	7.8	22	5 mins	0.5	>99.9%	10
Hepatitis A	7.0	5	3.6 mins	0.5	>99.9%	11
Infectious hepatitis	6.8	Room	30 mins	3.25	Protected all 12 volunteers	1
Simian rotavirus SA11	6.0	5	15 secs	0.11-0.67	100%	12

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### OFF-GASSING

EVIDENCE THAT AFTER THE RECOMMENDED CYCLE, HOCL OFF-GASSING LEVELS REACH A SAFE LEVEL BEFORE RESPIRATORS/GOWNS/EYEWEAR CAN BE RE-DONNED.

Due to a compatible pH with human skin, and the fact that Hypochlorous acid is made by the human immune system to fight pathogens and is non-cytotoxic, HOCl can be safely fogged in the presence of people (1) and topically applied to skin and is used for wound disinfection and accelerated healing (2, 8) as well as used for the treatment of eyelids (3), as a nasal rinse (4), therefore at all recommended concentrations of HOCl are always at a safe level, and re-donning can be immediate once PPE has dried.

“Topical stabilized HOCl provides an optimal wound healing environment and, when combined with silicone, may be ideal for reducing scarring. Additionally, in contrast to chlorhexidine, HOCl, used as an antiseptic skin preparation, raises no concerns of ocular- or ototoxicity.” (5)

“We speculate that some ocular spray disinfectants that contain hypochlorous acid, usually applied to treat blepharitis in order to reduce bacterial and viral load on the skin and eye lashes, could be used as a measurement of prevention for the facial area where many other chemical agents cannot be applied.” (6)

“HOCl has been incorporated into topical formulations due to antimicrobial, anti-inflammatory, immunomodulatory, and wound healing properties...Topical formulations of stabilized, pH-neutral HOCl (e.g., solution, gel, spray) have been evaluated in several studies demonstrating both antimicrobial effects and therapeutic benefit in many cutaneous disorders, including seborrheic dermatitis, atopic dermatitis-associated pruritus, acne vulgaris, diabetic foot ulcers, and hypertrophic scars/keloids. Topical HOCl appears to be well tolerated and safe, without any major adverse events reported.” (7)

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VISUAL INSPECTION

RESPIRATORS/GOWNS/EYEWEAR MUST NOT SHOW ANY VISIBLE DAMAGE POST HOCL PROCESSING

“Due to the neutral pH of the HOCL, it is non-toxic, does not leave residue on environmental surfaces, and is not corrosive to hospital equipment as traditional bleach and phenolics have long demonstrated.” (1)

Electrolytically-produced HOCL has been used for many years to process endoscopy units without causing significant corrosive concern that would have a bearing on the processes we are proposing for the use of NaDCC-derived HOCl for PPE. (2)

“The material compatibility of the Sterilox germicide (HOCl) was evaluated by subjecting a variety of metallic, plastic, adhesive and rubber endoscope components to continuous immersion in Sterilox. The samples were inspected for, visible change, visible evidence of damage or any other change in appearance. Sterilox germicide does not produce any corrosion or other visible damage in the majority of endoscope components. Color changes and the "tack"\* of the coating of the outer endoscope sheaths were noted on some endoscopes. Corrosion was noted on anodized aluminum\*.” (2)

\*These issues were not considered relevant by Essex Rivers Healthcare NHS Trust when weighed up against the benefits of the use of Sterilox in place of glutaraldehyde for sterilising endoscopes. Also, this was resulting from continuous immersion in Sterilox. This is not the process that we are proposing for HOCl for PPE in a hospital setting.

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## PERFORMANCE TESTING

### RESPIRATORS/GOWNS/EYEWEAR MUST STILL BE FIT FOR PURPOSE AFTER PROCESSING

Electrolytically-produced HOCL has been used for many years to process endoscopy units without corrosive concern, despite being submerged for long periods of time in solution. (1)

We are unable to locate tests evidencing use of NaDCC-generated HOCl on common PPE materials, however we have found references to testing on metals. (2).

“Electrochemically activated saline solutions ...are commonly used for disinfection of dental equipment and endoscopes, and swimming pool sanitation.” (3)

“This ECA process produces a powerful “free available chlorine (FAC)” sterilising and disinfection liquid – Anolyte ANK – at a concentration of 500–700ppm, within a neutral pH range of 6–7.5, making it ideal for use on equipment and surfaces within a cleanroom, for example.” (4)

We are unable to find any references to degradation of materials or plastics after fogging or spraying of HOCl.

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### QUANTITATIVE FIT TESTING

EVIDENCE TO SHOW THAT THE FIT OF A RESPIRATOR IS NOT COMPROMISED AFTER A STATED NUMBER OF CYCLES UNDERTAKEN BY THE HOCL PROCESS UNDER THE RECOMMENDED OPERATING CONDITIONS.

There is no reason to believe that fogging/misting with HOCl will compromise the fit of a respirator in any way, even after repeated cycles. There is no evidence to suggest that materials are in any way altered after spraying or fogging of HOCl.

### HEALTH AND SAFETY PROTOCOL

THERE IS A HEALTH AND SAFETY PROTOCOL THAT SUPPORTS THE SAFETY OF COLLEAGUES INVOLVED IN EXECUTING THE PROCESS.

Hypochlorous acid is made by the human immune system and does not pose any dangers either when in topical contact or when inhaled or consumed in drinking water (1).

As discussed in Off-Gassing, Hypochlorous acid is non-toxic and non-cytotoxic, can be safely fogged in the presence of people (2) and topically applied to skin (3) as well as used for the treatment of eyelids, as a nasal rinse (4), is used for wound disinfection (5) making it an all-round safe biocide to handle in a hospital setting.

“Toxicological Conclusion: For all practical purposes the health risks to patient and to operator arising from exposure to Sterilox germicide and diluted Sterilox germicide are minimal. The principal potential effect of the chemicals present in Sterilox would be irritation, but Sterilox does not exhibit this effect in tests. Sterilox is unlikely to pose a significant risk to either patient or user.” (6)

“Application of Sterilox HG did not result in production of noticeable noxious fumes but was described as producing an odor similar to that of swimming pool water. There were no reported complaints from nursing staff or patients.” (7)

“All chlorine products have some level of toxicity; this is what renders them such effective microbicides. When chlorinated water is ingested, however, the available chlorine is rapidly reduced by saliva and stomach fluid to harmless chloride ions salts (Kotiah et al., 1992). This is true for all

sources of chlorine, including both NaOCl and NaDCC... Chronic and sub-chronic toxicity studies also found no toxicity. Developmental toxicity studies have also established that the compound is not fetotoxic, teratogenic (causing birth defects), mutagenic or carcinogenic. Chlorinated isocyanurates are not metabolized in the body and do not bioaccumulate.” (8)

“The trust [Essex Rivers Healthcare NHS Trust] has removed a potentially harmful substance that was a health risk to staff and replaced it with a non-toxic substance that has no health, safety or environmental risks. Of the five substitute disinfectants examined, only Sterilox satisfied the trust’s rigorous microbiological, toxicological and scope compatibility criteria. Occupational health problems in endoscopy have been eradicated, endoscopes are turned around faster and the risk of cross-infection between patients has been reduced to a minimum.” (9)

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