

Application for Inclusion in the
2021 WHO Essential Medicines List

Hypochlorous Acid (HOCl)
for disinfection, antiseptis, and wound care in
Core Categories 15.1, 15.2, and 13

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1. Summary Statement

We request that **Aqueous Hypochlorous Acid (HOCl)** be added to the core Essential Medicines List to address priority conditions in Categories 15.1 and 15.2 (Disinfectant and Antiseptic products), and Category 13 (Wound Care).

HOCl has emerged in the current pandemic as the most potent and environmentally safe disinfectant available and with a wide range of efficacy against many human pathogens, including the SARS-CoV-2 coronavirus.^{8,36,37} In recent years compelling evidence of the potency of pure, stable preparations of HOCl in the inactivation of even the most resistant infectious agents, such as BSE Prions⁽⁵²⁾, and HPV16 viruses^{78,79} (both of which are completely unaffected by disinfectants currently on the EML list) has made it clear that HOCl deserves a place in every public health program as a fundamental instrument of infectious disease control.¹¹⁷

Manufacturing improvements for HOCl now enable reliable, consistent production of authentically pure, stable solutions of HOCl in industrial quantities, sufficient to meet the needs of large-scale institutional use patterns. Large volumes of cost-effective disinfectants are going to be necessary from now onwards for addressing pandemic requirements and in heightened awareness of infectious diseases worldwide.^{18,41} High purity HOCl products permit safe application of a highly effective disinfectant with no risks to patients or staff - even allowing operators to forego PPE use.^{24,29} These developments put HOCl in the hands of those most in need; they can shift the power balance in the confrontation with pandemic agents while at the same time matching the growing demand for wide-spectrum antimicrobial agents that do not contribute to resistance traits.^{59,61,64} HOCl availability is an especially appropriate resource in the face of natural disasters and the medical resources they require, not only for disinfection, but also for food safety, personal hygiene, and wound care.⁹³

In this field of Antisepsis (15.2) and Wound Infection Control (13), HOCl offers an unequalled combination of: 1) antimicrobial efficacy against all the common bacteria and yeasts/fungi that infect wounds⁶⁸, along with; 2) a convincing record of enhancing the rate of healing to reduce the high burden of wound care costs.¹⁰⁵ Both acute and intractable chronic wound infections, often treated with high value antibiotics in ways that inevitably contribute to multi-drug resistance traits, put great strains on healthcare delivery. HOCl can help ease that burden.^{9,10,76,89}

The availability of HOCl to provide both effective infection control and improved rates of healing⁹⁷ with a proven reduction in wound biofilms,¹⁰² will seriously reduce the costs of wound management. This can benefit national health systems globally, but particularly in the developing nations of the world. Tolerant of temperature extremes, and stable when packaged for up to several years,⁹⁶ pure HOCl is ideally suited to tropical region primary healthcare needs. Since the end products of its use are water and salt, the compound poses no personal or ecotoxicological hazards either in handling or routine deployment as a disinfectant, antiseptic or wound decontaminant.^{61,128}

2. WHO Technical Department and Focal Point supporting the application

There are several candidates expressing interest in serving as a WHO internal Focal Point for HOCl inclusion within the core Essential Medicines List. That discussion is continuing as of 16 December 2020 and a single point of contact will be coming under separate cover.

3. Supporting Organizations and Individuals

Hypochlorous acid (HOCl) for the purposes proposed in this application (EML Sections 15.1, 15.2 and 13) has been evaluated by regulatory agencies, healthcare institutions, professional organizations, independent laboratories, and academic institutions in more than 50 countries. More than 2600 publications in peer-reviewed scientific journals address the extraordinary potency and safety of HOCl as an antimicrobial agent in the fields of environmental disinfection and antisepsis. A further 3000 journal articles report on the benefits HOCl provides to wound healing enhancement when applied topically, and the biochemical and physiological mechanisms involved in those processes. Representative examples of the published literature supporting these uses and benefits are included in the list of References that start on page 42.

HOCl solutions are already included in the WHO list of coronavirus-effective biocides, and in the US EPA ‘N’ list of disinfecting agents able to control emerging pathogens like SARS-CoV-2.^{37,126} More than ten branded aqueous HOCl formulations have been cleared by the US FDA for topical use in wound management over the last decade. A Class III medical product approval for HOCl has been granted in the EU, and the Japanese Ministry of Health has approved use of HOCl for topical medical applications. The US FDA has approved HOCl for high level disinfection and sterilization of medical instruments, including those for use at critical (i.e., sterile) sites.

A number of consensus and guidance statements have been issued in recent years by governmental agencies responsible for healthcare product regulatory oversight, and by professional specialty organizations focused on advantageous technical innovations that better serve their members’ needs.^{24,55} Here is a sample:

* From the WHO Interim Guidance Document on “Cleaning and disinfection of environmental surfaces in the context of COVID-19”, page 2:

“The use of chlorine-based products:

Hypochlorite-based products include liquid (sodium hypochlorite), solid or powdered (calcium hypochlorite) formulations. These formulations dissolve in water to create a dilute aqueous chlorine solution in which undissociated hypochlorous acid (HOCl) is active as the antimicrobial compound.”¹²⁶

* From the US FDA Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards, Chapter 5:

“Hypochlorous acid (HOCl) is the form of free available chlorine that has the highest bactericidal activity against a broad range of microorganisms.”³⁹

* The Japanese National Institute of Technology and Evaluation determined that hypochlorous acid is effective at inactivating SARS-CoV-2 at virucidal levels:

“Based on these results, the Committee judged that HAW (hypochlorous acid water) of an ACC (active chlorine concentration) of 35 ppm or higher was effective, which showed a more than 99.9% reduction of viral titer in the evaluation study at NIID (the National Institute for Infectious Diseases) as well as a more than 99.99% reduction in the studies at OUAVM and QTEC (universities).”⁵⁵

* Also from the US FDA Guidance on minimizing microbial food safety hazards using chlorine-based disinfectants:

“For example, the antimicrobial activity of a chlorine-based disinfectant depends on the amount of hypochlorous acid (also called "free chlorine") present.”³⁹

* *Consensus Guidelines* within the Expert Recommendation on the use of Hypochlorous Acid (HOCl), published in “Wounds” (2016) included these statements:

“Wound complications such as infection continue to inflict enormous financial and patient quality-of-life burdens. The traditional practice of using antiseptics and antibiotics to prevent and/or treat infections has been questioned with increasing concerns about the cytotoxicity of antiseptics and proliferation of antibiotic resistant bacteria.

In the last 15 years, more advanced hypochlorous acid (HOCl) solutions, based on electrochemistry, have emerged as safe and viable wound-cleansing agents and infection treatment adjunct therapies.”²⁴

* Bongiovanni *et al.* in 2016 in a comprehensive review of use of HOCl in treatment of more than 1,000 venous leg ulcers (VLU) concluded:

“Perhaps the greatest advance in VLU care is the addition of HCA [hypochlorous acid, HOCl] to the treatment armamentarium. These aqueous solutions of hypochlorous acid, even in trace amounts, will kill most pathogens within 30 seconds of exposure. Additional actions of HCA include reduction of mast cell degranulation and active capillary dilation. The latter effect is of great importance in the diabetic VLU patient since one of the paradoxes in diabetes is the reduction of capillary perfusion via arteriovenous shunting at the microcirculatory level.”⁹

* Gold *et al.*, in 2020 reporting on the conclusions of an expert panel on surgery and wound care, wrote as follows:

“HOCl can be indispensable in pre and peri-procedures as an antiseptic and anti-inflammatory agent, and in post-procedures, including post-sutures, as a wound healing agent.

HOCl could become the first line pre and peri-procedure antiseptic for supporting wound healing and scar management.”⁴⁵

* DelRosso and Bhatia in 2020 published an updated status report on topical HOCl and concluded as follows:

“HOCl exhibits broad spectrum antimicrobial activity that is directly toxic to many bacteria and fungi.

Hypochlorous acid exhibits anti-inflammatory and immunomodulatory properties based on multiple laboratory analyses. These properties appear to correlate with potential therapeutic benefits of topically applied HOCl for a variety of skin disorders.”²⁹

* Block and Rowan in 2020 reviewed surgeons’ needs for disinfection in the face of the coronavirus pandemic and concluded that:

“It [HOCl] comprises many of the desired effects of the ideal disinfectant: it is easy to use, is inexpensive, has a good safety profile, and can be used to disinfect large areas quickly and with a broad range of bactericidal and virucidal effects.”⁸

* Rasmussen and Williams (2017) writing on the usefulness of HOCl for the demands of healthcare after natural disasters, concluded:

“Brio HOCl offers a significant ability to protect humanitarian aid workers, the populations they serve and the communities that receive those displaced families. It can help control the next Ebola outbreak, or SARS epidemic.

When used for area disinfection in tents, clinics, refugee camps, and hospitals Brio HOCl can significantly reduce pathogen bioloads, and when used for traumatic injuries it can eliminate microbial wound biofilms while decontaminating tissue, potentially reducing the risk of sepsis while improving the rate of healing.”⁹³

4. International Non-proprietary name (INN) and Anatomical Therapeutic Chemical (ATC) code

IUPAC name: Hypochlorous acid

CAS Registry number: 7790-92-3

FDA UNII: 712K4CDC10

European Community (ECHA) number: 232-232-5

EPA DSSTox Substance ID: DTXSID3036737

Human Metabolome Database: 14380-61-1

UNSPSC Code: 42312313 (Wound Cleaning Solutions)

5. Formulation and strengths

Base HOCl formulation in a saline solution (acceptable for disinfection, antisepsis, and wound care):

1. 150 parts per million or greater of hypochlorous acid (HOCl).
2. Isotonic solution of 0.9% saline (Normal Saline) preferred for clinical use.
3. pH less than 5.5, and greater than 99% pure HOCl
4. Oxidation-Reduction Potential (ORP) greater than 1000 mV.

Alternative HOCl formulation in a non-salt solution (also acceptable for disinfection, antisepsis, and wound care):

1. 150 parts per million or greater of hypochlorous acid (HOCl).
2. pH less than 5.5, and greater than 99% pure HOCl
3. Oxidation-Reduction Potential (ORP) greater than 1000 mV.
4. Salt-free water-based solution (rather than 0.9% saline) as an option for environmental use.

Aqueous solutions of HOCl approved for disinfection in the EU may contain up to 200 ppm of oxidative titratable chlorine (Cl). The Cl ppm content of approved disinfectant HOCl solutions in the US ranges from 180-460 ppm. Wound care solutions cleared for the US market by FDA range from 100-200 ppm Cl. A preparation of HOCl approved as a Class III medical product for wound care in the EU contains no more than 200 ppm Cl.

Potency, stability and safety of HOCl preparations depend on the purity of the solution, and the avoidance of contaminating molecular species of aqueous chlorine (such as hypochlorite, chlorate, chlorite, perchlorates, and elemental chlorine.)^{1,13,17,69,71} Authentic, pure HOCl therefore provides a higher level of security for users in all applications. Its production is achievable by improved process-controlled manufacturing advances introduced in the last several years.

Absent careful manufacturing controls HOCl products may lack stability on storage, show declining efficacy, or introduce toxicity in the form of contaminants that may not be optimal for either environmental or topical applications. Attempts to make crude HOCl solutions by acidification of sodium hypochlorite run the risk of generating hazardous amounts of chlorine gas.⁶⁹ Nevertheless, plentiful historical medical literature found in the References^{5,12,25,33} supports the many benefits that may flow from HOCl uses in healthcare. This body of literature attests to the advantages that emerge from its routine adoption, even in suboptimal, impure, short-lived unstable products, identified variously as electrolyzed water, superoxidized water, acid electrolyzed water, superoxidized saline, and many other variants apparent from the Reference list below.

Historically, carefully controlled HOCl manufacturing methods brought with them cost implications that impeded the uptake and use of HOCl products. However, now that HOCl is available in more well-defined, reliable and economically attractive forms at industrial volumes, the superiority of its performance in disinfection, antisepsis and wound care argues for its deployment on a much wider scale worldwide.

6. Individual medicine

Branded HOCl solutions, including Briotech HOCl, are identified in the US EPA “List N” of disinfectants appropriate for environmental use in emerging infectious diseases such as the current SARS-CoV-2 pandemic.^{36,37} These are registered for commercial use in the US and in many other countries worldwide, including within the EU, ASEAN, and the AU.

More than ten HOCl solutions are cleared for use in wound care by the US FDA. One HOCl product is approved as a Class III medical product in the EU, and solutions of comparable composition are approved for topical uses within countries in Asia, Latin America, and the Middle East.

Product claims relating to details of composition and efficacy depend on the care with which solutions of HOCl are prepared in order to optimize the power and stability of the oxidative

chlorine content. Some products offer very short shelf life and need to be used as soon as possible after production, preferably on-site. Pure homogeneous preparations such as Briotech HOCl offer extended shelf life, optimal pH, and high Oxidation-Reduction Potential (ORP). These products ensure reliable potency in the inactivation of microbes, in addition to enhanced safety in the topical treatment of wounds.

7. Treatment details

Hypochlorous acid solutions are routinely applied for environmental disinfection (EML 15.1) through use of conventional hand-held spray devices. Rigorous evaluations published in recent years have proven the usefulness of large-scale misting equipment for HOCl dispersion into enclosed spaces in institutions and healthcare facilities.^{40,88,92}

For the purposes of antisepsis (EML 15.2) and wound decontamination (EML 13.0) HOCl solutions are applied topically. Applications are often repeated during the day in the early stages of wound management and are adjunctive to debridement and other procedures aimed at adherent soil and biofilm removal. HOCl-soaked wound dressings are relied upon for continued delivery of antimicrobial and healing enhancement benefits over time.

There are no reports of adverse reactions to topical applications by these methods based on the US EPA's Toxicology Database *DSSTox*, the US CDC *Toxic Substances and Disease Registry*, nor at either the *Development and Reproductive Toxicology Database*, or the *European Bioinformatics Institute* of EMBL. This exemplary record supports use of HOCl as a safe and effective component of wound care for a wide range of dermal lesions, including chronic non-healing wounds.

8. Public Health relevance

8.a Disinfection

Environmental disinfection measures have acquired a much-deserved higher level of appreciation in 2020 as a result of the coronavirus pandemic. Regular decontamination of surfaces and air have become imperatives to assure the public of appropriate infection control measures being taken to provide for their security and safety. This trend is likely to continue with heightened awareness of infectious disease hazards, and wider recognition of the need for infection risk reduction in healthcare facilities. Many disinfectant formulations relied upon in the past (including examples in EML 15.1) involved less stringent and more intermittent use patterns. Those have proven inappropriate for the more demanding, high frequency applications adopted in the face of the pandemic and, as Choi and others have described below, the choices made for area disinfection have been problematic. Bleach, quats, and peroxide are not only more toxic than HOCl, but they are also considerably less effective:

Table 1: Comparison of kill times for HOCl, bleach, and hydrogen peroxide. HOCl is far superior.¹²⁴

Table 3. Comparative time kill studies of HOCl, NaOCl, and H₂O₂ against 3 test organisms at room temperature for a total of 90 min

Pathogen	ATCC	Time kill (min)		
		HOCl	OCl ⁻	H ₂ O ₂
<i>Escherichia coli</i>	25922	0	<5	>90
<i>Pseudomonas aeruginosa</i>	27853	<1	<20	<15
<i>Staphylococcus aureus</i>	29213	0	<10	>90

ATCC indicates American Type Culture Collection.

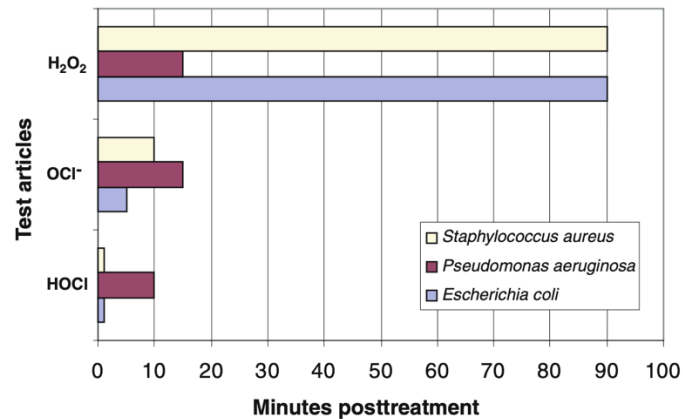


Figure 3. Comparative time kill studies of HOCl, NaOCl, and H₂O₂ against 3 test organisms—*Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, and *Staphylococcus aureus* 29213—at room temperature for total of 90 minutes.

The options available for antiseptics and disinfection have therefore become narrowed by:

- 1) evidence of environmental degradation through corrosiveness, ecological toxicity, extreme flammability, resistant trait encouragement, or persistence of toxic residues; and
- 2) adverse reactions to contact and inhalation of chemical solutions and aerosols on the part of staff, patients, or bystanders.

For example, the US National Toxicology Program, NIOSH, and OSHA have each determined that the acceptable Occupational Exposure Level, or OEL, for quaternary ammonium compounds and for bleach – both common in COVID-19 disinfection and conventional cleaning, is **zero**.³⁰

Table 1. OELs for active ingredients of antimicrobial products under investigation by the National Toxicology Program (NTP 2019a).

Common name (chemical name)	CAS #	ACGIH (2019)	NIOSH (2020)	OSHA (2020)	OARS-WEEL (2020)
Alkyl dimethyl benzyl ammonium chloride (ADBAC QUAT)	68424-85-1	None	None	None	None
Bleach (sodium hypochlorite)	7681-52-9	None	None	None	None
Chlorinated isocyanurate (trichloro-s-triazinetrione)	87-90-1	None	None	None	None
Chlorine dioxide in solution (chlorine dioxide)	10049-04-4	TLV TWA (0.1 ppm) TLV STEL (0.3 ppm)	REL TWA (0.1 ppm)	PEL TWA (0.1 ppm) PEL STEL (0.3 ppm)	None
1-Decanaminium, N-decyl-N, N-dimethyl chloride (DDAC QUAT)	7173-51-5	None	None	None	None
Hydrogen peroxide (hydrogen peroxide)	7722-84-1	TLV TWA (1 ppm)	REL TWA (1 ppm)	PEL TWA (1 ppm)	None
OPP	90-43-7	None	None	None	None
PAA	79-21-0	TLV STEL (0.4 ppm)	None	None	None
PCMC	59-50-7	None	None	None	None
PHMB	32289-58-0	None	None	None	None

PHMB: poly(hexamethylene biguanide) hypochloride; PCMC: *p*-chloro-*m*-cresol; PAA: peracetic acid; OPP: ortho phenyl phenol; OEL: occupational exposure limit; ACGIH: American Conference of Governmental Industrial Hygienist; CAS #: chemical abstract number; NIOSH: National Institute for Occupational Safety and Health; OSHA: Occupational Safety and Health Administration; PEL: permissible exposure limit; REL: recommended exposure limit; STEL: short-term exposure limit (15 min); TLV: threshold limit value; TWA: time weighted average; OARS-WEEL: Occupational Alliance for Risk Science-Workplace Environmental Exposure Level.

Similarly, a common quat, benzalkonium chloride, was evaluated by Choi et al¹⁹ and they found significant pulmonary damage at extremely low levels in a mammal exposure model. They determined that the safe level of exposure was 0.000062 mg/m³ and therefore unlikely to be achieved with existing disinfection protocols. The implication is that damage from quat exposure is routinely done to the protectors as well as those being protected.

Hypochlorous acid solutions bring high level potency to bear on this need, with resultant rapid inactivation of the entire spectrum of infectious disease agents (for an example see Table 1 below for findings with Briotech's HOCl). Some pathogens susceptible to pure HOCl are resistant to all other known disinfectants.^{78,79} Unprecedented efficacy is matched with a benign safety profile that makes authentic homogeneous HOCl eminently suited to contemporary public health needs.

Available now in homogeneous pure and optimally stable solutions that can be produced at industrial scale, the former shortcomings of instability (requiring production at point-of-use in some cases) and cost have been overcome. Use of HOCl for the purposes identified in the EML 15.1 category will provide healthcare authorities with a superior tool for interventions aimed at environmental sanitation while avoiding the hazards of conventional chemical measures.

The efficacy table below is assembled from independent laboratory reports submitted to Briotech about BrioHOCl. The submissions are from US FDA-certified cGLP laboratories, academic laboratories, and national and reference laboratories in Canada, Australia, Peru, the United Arab Emirates, Europe, South America, and Asia. Similar kill rates and inactivation LRVs would be seen in any equivalent HOCl formulation.

Table 1: Independent laboratory evaluations of HOCl (Briotech HOCl) against a range of pathogenic organisms:

Pathogen*	Elimination	Testing Date	Testing Laboratory
<i>Acinetobacter baumannii</i>	>99.999%	06-02-2016	NW Regional COE for Biodefense & Emerging Infectious Diseases Research at the University of Washington
<i>Acinetobacter baumannii</i>	>99.9999%	06-15-2016	Pacific Northwest Microbiology Services
<i>Aspergillus brasiliensis</i>	99.999%	07-07-2020	Laboratorio Baltic Control
<i>Aspergillus niger</i>	>99.9999%	08-03-2016	Pacific Northwest Microbiology Services
<i>Aspergillus niger</i>	>99.99%	11-20-2015	Pacific Northwest Microbiology Services
<i>Aspergillus spp.</i> (black mold spores)	>99.999%	02-01-2017	University of Costa Rica, Faculty of Microbiology
<i>Bacillus cereus</i> spores	99.999%	02-01-2017	University of Costa Rica, Faculty of Microbiology
<i>Bacillus sp.</i>	99%	05-11-2016	Scientific Clinical Labs, Dubai
<i>Bacillus subtilis</i>	>99.999%	11-20-2015	Pacific Northwest Microbiology Services
<i>Bacillus subtilis</i>	>99.9999%	08-03-2016	Pacific Northwest Microbiology Services
<i>Candida albicans</i>	>99.999%	11-20-2015	Pacific Northwest Microbiology Services
<i>Candida albicans</i>	>99.9999%	02-01-2017	University of Costa Rica, Faculty of Microbiology
<i>Candida albicans</i>	99.999%	07-07-2020	Laboratorio Baltic Control
Coronavirus (Human, OC43)	>99.999%	03-04-2016	School of Public Health, University of Washington
Ebolavirus	>99.99%	—	**See Below
<i>Enterobacter cloacae</i>	>99.9999%	06-15-2016	Pacific Northwest Microbiology Services
<i>Enterococcus faecalis (VRE)</i>	>99.9999%	11-20-2015	Pacific Northwest Microbiology Services
<i>Enterococcus faecalis (VRE)</i>	99.999%	07-07-2020	Laboratorio Baltic Control
<i>Escherichia coli</i>	>99%	03-02-2015	Cascade Analytical Inc.
<i>Escherichia coli</i>	>99.99999%	08-03-2016	Pacific Northwest Microbiology Services
<i>Escherichia coli</i>	99%	05-11-2016	Scientific Clinical Labs, Dubai
<i>Escherichia coli</i>	>99.9999%	2-15-2017	University of Costa Rica, Faculty of Microbiology
<i>Escherichia coli</i>	99.999%	07-07-2020	Laboratorio Baltic Control
<i>Escherichia coli</i> NDM-1	>99.99999%	06-15-2016	Pacific Northwest Microbiology Services
<i>Escherichia coli</i> 0157	>99.999%	11-20-2015	Pacific Northwest Microbiology Services
<i>Feline calicivirus,</i> (Human Norovirus)	>99.99%	04-06-2017	Microchem Laboratory
<i>Human papillomavirus</i> (HPV 16 & 18)	>99.999%	02-01-2016	Pennsylvania State University, Hershey Medical Center
<i>Influenza H1N1 Swine</i>	>99.99%	07-12-2017	Microchem Laboratory
<i>Klebsiella pneumoniae</i>	>99.99%	11-20-2015	Gibraltar Laboratories
<i>Klebsiella pneumoniae</i>	>99.99999%	11-20-2015	Pacific Northwest Microbiology Services
<i>Klebsiella sp.</i>	99%	05-11-2016	Scientific Clinical Labs, Dubai

Pathogen*	Elimination	Testing Date	Testing Laboratory
<i>Listeria monocytogenes</i>	>99%	03-02-2015	Cascade Analytical Inc.
<i>Listeria monocytogenes</i>	>99.9999%	02-25-2014	Gibraltar Laboratories
<i>Methicillin-Resistant S. aureus (MRSA)</i>	>99.999%	02-25-2014	Gibraltar Laboratories
<i>Methicillin-Resistant S. aureus (MRSA)</i>	>99.999%	06-02-2016	NW Regional COE for Biodefense & Emerging Infectious Diseases Research at the University of Washington
<i>Mold (NOS)</i>	>99%	04-15-2015	Cascade Analytical Inc.
<i>Poliovirus</i>	99.99%	06-02-2020	Cantacuzino Laborator Infectii Enterice Viral
<i>Polymicrobial biofilm</i>	99.96%	11-15-2016	Pacific Northwest Microbiology Services
<i>Polymicrobial cafeteria table</i>	99%	02-08-2016	P.A. Benjamin Manufacturing
<i>Prions (vCJD, others)</i>	>99.9999%	09-29-2016	Rocky Mountain Labs, The National Institutes of Health (NIH)
<i>Proteus vulgaris</i>	>99.9999%	06-15-2016	Pacific Northwest Microbiology Services
<i>Pseudomonas aeruginosa</i>	>99.999%	06-01-2017	Microchem Laboratory
<i>Pseudomonas aeruginosa</i>	>99.999%	11-20-2015	Pacific Northwest Microbiology Services
<i>Pseudomonas aeruginosa</i>	>99.9999%	2-15-2017	University of Costa Rica, Faculty of Microbiology
<i>Pseudomonas aeruginosa</i>	>99%	03-02-2015	Laboratorio Baltic Control
<i>Salmonella (NOS)</i>	99.999%	07-07-2020	Cascade Analytical Inc.
<i>Salmonella choleraesuis</i>	>99.9999%	11-20-2015	Pacific Northwest Microbiology Services
<i>Salmonella enterica</i>	>99.999%	11-20-2015	Gibraltar Laboratories
<i>Salmonella enterica</i>	99.999%	11-20-2015	Microchem Laboratories
<i>Salmonella spp.</i>	>99.999%	2-15-2017	University of Costa Rica, Faculty of Microbiology
<i>Salmonella typhi</i>	>99.999%	01-29-2014	Gibraltar Laboratories
<i>Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2 / COVID-19)</i>	99.96%	07-15-2020	Applied Biosafety Research Program, National Microbiology Laboratory, Public Health Agency of Canada
<i>Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2 / COVID-19)</i>	99.98%	07-18-2020	Human Microbiology Institute
<i>Shigella flexneri</i>	>99.9999%	06-15-2016	Pacific Northwest Microbiology Services
<i>Staphylococcus aureus</i>	>99.999%	01-24-2014	Gibraltar Laboratories
<i>Staphylococcus aureus</i>	>99.999%	06-01-2017	Microchem Laboratory
<i>Staphylococcus aureus</i>	>99.999%	02-01-2017	University of Costa Rica, Faculty of Microbiology
<i>Staphylococcus aureus</i>	99%	05-11-2016	Laboratorio Baltic Control
<i>Staphylococcus epidermidis</i>	99.999%	07-07-2020	Scientific Clinical Labs, Dubai
<i>Trichophyton interdigitale</i>	99.999%	06-01-2017	Microchem Laboratory
<i>Yersinia enterocolitica</i>	99.999%	06-15-2016	Pacific Northwest Microbiology Services

8.b Antisepsis

Antimicrobial efficacy results for HOCl tested by the Cantacuzino Institute in Romania (Table 2) and the Functional Water Association of Japan (Tables 3 and 4) reinforce the scope and speed of microbial inactivation. They also emphasize the exceptionally high level of performance of HOCl against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, both regularly implicated in both acute and chronic wound infections.

Table 2: Efficacy of HOCl manufactured by electrolysis (Briotech HOCl). Independent evaluation by the Cantacuzino National Institute of Research and Development in Microbiology and Immunology - the EU's EUPEHM reference laboratory in Bucharest, Romania. Unpublished; Romanian Lab POC on request.

Summary	Tested	Briotech's		
European Union data available on request	June 2020	Multi-Surface Sanitizer		
Microbe	LRV	Percent Reduction	Exposure	Test Condition
Bacteria				
E. coli	5.13	99.9993%	30 seconds	Clean
Enterococcus	5.12	99.9992%	30 seconds	Clean
Pseudomonas	5.20	99.9994%	30 seconds	Clean
Staph aureus	5.25	99.9994%	30 seconds	Clean
Enterococcus	4.00	99.9900%	2 minutes	With bioload
Pseudomonas	4.15	99.9929%	2 minutes	With bioload
Staph aureus	4.23	99.9941%	2 minutes	With bioload
Fungus				
Aspergillus	2.94	99.8852%	2 minutes	Clean
Candida	4.23	99.9941%	30 seconds	Clean
Aspergillus	2.93	99.8825%	2 minutes	With bioload
Candida	4.23	99.9941%	2 minutes	With bioload
Virus				
Poliovirus	4.10	99.9921%	30 seconds	Clean

Table 3: Efficacy of HOCl manufactured by electrolysis (independent evaluation in support of a Japanese regulatory application; original Japanese below with links)

	microorganism	HOCl (40ppm)
	virus	
Gram positive	Staphylococcus aureus	◎(10sec)
	MRSA	◎(10sec)
	Bacillus cereus	△(3~5min)
	Mycobacterium tuberculosis	△(2.5min)
	Others	△(1~2.5min)
Gram negative	Salmonella Enteritidis	◎(10sec)
	Vibrio parahaemolyticus	◎(10sec)
	Escherichia coli O157:H7	◎(10sec)
	Campylobacter jejuni	◎(10sec)
	緑膿菌 Pseudomonas aeruginosa	◎(10sec)
Virus	Norovirus	◎
	Influenzavirus	◎(10sec)
	Enterovirus	◎(10sec)
	Herpesvirus	◎(10sec)
Fungi	Candida albicans	◎(10sec)
	Aspergillus niger	△(5min)
	Penicillium cyclopium	△(5min)

◎ > o > △ > ▲ > x, where ◎ = Complete kill

Table 4: <http://www.fwf.or.jp/kinousui.html>

微生物・ウイルス		次亜塩素酸水 (40ppm)
グラム陽性菌	黄色ブドウ球菌 (<i>Staphylococcus aureus</i>)	◎ (10秒)
	MRSA (メチシリン耐性ブドウ球菌)	◎ (10秒)
	セレウス菌 (<i>Bacillus cereus</i>)	△ (3~5分)
	結核菌 (<i>Mycobacterium tuberculosis</i>)	△ (2.5分)
	その他の抗酸菌	△ (1~2.5分)
グラム陰性菌	サルモネラ菌 (<i>Salmonella</i> Enteritidis)	◎ (10秒)
	腸炎ビブリオ菌 (<i>Vibrio parahaemolyticus</i>)	◎ (10秒)
	腸管出血性大腸菌 (<i>Escherichia coli</i> O157:H7)	◎ (10秒)
	カンピロバクター菌 (<i>Campylobacter jejuni</i>)	◎ (10秒)
	緑膿菌 (<i>Pseudomonas aeruginosa</i>)	◎ (10秒)
ウイルス	ノロウイルス (ネコカリシウイルス)	◎
	インフルエンザウイルス (2009年新型含む)	◎ (10秒)
	エンテロウイルス	◎ (10秒)
	ヘルペスウイルス	◎ (10秒)
真菌	カンジダ (<i>Candida albicans</i>)	◎ (10秒)
	黒カビ (アスペルギルス, <i>Aspergillus niger</i>)	△ (5分)
	青カビ (ペニシリウム, <i>Penicillium cyclospium</i>)	△ (5分)

殺菌効果または不活化効果: ◎ (即効) > ○ > △ > ▲ > × (無効)

引用: 強電解水企業協議会編 「強酸性電解水使用マニュアル2002」
 // 「微酸性電解水使用マニュアル2002」
 機能水研究振興財団発行 「次亜塩素酸水生成装置に関する指針 第2版-追補」

Infected wounds and the rise of antibiotic resistant organisms are responsible for significant increases in morbidity, mortality, and the costs of health care, especially in the aged.^{14,18,20,23,31} HOCl is particularly suited to meet the pressing need for improved approaches to these growing problems worldwide. The urgency of this issue is emphasized by these recent public comments:

November 23, 2020, Dr. Tedros Ghebreyesus commenting on a new international initiative to address the problem, said “*Antimicrobial resistance is one of the greatest health challenges of our time, and we cannot leave it for our children to solve.*”⁴⁴

On the same occasion Dr. Monique Eloit, DG OIE, said “*Antimicrobial resistance is a problem affecting animal and human health and the environment and we need to act today to protect the efficacy of antibiotics.*”³⁴

Adoption of HOCl for the purposes of antisepsis (EML 15.2) can bring immediate and important benefits. In a modern and pure form, HOCl brings none of the cytotoxicity associated with hypochlorite bleach in earlier crude mixed oxidant HOCl products.^{13,59} Nor does it bring the cytotoxic effects and discoloration caused by povidone-iodine antiseptic products.^{56,90}

The use of HOCl avoids entirely the selective pressures that can lead to resistance to silver and biguanide antiseptic formulations. It does not encourage antibiotic resistance traits in the way

chlorhexidine has been proven to act. Moreover, despite the popularity of silver-based antiseptic products, Storm-Versloot *et al* published a Cochrane meta-analysis of topical antimicrobial agents that included silver sulfadiazine, and found that:

“There is insufficient evidence to establish whether silver-containing dressings or topical agents promote wound healing or prevent wound infection.”¹¹¹

8.c Wound care

In addition to the antiseptic contributions that HOCl can bring to wound management there is compelling evidence that exogenous HOCl applied topically triggers a cascade of events leading to speedier healing, and faster restoration of normal tissue architecture with minimal scarring.^{45,105} HOCl is, after all, a natural product of the human body, relied upon as the first line of chemical defense in response to injury and infection. Generated enzymatically on demand in phagocytes, endogenous HOCl brings about rapid attack on infectious microorganisms of many types, including bacteria, yeasts, fungi, and viruses.^{10,45,104}

The end products of HOCl reactions with surrounding molecular substrates in tissues are responsible for the beneficial effects on physiological processes needed to enhance healing. These include:

- local coagulation to seal off the damaged tissues
- the proliferation and migration of new endothelial cells
- inhibition of harmful metalloproteases
- faster migration of dermal fibroblasts
- down-regulation of mast cell degranulation
- faster processing of microbial antigens in local lymph nodes

Plentiful biochemical evidence in support of these pathways appears in the peer-reviewed literature on wound healing and the pathophysiology of tissue repair.^{2,4,8,11,12,16,22,27,28,46,62,70,73,75,83,85,87,91,94,98,102,105,124} In contrast, certain currently used antiseptics, such as povidone iodine and chlorhexidine, have been shown to inhibit many of these healing processes, and are often applied to wounds in formulations that are seriously cytotoxic.^{32,106}

Beneficial enhancement of healing by exogenous HOCl has been described for both acute and chronic wound management. Documented treatment successes in the References below range from use on minor lacerations, to serious traumatic injury, thermal and chemical burns, diabetic and pressure ulcers, deep VLU, and surgical site infections.^{2,5,8,9,10,25,26,43,45,65,67,68,76,86,89,90,95,102,104,112} Topical HOCl significantly improves wound perfusion, measurable as TcPO₂ in tissues adjacent to ulcerous lesions, for example, and these improvements persist for up to 36 hours post treatment.^{9,86} Of the modified tissue constituents responsible for continued stimulation of wound healing after the short lived exogenous HOCl has

all disappeared, N-chlorotaurine (NCT) is clearly the most important. Its role has been thoroughly studied and reported in primary scientific journals.^{4,73,87,98,125}

8.d. Product chemistry

Modern, carefully controlled manufacturing processes allow production of stable solutions with no detectable constituents other than HOCl. These differ markedly from former marketplace product offerings of ‘mixed oxidants’ that were nevertheless frequently described as HOCl. Those products often contained variable amounts of HOCl in the presence of other molecular species of chlorine in water and risked the production of dangerous chlorine gas. They also suffer from:

- 1) instability of the final product in storage
- 2) unreliable efficacy in all applications; and
- 3) significantly more toxicity to tissues and environmental surfaces; their higher pH led to more hypochlorite, which make the solutions more toxic.

However, even suboptimal preparations have given rise to a compelling body of evidence in the biomedical literature that supports the much-safer HOCl use in the ways proposed herein.^{5,25,49,110}

Homogeneous solutions of HOCl for the purposes designated in this proposal for EML inclusion are optimally prepared to ensure stability over a narrow pH range (the green zone illustrated in the figure below). Within that zone the solution will show maximum potency, reliably displaying an efficacy approximately 100-fold higher than the equivalent amount of chlorine in the form of hypochlorite bleach (i.e., the red zone form). The degree of disinfecting power of such HOCl preparations is a function of the Oxidation-Reduction Potential (ORP) measured in millivolts (most effectively 1020 mV or higher). The capacity of that power is provided by the active Cl content in ppm, with ranges in commercially available formulations as described on page 8 above.

It should also be apparent then, that any products designated as “neutral pH” are improperly constituted, contain a large fraction of toxic hypochlorite (see Figure 3 on page 33 below), and will be far less valuable in the use patterns described in this proposal. They will inevitably contain a much lower proportion of the optimally active HOCl needed for best performance.

Deterioration upon storage is also an inevitable consequence of impure mixed oxidant solutions. Pure HOCl products made at the proper pH with adequate process controls, however, will maintain their properties for months to years, depending on the storage container characteristics.⁹⁶

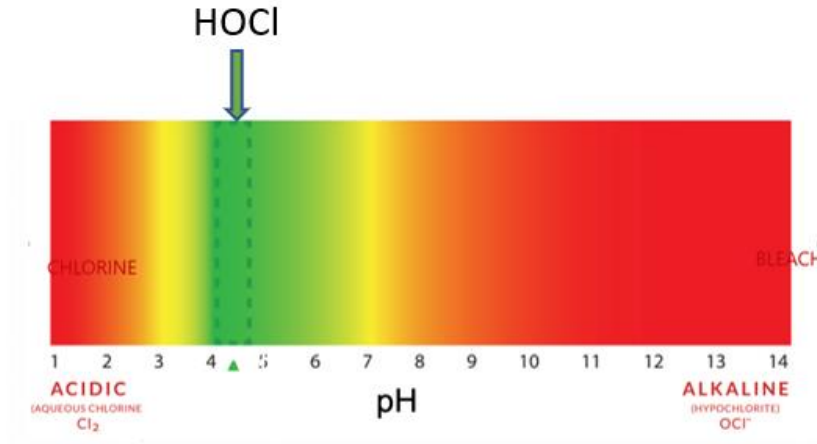


Figure 1: Chlorine species in water (diagram courtesy of Briotech Inc.)

Figure 1 above is a diagrammatic representation of the spectrum of chlorine species in water at various pH values. In the green zone HOCl predominates. In the region between the dashed lines, HOCl is the only molecular form of chlorine that can exist, ensuring homogeneity of products made to this specification.

Raman spectroscopy is an analytical technology that can identify the unique energy resonance signature of every molecular species – a structural fingerprint, in a sense. In the figure below the singular peak for HOCl at wavenumber 732 in a pure solution (made by Briotech) is clearly evident over the background of water molecules. HOCl in this form offers the most consistent and reliable utility for disinfection, antisepsis and wound care.

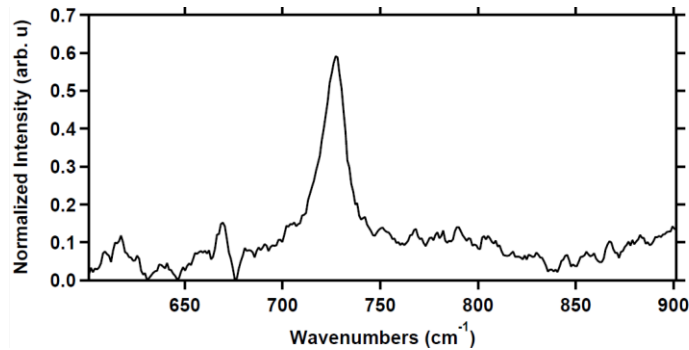


Figure 2: Raman spectrograph of Brio HOCl detecting only hypochlorous acid at 732 and no other chlorine species [Courtesy Molecular Engineering Laboratory, School of Engineering, University of Washington, 2017]

9. Review of Benefits: Efficacy

The table below contains highlights from 25 publications summarized below the table and listed in the References, each of which independently document the safety and efficacy of HOCl (under various chemical names) for each of the WHO EML categories proposed for HOCl inclusion:

	First author	Study type	Key result
1	Hughson	Bench research	HOCl inactivates prions at STP
2	Rasmussen	Review	HOCl use in highly vulnerable populations
3	Gray	Cohort study	HOCl decolonization reduced Burn Unit MRSA
4	Williams	Review	HOCl against emerging and resistant pathogens
5	Robins	Bench research	HOCl evaluation for purity and stability
6	Meyers	Bench research	HOCl rapidly inactivates HPV 16 at STP
7	Mendez-Duran	RCT	HOCl reduces dialysis infections
8	Day	Clinical model	HOCl disrupts wound and ulcer biofilms
9	Khan	Clinical trial	HOCl effective in pre-op peritoneal lavage
10	Mohd	RCT	HOCl reduces sternotomy infections
11	Garg	RCT	HOCl effective in peritonitis lavage
12	Mekkawy	RCT	HOCl improves septic traumatic wounds
13	Prabhakar	RCT	HOCl better for infected diabetic ulcers
14	Landsman	RCT	HOCl best of 3 in tx of diabetic foot infections
15	Pandey	RCT	HOCl better than Betadine for infected wounds
16	Piaggessi	RCT	HOCl better for post-surgical diabetic wounds
17	Bongiovanni	Clinical Review	HOCl better for venous leg ulcers
18	Suri	RCT	HOCl better for diabetic foot wounds
19	Martinez-DeJesus	RCT	HOCl safe and effective in severe wounds
20	Ricci	RCT	HOCl best for infected chronic wounds
21	Gold	Panel Review	HOCl best for wound care and scar management
22	Sakarva	Clinical model	HOCl for biofilm disruption and wound healing
23	Hadi	RCT	HOCl effective as adjunct wound therapy
24	Dalla-Paola	NR/OL Trial	HOCl better than Betadine for DM foot ulcers
25	Kapur	RCT	HOCl better than Betadine for all wounds

EML Section 15.1 Disinfection

Hughson AG, Race B *et. al.* Inactivation of prions and amyloid seeds with hypochlorous acid. *PLoS Pathog* 12(9): e1005914.doi:10.1371/journal.ppat.1005914⁵²

Objective: To test the anti-prion activity of a weakly acidic aqueous formulation of HOCl (BrioHOCl) that poses no apparent hazard to either users or many surfaces.

Design: To test the effects of HOCl and other disinfectants on steel-bound prion seeding activity, wires coated with a 10–3 dilution of ScBH were immersed in BrioHOCl, 40% bleach, 1 N NaOH, 2% Environ LpHTM, or saline for 1 h, rinsed, and added to RT-QuIC reactions.

Abstract: Anti-prion activity of pure stable HOCl from Briotech Inc. was demonstrated using both intracerebral infectivity of treated prions of Scrapie, as well as with an *in vitro* fluorescent chemistry method showing HOCl efficacy versus BSE, CJD, CWD prions and amyloid prion-like proteins. Efficacy was shown to reach levels of almost 6 LRV after exposures of 60 minutes at room temperature, but significant reductions were achieved up to 3–4 LRV in only 5 minutes of contact with HOCl. High efficacy was also demonstrated against *Bacillus* spores.

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Rasmussen ED and Williams JF, "Stabilized hypochlorous acid disinfection for highly vulnerable populations: Brio HOCl wound disinfection and area decontamination," 2017 IEEE Global Humanitarian Technology Conference (GHTC), San Jose, CA, 2017, pp. 1-8, doi: 10.1109/GHTC.2017.8239259.⁹³

Objective: To assess the value proposition of HOCl in wound disinfection and area decontamination in conflict zones, natural disaster response, displacement camps, and slums.

Design: A literature review and description of the documented safety and efficacy profile of HOCl as determined by independent laboratories over more than 100 years of research.

Abstract: The US Food and Drug Administration regards HOCl as the most effective form of all chlorine-based antimicrobial compounds. Historically, conventional production pathways generate HOCl solutions that are flawed by their instability, and by their inclusion of heterogeneous mixtures of aqueous chlorine species and other oxidants. These detract from the intrinsic safety and efficacy of HOCl for a wide range of applications. Large scale manufacture of HOCl in a novel, stable form, uncontaminated with uncharacterized components or extraneous stabilizing additives, has now become possible through an innovative chemo-electrolytic process. It provides HOCl tolerant of elevated temperatures, with a practical storage shelf life of years, and disinfecting potency against viruses, bacteria, endospores, fungi, and even infectious proteins (prions). The high Oxidation/Reduction Potential (ORP) is sustained over years of storage, and the end products of its practical deployment are water and NaCl. Further evaluation is proposed for this form of HOCl for field hospitals, remote clinics, and disaster zones as a safe, effective,

and affordable new disinfectant. HOCl offers a significant ability to protect humanitarian aid workers, the populations they serve, and the communities that receive those displaced families. When used for area disinfection in tents, clinics, refugee camps, and hospitals HOCl can significantly reduce pathogen bioloads, and when used for traumatic injuries it can eliminate microbial wound biofilms while decontaminating tissue, potentially reduce the risk of sepsis while improving the rate of healing. It is stable for months at temperatures above 150°F, is unaffected by vibration or shock, and can be stored and used at ordinary temperatures without the need for personal protection.

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Gray D *et al.*, Universal decolonization with hypochlorous solution in a burn intensive care unit in a tertiary care community hospital. *Am J Infect Cont.* 2016⁴⁷

Objective: To evaluate the efficacy of universal decolonization to decrease health care–associated infections caused by MRSA in patients admitted to a burn intensive care unit (BICU) using mupirocin and hypochlorous solution for skin decolonization.

Design: A retrospective, single institution cohort study.

Results: Global MRSA infection rates per 1,000 patient days were 7.23 pre-intervention and 2.37 post-intervention, resulting in an incidence rate ratio of 0.328, favoring post-intervention (95% confidence interval, 0.167-0.646; P = .001).

BICU patients without universal decolonization had 3.05 times higher risk of acquiring an MRSA infection than those with universal decolonization. No complications were noted from use of hypochlorous acid solution for skin decolonization.

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Williams JF, Rasmussen ED, and Robins L, *Hypochlorous Acid: Harnessing an Innate Response*

<https://infectioncontrol.tips/2017/10/06/hypochlorous-innate-response/>

Published online, October 6, 2017¹²⁸

Objective: We review here an emerging technology based on hypochlorous acid (HOCl), with emphasis on a novel, stable form (Brio HOCl), that inactivates viruses, bacteria, endospores, and fungi, is safe for human tissues, is environmentally benign requiring no toxic waste disposal or hazardous material management, and yet is capable of degrading the infectivity of prions.

Design: Review of the HOCl literature associated with disinfection, with particular attention to human biocompatibility, immune-conservation, and the inactivation of a possible etiologic agent in Alzheimer’s Disease.

Abstract: Human pathogens are becoming resistant to antibiotics developed over the past century, and common infections once routinely managed by conventional antibiotics can now become fatal even with best-practice therapy. One method for addressing that developing risk is to attack pathogens before they become life-threatening infections using area and wound decontamination and disinfection techniques. Current methods for disinfection, however, can contribute to the development of resistance, prove toxic to tissues, and damage the environment. We review here an emerging technology based on hypochlorous acid (HOCl), with emphasis on a novel pure and stable form (BrioHOCl), that inactivates viruses, bacteria, endospores, and fungi, is safe for human tissues (including eye, lung, and skin), is environmentally benign requiring no toxic waste disposal or hazardous material management, and yet is capable of degrading the infectivity of prions at a Log Reduction Value (LRV) of >5, equating to roughly a 99.999% elimination.

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Robins, L et. al. Purity and Stability of an Electrolytically-Generated Hypochlorous Acid Solution, Annual Meeting of the American Chemical Society, August 2017, Washington DC⁹⁶

Objective: To evaluate the decomposition kinetics of HOCl when generated electrolytically.

Design: We used Raman Spectroscopy, iodometric titrations, UV-visible spectrophotometry, and ORP measurements to characterize an electrolytically-generated, solution of pure HOCl (pH 4) made from only NaCl and water.

Abstract: This unbuffered solution was unexpectedly stable, even at elevated temperatures, sealed in a variety of vessels. There were no detectable changes in oxidative Cl levels (ppm), ORP (+mv), or pH in HOCl solutions maintained in glass containers at 52 °C for 38 days. After 30 days at 70 °C in glass, active Cl declined from 190 ppm to 151 ppm, but ORP remained constant, while pH rose to 4.3. No oxidative aqueous Cl species other than HOCl were detected in any stored samples. The results demonstrate that long-lived HOCl solutions can be prepared that undergo little or no change while in storage, even at high temperatures. The findings open up prospects for wider use of HOCl in environmental disinfection, with avoidance of on-site production, or concerns about the efficacy of stored solutions.

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Meyers, C et al, Efficacy Testing of HOCl as a Disinfectant for High-Risk HPV, The International Conference on Prevention & Infection Control (ICPIC) 2019 ICPIC Conference, Geneva, Switzerland, September 2019⁷⁸

Objective: Evaluation of HOCl against human papilloma virus (HPV16), a virus known to cause extensive morbidity as a sexually transmitted disease and implicated in causing two of the top ten fatal cancers globally, cervical and oropharyngeal.

Design: Infectious high-titer stocks of HPV16 and HPV18 were produced, titred, and infectivity-tested. Both suspension and carrier tests were performed with contact times spanning 15 seconds to 20 minutes. Following contact any remaining HOCl was neutralized. Residual HPV was isolated and measured by our published infectivity methods.

Results: All HOCl treatment contact times produced >99.99% reduction in infectivity of HPV16 and HPV18, comparable to the efficacy of concentrated (0.87%) sodium hypochlorite. HOCl is a highly effective disinfectant for HPV even at 15 seconds, the fastest process-measurable time.

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EML Section 15.2 Antisepsis

Méndez-Durán A. Efficacy and safety of the use of superoxidized solution in the prevention of dialysis-related infections. Dial Transpl. 2013; 34(4); 160-165.⁷⁷

Objective: To evaluate the efficacy and safety of super-oxidized solution in reducing the frequency of dialysis-associated infections.

Design: Randomised controlled trial in one hundred and eleven intraperitoneal dialysis patients treated with either povidone- iodine or super-oxidized solution following catheter placement.

Results: After 8 weeks follow-up, 24.5% of the povidone-iodine group had experienced catheter-related infections compared with 6% in the super-oxidized solution group ($p<0.05$). The mean time resolution of infection in the povidone-iodine group was 12 days compared with 4 days for the super-oxidized solution group ($p<0.05$).

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Day, A., Alkhalil, A., Carney, B. C., Hoffman, H. N., Moffatt, L. T., & Shupp, J. W. (2017). Disruption of Biofilms and Neutralization of Bacteria Using Hypochlorous Acid Solution: An In Vivo and In Vitro Evaluation. *Advances in skin & wound care*, 30(12), 543-551. doi:10.1097/01.ASW.0000526607.80113.66²⁸

Objective: The aims of this study were to assess the effectiveness of a hypochlorous acid-based wound cleanser in disrupting methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms relative to other cleansers using an in vitro collagen biofilm model and to evaluate cleansers' cytotoxicity.

Design: Plates coated with collagen films incubated with active bacteria cultures to yield biofilm mimics were treated with VWS, 1% and 10% povidone-iodine (PI), 0.05% chlorhexidine wound solution (CWS), or normal saline for 3 or 10 minutes. Biofilms were then analyzed for biomass density using a crystal violet assay, quantitative cultures, and fluorescent microscopy.

Cytotoxicity was measured using neutral red uptake by primary human dermal fibroblasts. Pre- and post-cleansing exudates and swab samples obtained from venous stasis wounds of patients were processed and plated on a series of selective agar plates for bacteria typing and quantification.

Results: All agents tested significantly neutralized methicillin-resistant *S. aureus* and *P. aeruginosa* biofilms compared with saline control as assessed by crystal violet assay and fluorescent microscopy assays. Undiluted HOCl was significantly less cytotoxic compared with 1% PI, CWS, and 10% PI (in increasing order of cytotoxicity). There was no significant difference in bacterial reduction in wounds after treatment with HOCl for any type of bacteria examined using selective media. In wounds that were treated with HOCl, there was a similar percentage reduction in bacterial colony-forming units from pre-cleansing levels when plated on tryptic soy agar, MacConkey, streptococcal, and mannitol salt agar plates.

Conclusion: These findings support the use of HOCl in the treatment of wounds with biofilms and to reduce the bioburden of venous stasis ulcers. While HOCl-treated biofilms had higher biomass than saline-treated biofilms, most of the cellular component was not viable. Ultimately, HOCl had a similar effectiveness to CWS in eliminating bacteria but with lower cytotoxicity.

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Khan S M et al. Evaluation of pre-operative peritoneal lavage by super-oxidized solution [HOCl] in peritonitis. *Mid East J Int Med.* 2009; 2(3); 15-35.⁵⁷

Objective: To assess the role of intraperitoneal lavage with super-oxidized solution in patient peritonitis.

Design: Eighty patients with peritonitis were randomly assigned to either 1 hour gastric lavage with saline or super-oxidized solution following surgery.

Results: Purulent discharge occurred in 20% of patients receiving super-oxidized solution lavage versus 52.5% of patients receiving saline lavage ($p < 0.01$). The incidence of burst abdomen among the super-oxidized solution lavage patients was significantly less than those receiving saline lavage (27.5% versus 47.5%, $p < 0.05$).

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Mohd A R R. Dermacyn® [a neutral pH superoxidized solution, HOCl] Irrigation in Reducing Infection of a Median Sternotomy Wound. *Heart Surg Forum.* 2010; 13(4); 228-232.⁸¹

Objective: To compare effectiveness of a neutral pH superoxidized solution (HOCl) irrigation and povidone-iodine with respect to reducing the incidence of sternotomy wound infection following CABG.

Design: A prospective randomized trial of 178 post-CABG patients who were treated with either HOCl or povidone-iodine wound irrigation.

Results: The incidence of sternotomy wound infection was five (5.7%) of these cases were from the HOCl group, and 14 (15.6%) were from the povidone-iodine group (P = 0.033). No HOCl-related complication was identified.

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Garg P K *et al.* Evaluation of intraoperative peritoneal lavage with super-oxidized solution and normal saline in acute peritonitis. Arch Int Surg. 2013; 3(1); 43-48.⁴³

Objective: To evaluate the role of intraperitoneal lavage with normal saline and normal saline followed by NEW in patients with acute peritonitis.

Design: One hundred patients were randomly allotted by slip method into two groups of 50 each. In the control group, after the definitive surgery for the pathology of peritonitis, the peritoneal cavity was lavaged with normal saline and closed after putting in drains. In the study group, after the definitive surgery the peritoneal cavity was lavaged with saline followed by 100 ml of super-oxidized solution and drains were closed for 1 h after abdominal closure. The patients were followed-up for morbidity and mortality.

Results: Surgical site infection occurred in 14% of super-oxidized solution lavage patients vs. 40% of saline lavage patients (p=0.0034). Eight (16%) patients in the control group died (saline lavage) compared to 2 (4%) patients in the study group (super-oxidized solution lavage).

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EML Section 13.0 Wound Care

Mekkawy M M and Kamal A. A Randomized Clinical Trial: The Efficacy of Hypochlorous Acid on Septic Traumatic Wound. J Ed Prac. 2014; 5(16); 89-100.⁷⁶

Objective: To evaluate the efficacy of hypochlorous acid as a wound care agent in a septic traumatic wound.

Design: A randomized clinical trial of sixty patients with septic trauma wounds at a single centre to treatment with either daily hypochlorous acid washes or daily povidone-iodine (P-I) washes.

Results: Wound pain (no pain at day 14, 100% hypochlorous acid versus 16.6% P-I, p=0.004), odour (no odour at day 14, 100% hypochlorous acid versus 13.3% P-I, p=0.001), no discharge (serous at day 14, 100% hypochlorous acid versus 10% P-I, p=0.004) and bacterial count (reduction in day 14 quantitative count, p=0.0001) were dramatically reduced by using

hypochlorous acid compared to povidone-iodine. At day 14, 90% of the hypochlorous acid treated group had wounds ready for surgical reconstruction compared with 0% for the P-I group.

Hypochlorous acid was an effective, easy to perform, comfortable, inexpensive and safe in treatment for infected acute traumatic wounds and allows for earlier surgical closure and hospital discharge. Hypochlorous acid controls the tissue bacterial bioburden without inhibiting the wound healing process rapidly relieving pain with the area becoming well-prepared for skin flap or graft.

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Prabhakar K B S *et al.*, Comparison of Super-oxidized Solution [HOCl] versus Povidone Iodine in Management of Infected Diabetic Ulcers: Our Experience. Int Arch Integ Med. 2016; 3(5); 151-158.⁹⁰

Objective: To compare the efficacy of dressings with super-oxidized solution [HOCl] versus povidone iodine in the management of infected diabetic ulcers.

Design: This one-year randomized controlled trial was conducted on a total of 60 patients presenting with infected diabetic ulcers. Patients were divided into two groups of 30 each based on computer generated randomization that is, Group A (super-oxidized solution dressing) and Group B (topical povidone-iodine dressing). Wound was observed for decrease in size of the ulcer, granulation, tissue quality and discharge from the wound at the end of each week for two weeks.

Results: The mean percentage *reduction in ulcer area* among patients with group A (super-oxidized [HOCl] solution) was significantly better (58.90 ± 5.21 percent vs. 40.90 ± 8.76 percent; $p=0.024$).

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Landsman A *et al.* An open-label, three-arm pilot study of the safety and efficacy of topical Microcyn Rx [HOCl] wound care versus oral levofloxacin versus combined therapy for mild diabetic foot infections. J Am Podiatr Med Assoc. 2011; 101(6); 484-496⁶⁷

Background: This randomized, prospective, multicenter, open-label study was designed to test whether a topical, electrolyzed, superoxidized solution (Microcyn Rx, HOCl) is a safe and effective treatment for mildly infected diabetic foot ulcers.

Methods: Sixty-seven patients with ulcers were randomized into three groups. Patients with wounds irrigated with HOCl alone were compared with patients treated with oral levofloxacin plus normal saline wound irrigation and with patients treated with oral levofloxacin plus [HOCl] wound irrigation. Patients were evaluated on day 3, at the end of treatment on day 10 (visit 3), and 14 days after completion of therapy for test of cure (visit 4).

Results: In the intention-to-treat sample at visit 3, the clinical success rate was higher in the [HOCl] alone group (75.0%) than in the saline plus levofloxacin group (57.1%) or in the [HOCl] plus levofloxacin group (64.0%). Results at visit 4 were similar. In the clinically evaluable population, the clinical success rate at visit 3 (end of treatment) for patients treated with [HOCl] alone was 77.8% versus 61.1% for the levofloxacin group. The clinical success rate at visit 4 (test of cure) for patients treated with [HOCl] alone was 93.3% versus 56.3% for levofloxacin plus saline-treated patients. This study was not statistically powered, but the high clinical success rate (93.3%) and the P value ($P = .033$) suggest that the difference is meaningfully positive for [HOCl]-treated patients. [HOCl] is safe and at least as effective as oral levofloxacin for mild diabetic foot infections.

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Pandey P K *et al.* Outcomes of superoxide solution dressings in surgical wounds: a randomized case control trial. *Int J Biol Med Res.* 2011; 2(4); 965-968.⁸⁶

Objective: To evaluate the role of superoxide solution in wound healing. At the same time an effort was made to compare the efficacy and outcomes of superoxide solution dressings and those with povidone iodine solution.

Design: Randomized controlled trial of one hundred patients with a variety of wounds treatment with either superoxide solution-saturated dressings (Group A) or povidone-iodine saturated dressings (Group B).

Results: The incidence of infection in primarily sterile cases was 15% in group A and 36% in group B, respectively. The most common infecting organism isolated in the study was *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* and *Klebsiella* spp. Decrease in surface area of wounds at the end of 1, 2, 3, and 4 weeks, which was statistically significant, was more in the superoxide solution group compared to the povidone-iodine group ($p=0.005$, 0.002 , <0.001 , and 0.001 , respectively). This study revealed less induration in wound margins when superoxide solution was used. This finding appears to be consistent with the fact that this solution does not damage cellular elements or restrict microcirculation of wound. Thus, the solution ensures the wellbeing of surrounding healthy tissues.

In addition, this study also revealed the early reduction in discharge from wounds dressed with superoxide solution as compared to povidone-iodine solution. Granulation tissue formation was earlier in the superoxide solution group as compared to the povidone-iodine group and also covered a greater wound surface area as compared to povidone-iodine.

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Piaggessi A *et al.* A randomized controlled trial to examine the efficacy and safety of a new super-oxidized solution for the management of wide postsurgical lesions of the diabetic foot. *Int J Low Extrem Wounds.* 2010;9(1); 10-15.⁸⁹

Objective: A study of the safety and efficacy of super-oxidized solution compared with standard treatment in the management of wide post drainage lesions of the infected diabetic foot ulcers.

Design: Non-randomised cohort study involving forty patients with >5 cm postsurgical wounds secondary to infected DFU treated with either super-oxidized solution or povidone-iodine as adjuncts to systemic antibiotics and debridement as needed. Patients were followed for 6 months.

Results: Healing as measured by complete re-epithelisation at 6 months occurred in 90% of the super-oxidized solution-treated group compared with 55% of the povidone-iodine-treated the group ($p < 0.01$). The super-oxidized solution-treated group also experienced significantly shorter period of antibiotic treatment (10.1 weeks vs. 15.8 ($p = 0.016$) and interventions (4 vs. 11, $p = 0.022$). The super-oxidized solution-treated group also had fewer episodes of reinfection ($p < 0.01$).

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Bongiovanni C M. Effects of hypochlorous acid solutions on venous leg ulcers (VLU): experience with 1249 VLUs in 897 patients. *J Am Coll Clin Wound Spec.* 2016: 10.1016/j.jccw.2016.01.001⁹

Objective: To assess the impact of comorbidities and identify factors that accelerate the healing rate of venous leg ulcers.

Design: An extensive, retrospective analysis of our experience in a diverse population.

Results: Initial treatment of all venous leg ulcers involved cleaning and debriding foreign matter, debris, and necrotic material via application of copious hypochlorous acid solutions, and under pressure if necessary. Where needed, this was accompanied by abrasion using sterile gauze soaked with hypochlorous acid solutions. In all cases requiring sharp debridement, this was performed in an appropriate surgical facility and within 10 days of presentation.

Following initial treatment, all ulcers were dressed and/or loosely packed with sterile gauze soaked with hypochlorous acid solutions. An appropriately compressive, multi-layered, overlying bandage system, utilizing short-stretch or non-stretch materials was constructed such that the greatest compression was at the ankle level. Light abrasion utilizing sterile cotton gauze soaked with hypochlorous acid solutions, followed immediately by flushing the wound with more of the solution effectively destroyed the extant biofilm in situ. With several repetitions over several days, it also prevented biofilm from re-establishing.

All 1249 venous leg ulcers reported in this data set were healed completely. The longest healing times were encountered by 10 patients for whom compression therapy was contraindicated: diabetic patients with severe arterial occlusive disease [$ABI < 0.6$]. Nonetheless, aggressive management with hypochlorous acid solutions resulted in complete wound closure within 180

days for this treatment refractory cohort. Perhaps the greatest advance in Venous Leg Ulcer (VLU) care is the addition of hypochlorous acid solutions to the treatment armamentarium.

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Suri A P S. The effectiveness of stable pH-neutral super-oxidized solution [HOCl] for the treatment of diabetic foot wounds. Poster at the Diabetic Foot Global Conference, Los Angeles, 2008.¹¹²

Objective: To evaluate the clinical efficacy and microbial load reduction of stable pH-neutral super-oxidized solution compared with saline in patients with diabetic foot wounds.

Design: A randomized trial of 100 patients with diabetic foot ulcers 2-15 cm in diameter treated with a once daily bath of either stable pH-neutral super-oxidized solution or saline for 16 weeks.

Results: The higher proportion of the stable pH-neutral super-oxidized solution treated group had a significant reduction in bioburden (76% vs 32%) and healed wounds (78% vs 40%).

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Martinez-de Jesus F *et al.* Efficacy and safety of a neutral pH superoxidized solution in severe diabetic foot infections. Int Wound J. 2007;4(4):353-362⁷⁴

Objective: To assess the efficacy of neutral pH superoxidized solution for infection control, odour reduction and surrounding skin and tissue damage on infected diabetic foot ulcerations.

Design: A randomized, single-blind trial of forty-five patients with DFU treated with standard care with or without neutral pH superoxidized solution, which was applied as a foot soak followed by spray application. Standard care consisted of broad-spectrum IV antibiotics, surgical debridement, and glycaemic control.

Results: Odour reduction was achieved in all neutral pH superoxidized solution-treated patients compared to patients treated without neutral pH superoxidized solution (100% versus 25%; $p<0.01$) and surrounding cellulitis diminished in 17 patients (80.9% versus 43.7%; $p<0.001$). Nineteen patients in the neutral pH superoxidized solution group showed advancement to granulating tissue stage (90.4% versus 62.5%; $p<0.05$) with significantly less tissue toxicity (94% versus 31.2%; $p<0.01$).

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Ricci E *et al.*, Clinical results about an antimicrobial solution (Dermacyn® Wound Care) [HOCl] in the treatment of infected chronic wounds. Poster presented at: 17th Conference of the European Wound Management Association (EWMA); 2007 May 2-4; Glasgow, UK⁹⁵

Objective: To evaluate the clinical performance of the antiseptic solution Dermacyn [HOCl].

Design: Forty patients with infected wounds were randomly assigned twice daily dressings of either [HOCl] or povidone-iodine. All patients received standard antibiotic therapy.

Results: The [HOCl] group had fewer days on average with clinical signs of infection (7.9 vs 5.4), odour (19 vs 2) and hospitalized (12.3 vs 8.2).

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Gold, M. H., Andriessen, A., Bhatia, A. C., Bitter, P., Chilukuri, S., Cohen, J. L., & Robb, C. W. (2020). Topical stabilized hypochlorous acid: The future gold standard for wound care and scar management in dermatologic and plastic surgery procedures. *Journal of Cosmetic Dermatology*, 19(2), 270-277. doi:10.1111/jocd.13280⁴⁵

Objective: This paper focuses on the use of topical stabilized HOCl in wound and scar management for pre-, peri-, and post-procedures—including its ability to reduce the occurrence hypertrophic scars and keloids.

Design: A panel comprising clinicians with experience in cosmetic and surgical procedures met late 2018 to discuss literature search results and their own current clinical experience regarding topical stabilized HOCl. The panel of key opinion leaders in dermatology and plastic surgery defined key insights and consensus statements on the direction of use for the product.

Results: 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.

Conclusion: For wound care and scar management, topical stabilized HOCl conveys powerful microbicidal and antibiofilm properties, in addition to potency as a topical wound healing agent. It may offer physicians an alternative to other less desirable wound care measures.

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Sakarya, S., Gunay, N., Karakulak, M., Ozturk, B., & Ertugrul, B. (2014). Hypochlorous Acid: an ideal wound care agent with powerful microbicidal, antibiofilm, and wound healing potency. *Wounds*, 26(12), 342-350¹⁰²

Objective: The aim of this study was to investigate the effect of stabilized hypochlorous acid solution (HOCl) on killing rate, biofilm formation, antimicrobial activity within biofilm against frequently isolated microorganisms and migration rate of wounded fibroblasts and keratinocytes.

Design: Minimal bactericidal concentration of stabilized HOCl solution for all standard microorganisms was 1/64 dilution and for clinical isolates it ranged from 1/32 to 1/64 dilutions.

Results: All microorganisms were killed within the first minute and accurate killing time was 12 seconds. The effective dose for biofilm impairment for standard microorganisms and clinical isolates ranged from 1/32 to 1/16. Microbicidal effects within the biofilm and antibiofilm concentration was the same for each microorganism.

Conclusion. The stabilized HOCl solution had dose-dependent favorable effects on fibroblast and keratinocyte migration compared to povidone iodine and media alone. These features lead to a stabilized HOCl solution as an ideal wound care agent.

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Hadi S F et al. Treating infected diabetic wounds with superoxidized water as antiseptic agent: a preliminary experience. JCPSP. 2007; 17(12); 740-743⁴⁹

Objective: To evaluate the effectiveness of superoxidized water in diabetic patients with different wounds.

Design: One hundred patients with diabetic foot ulcer (DFU) wounds randomized to treatment with either daily superoxidized water [HOCl] or saline soaked gauzes. All patients received IV antibiotic therapy and surgical debridement as necessary.

Results: Patients treated with superoxidized water [HOCl] had a significantly shorter period of hospitalization than saline-treated patients (1-7 days hospitalization of 68% vs. 20%, $p < 0.05$) and a higher proportion experienced a down-grading of their DFU (IV to I, 62% versus 15%, $p < 0.05$).

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Dalla Paola L, Carone A, Ricci S, Russo A. Advances in treatment of diabetic foot ulcers. Avances en diabetología. 2010;26(5):296-305.²⁶

Objective: To evaluate the efficacy of a novel HOCl product compared with standard treatment (10% povidone-iodine) in the treatment of diabetic foot ulcers.

Design: An open-label, non-randomized trial involving two hundred and twenty consecutive patients with stage II/III infected diabetic foot ulcers (DFU) treated with either Dermacyn dressings or povidone-iodine dressings for 10 days plus oral or parenteral antibiotics as necessary. The mean follow-up time was 94.8 days.

Results: At the time of surgical closure, 75% of the Dermacyn [HOCl] group and 48% of the povidone-iodine group were microbiologically negative ($p < 0.005$).

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Kapur V and Mawaha A K. Evaluation of effect and comparison of superoxidized solution (Oxum) vs. povidone-iodine (Betadine). *Ind J Surg.* 2011; 73(1); 48-53.⁵⁶

Objective: To evaluate the effect of superoxidized solution vs povidone iodine (Betadine) on similar types of wounds.

Design: 200 patients having wounds (acute and chronic ulcers, diabetic foot ulcers, venous stasis ulcers, cellulitis, carbuncles, abscesses of different types, burns, traumatic wounds, post-surgical wounds, pressure sores, bed sores, *fistula in ano*, gangrenous wounds, and internal irrigation like peritoneal lavage in peritonitis) prospectively randomized to treatment with either NEW-saturated gauzes or povidone iodine-saturated gauzes. All patients received antibiotics.

Results: The mean follow-up of 21 days showed that the average reduction in Diabetic Foot Ulcer (DFU) wound size in the superoxidized solution-treated group was 70% compared to 50% in the povidone iodine-treated group. Pus discharge in patients with abscesses was reduced earlier in the superoxidized solution-treated group (100% vs. 90% at day 12 for superoxidized solution vs. povidone-iodine) and there was an earlier appearance of granulation and epithelization (100% versus 85% at day 18 for superoxidized solution versus povidone- iodine). Superoxidized solution was safe and efficient as a wound care product and superior to povidone-iodine.

10. Review of Benefits: Safety

The safety of hypochlorous acid for human use has been evaluated multiple times over the past century, with increasingly pure solutions proving to be increasingly safe for human contact. Hypochlorous acid is a natural compound produced by human neutrophils so, when pure, there are teleological reasons to suppose HOCl might be safe for human use. Rigorous studies have justified that supposition, though the terminology associated with hypochlorous acid studies over the past decade, as noted above, can be confusing. Evaluating the full body of research can make the case for HOCl seem less compelling than it actually is.

The research results for HOCl have been consistent, and its killing potency is only one part of the historical record. Equally compelling have been the human safety trials and the absence of ANY resistance from any class of biological pathogen. There has not been a single verified claim of clinical resistance over more than 100 years of careful evaluation.

Table 2: From Wang et. al.¹²⁴

Table 5. Safety studies with control vs stabilized HOCl in 4 different animal species

Studies	Species	Site applied	NVC-101 (% w/v)	Results
Eye irritation	Rabbits	Eye, Q8 for 72 h	Saline	No irritation
			0.013 Betadine	No irritation Progressive irritation
Eye irritation	Rabbits	Eye, single instillation	Saline 0.01, 0.03, and 0.1	No irritation No irritation at any dose
Skin sensitization	Hartley-derived albino guinea pig	Skin	Saline 0.01, 0.03, and 0.1	No sensitization No irritation at any dose
28-Day toxicology	Rat	Full-thickness wound	Saline 0.01, 0.03, and 0.1	No systemic toxicity at any dose and histopathology consistent with wound healing
28-Day toxicology	Mini-pig	Full-thickness wound	Saline 0.01, 0.03, and 0.1	No systemic toxicity at any dose and histopathology consistent with wound healing

Clinical adverse events from exposure to pure HOCl (present at a pH between 4.0 and 5.33) have not been recorded in the medical literature. However, there are reports of incidents following exposure to relatively high pH, crude formulations (>6.5) containing mixed-oxidants, including hypochlorite, resulting from poorly controlled manufacturing processes. Similar problems may arise when modern HOCl formulations are deliberately pH-adjusted into the neutral or higher zone. Eye and skin inflammation and respiratory irritation are common with hypochlorite (bleach), which can be present at levels of 30% or more in “HOCl” solutions made or adjusted to pH 7, or in swimming pools that are improperly managed, allowing pH to rise into the alkaline range. (See figure 3 below).

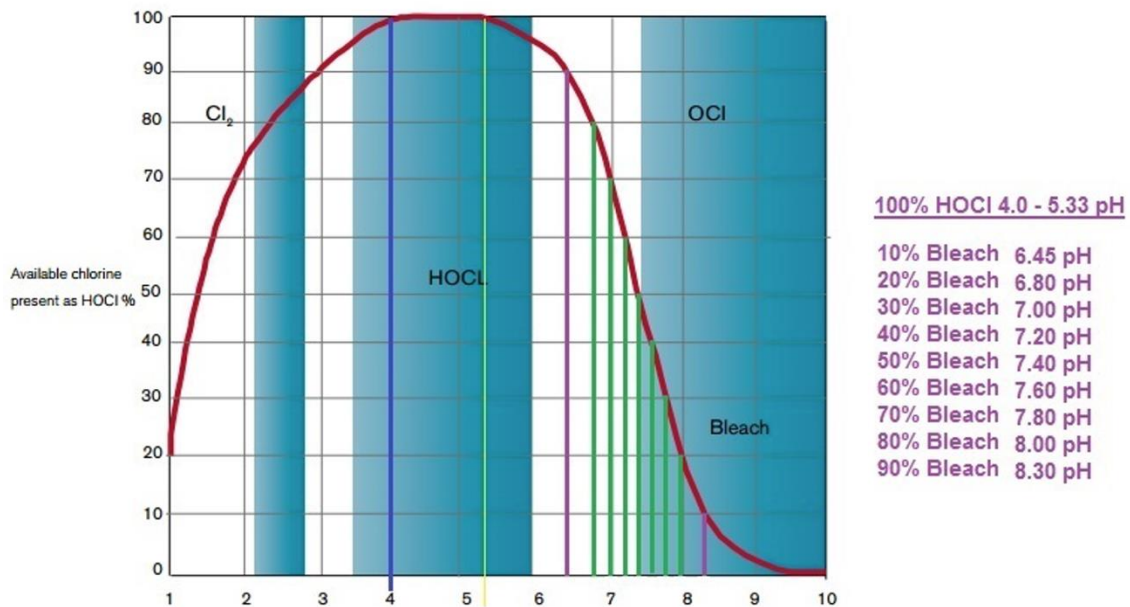


Figure 3: HOCl species concentration as a function of pH (graph courtesy of Briotech, Inc.)

We noted above the superior efficacy of HOCl over bleach and peroxide, and the significant toxicity present in the use of hypochlorite or peroxide when compared to HOCl. This section on Safety is an appropriate location to summarize that difference in a chart of the Therapeutic Index across those three disinfecting agents.

A Therapeutic Index, as is perhaps most familiar from cancer therapies, is a ratio between doing good and doing damage in the use of a biologically active chemical. Table 3 below documents the remarkable safety of HOCl against common infectious pathogens, particularly in comparison to the much more toxic peroxide and bleach:

Table 3: Comparative safety vs. efficacy between hydrogen peroxide, bleach, and HOCl.

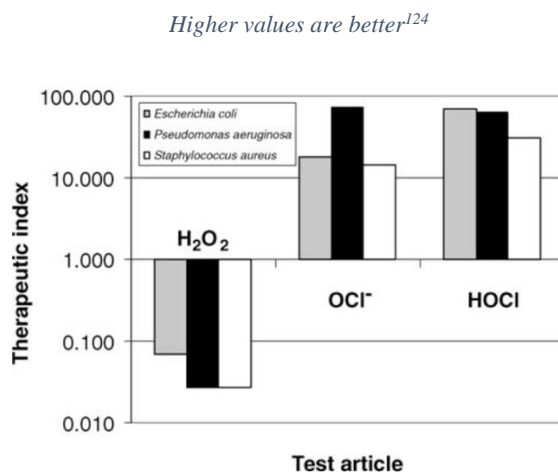


Figure 5. Relative therapeutic index of hypochlorous acid (HOCl; pH 4.0), hypochlorite (OCl⁻; pH 10.5), and hydrogen peroxide (H₂O₂; pH 7.0). Therapeutic index is expressed as a ratio of the CT₅₀ concentration (μg/mL) on L929 cells divided by the minimum bactericidal concentration (μg/mL) for *Staphylococcus aureus* 29213, *Pseudomonas aeruginosa* 27853 and *Escherichia coli* 2592. The higher the therapeutic index, the safer the test article will be.

10a. Clinical safety

A review by Thorn and colleagues in 2012 described both the exceptional antimicrobial efficacy of HOCl (particularly against *Cryptosporidium*) and its widely studied safety profile.¹¹⁷ They cite a large body of evidence available on safety, including single oral dose and 28-day repeated oral doses showing (1) no adverse effects in rats, (2) studies showing no toxicity to mucous membranes, and (3) accumulation-irritation tests and sensitization tests demonstrating biocompatibility at doses well above expected exposures. *In vitro* mutagenicity studies, too, have shown no evidence of genotoxicity on either Ames or micronucleus tests. Another broad safety study showed HOCl did not degrade nucleic acids or induce oxidative damage in dermal fibroblasts.

A 2011 study by Morita, *et. al.*⁸² evaluated the risk of biological toxicity in a mouse model when HOCl was ingested as drinking water for eight weeks. Visual inspection, gastrointestinal histology, inflammatory markers, mucosal thickness, periodontal tissue, tooth enamel, and other metrics were evaluated and no changes from controls were seen. Their conclusion was that HOCl has no systemic effects and would be safe if used as a mouthwash, even if ingested.

In 2015 Kubota *et.al.*⁶⁵ published a review of HOCl as a peritoneal lavage to prevent post-surgical infections after perforated appendicitis in children. The control lavage fluid was Normal Saline. There was no evidence of toxicity and they found a statistically significant reduction in surgical site infections with HOCl.

A 2017 paper by Aras *et. al.*³ looked at the potential toxicity associated with infusions of HOCl into the intraperitoneal cavity of rats. Evaluations seven days after infusion showed no evidence of pathology, no evidence of toxicity, and no statistical difference in blood biochemistry, renal function, or liver function between HOCl-infused rats and two sets of controls.

A 2019 review of the efficacy and safety profile of HOCl was published by Sipahi and colleagues.¹⁰⁹ That review evaluated *in vitro* toxicology, cytotoxicity, skin irritation, and eye irritation, and found HOCl exposure caused no harm and was deemed safe for skin and eyes. The authors recommended expanded use in food decontamination and medical care.

Block and Rowan⁸ have published a 2020 review of hypochlorous acid with a focus on its use within dental offices. Dental and oral surgery practices have been severely curtailed since the advent of SARS-CoV-2 and the article determined that, for safety and efficacy, HOCl may be the disinfectant of choice for restarting dental practices.

Unpublished

Two unpublished studies are also available for review:

1. A 2020 HOCl inhalational study at Stillmeadow Laboratories in the US exposed rats to four hours breathing a dense fog of Briotech's HOCl in a small chamber. Subsequent evaluations over two weeks showed no visible change in behavior and there were no detectable pathological changes at necropsy.
2. A second 2020 unpublished evaluation is a survey of human volunteers already choosing to inhale a dense fog of Brio HOCl for two minutes a day as COVID-19 prophylaxis, despite no evidence at the time of the survey (April-May 2020) that Brio HOCl would have any effect on SARS-CoV-2.¹ Permission was requested to have the volunteers describe on a survey form anything that happened during or after their inhalation.

¹ Two subsequent independent evaluations, however, have confirmed virucidal activity against SARS-CoV-2.

More than 450 voluntary exposures of two minutes or greater were documented, with a total inhalational exposure accumulation of more than 23 hours. Survey participant ages ranged from 18 to 77 and 84% were male. The results of the survey showed zero serious side-effects reported and 2.6% minor side effects (“nose tingling”, “runny nose”, “tickling feeling” and similar). No side effects persisted outside of the actual minutes of inhalation.

10b. Environmental safety

HOCl is a highly reactive molecule and short-lived when exposed to pathogens or other bioload. On exposure, pure HOCl degrades within minutes to NaCl and H₂O, becoming a benign and non-reactive salt water closely analogous to human tears.⁷¹

As a consequence of that rapid reactivity, pure HOCl at a label concentration of 180ppm poses no risk of environmental contamination (except perhaps as a mild 0.9% salt solution) and:

- Does not require Personal Protective Equipment (PPE),
- Can be stored with no hazardous materials protocol, and
- Can be disposed of with no risk of generating a toxic waste stream.

In contrast, impure HOCl/hypochlorite solutions, like hypochlorite (bleach) itself, require PPE, require hazardous material storage, and must be disposed of as both a toxic materials risk and an environmental hazard. Those same hazard considerations are also present for other classes of antiseptics and disinfection agents. See Figure 4 below:

	BrioHOCl	Alcohol	Bleach	Peroxide	Quaternary Ammonium	Chlorhexidine	Glutaraldehyde
Kills bacteria	✓	✓	✓	✓	✓	✓	✓
Inactivates viruses	✓	✓	✓	✓	✓	✗	✓
Kills spores	✓	✗	✓	✗	✓	✗	✓
Kills fungus	✓	✗	✓	✓	✓	✗	✓
Inactivates HPV	✓	✗	✓	✓	✗	✗	✗
Inactivates prions	✓	✗	✗	✗	✗	✗	✗
Remains stable for years	✓	✓	✗	✗	✓	✓	✓
Non-toxic	✓	✗	✗	✗	✗	✗	✗
Safe for skin	✓	✗	✗	✗	✗	✓	✗
Safe for eyes	✓	✗	✗	✗	✗	✗	✗
Safe for mucosa	✓	✗	✗	✗	✗	✗*	✗
Safe for inhalation	✓	✗	✗	✗	✗	✗	✗
Hypo-allergenic	✓	✗	✗	✗	✓	✗	✗
Safe for use (no PPE needed)	✓	✗	✗	✗	✗	✗	✗
Safe for food contact	✓	✗	✗	✗	✗	✗	✗
Rinse-free	✓	✓	✗	✗	✗	✗	✗
Environmentally friendly	✓	✗	✗	✓	✓	✗	✗
Biodegradable	✓	✓	✓	✓	✗	✗	✗
Not explosive	✓	✗	✗	✗	✓	✓	✓
Not flammable	✓	✗	✓	✗	✗	✓	✓
Does not stain or discolor	✓	✓	✗	✗	✓	✗	✓
No resistant organisms	✓	✗	✓	✓	✗	✓	✓
Odor control	✓	✗	✓	✓	✗	✗	✗
Biofilm slime disruption	✓	✗	✗	✓	✗	✗	✗

* With the exception of oral contact

Figure 4: Pure HOCl comparison chart for safety and efficacy [SDS matrix courtesy of Briotech, Inc.]

11. Cost analysis

HOCl was first synthesized before WW1 and for the past century has been prohibitively complex, impure, and unstable to allow for its broad use. Despite thousands of research papers showing exceptional efficacy, remarkable safety, and no resistance across a wide range of human pathogens, existing manufacturing processes generated impure mixed-oxidant solutions with toxic chlorine species present and proved unacceptable. Even relatively recent production methods dating from the 1970s that could generate HOCl in a more pure form had proved too expensive for widespread use, and had only limited capability for scalable production. Each of those flaws has now been overcome through new production methods and a scalable manufacturing system.

Modern manufacturing now permits the generation of pure, stable, authentic HOCl in large volumes for roughly one-eighth the cost of previous methods. **Current HOCl product pricing at scale can now be less than US\$2 (two dollars) per wholesale liter at the manufacturing facility**, with small regional variations. For historical comparison, a previous submission to the EML for exclusive inclusion of a proprietary electrolytically generated HOCl formulation (Electrocyn, 2017) had specified the cost at more than US\$11 (eleven dollars) per liter wholesale.

11a. Local production

As a further advance over previous capabilities, the capacity to produce HOCl from small, localized, and networked manufacturing facilities is now available. That eliminates the cost of transporting an HOCl solution essentially the density of water (at 1kg/liter) and allows even very remote locations to produce a pure and stable HOCl that meets the quality standards of a major production facility. Those small and local manufacturing facilities are designed to produce between 5,000 and 40,000 liters a day. Process control is ensured via remote operation overseen by centralized Operation Centers using low-bandwidth cell and satellite connections. Operations include Quality Control sensors that ensure rigorous production standards from readily available ingredients (salt and water). These local manufacturing systems create a local HOCl supply, local jobs, and consistent availability of high-quality product.

11b. Charitable contributions

Briotech, Inc. is the leading manufacturer of pure and stable HOCl at scale in the United States and has established a charitable arm. That division of the company donates both HOCl product and 5% of gross profits to vulnerable populations in Latin America, Africa, Southeast Asia, and India. It has also provided *gratis* HOCl for use on Native American reservations in North America.

Additionally, Briotech's remote manufacturing partners are obligated, by contract, to donate 10% of their entire HOCl production volume to registered charitable organizations in their region. Briotech is aware that HOCl, even at a significantly reduced cost resulting from improved manufacturing processes, is still financially inaccessible to many who would most benefit from both its area disinfecting and its wound-healing properties. Briotech is working with trusted NGO and commercial partners to identify those most in need (including within the Ebola Clinics of eastern Congo), and providing both in-kind and financial support.

Other global HOCl manufacturers may choose to make similar decisions on accessibility.

12. Summary of regulatory status and market availability

US EPA:

- There are 17 approved HOCl products for COVID-19 disinfection alone, including BrioHOCl (#93108-1).
- HOCl is approved by the EPA for use in drinking water.
- HOCl is approved for use as a no-rinse food sanitizer.

US FDA:

- HOCl is approved for use on meat, poultry, fish, and other seafood, fruits, and vegetables as a no-rinse sanitizer under:
 - FCN 1811, 13 October 2017
 - 21 CFR 173.315
 - Decision #692
 - 21 CFR 178.1010
 - 21 CFR 7120.1
- HOCl may be applied to food-contact surfaces in public eating places, dairy processing equipment, food-processing equipment, and utensils.

US Department of Agriculture:

- HOCl is approved as an organic substance in the USDA's National Organic Program.

Australian Government, Department of Health, Therapeutic Goods Administration:

- HOCl approved for use against COVID-19:
 - http://search.tga.gov.au/s/search.html?collection=tga-artg&profile=record&meta_i=343431

Health Canada:

- HOCl is approved as a COVID-19 disinfectant under DIN #024-957-16

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There is substantial international academic and regulatory evidence that hypochlorous acid is safe for use on humans, and that significant antimicrobial and wound management goals can be achieved through its use.

We respectfully request hypochlorous acid (HOCl) be added to the World Health Organization's Essential Medicines List core for 2021. //

Brio HOCl Recent Presentations and Papers

1. **254th (ACS) American Chemical Society Conference** | Washington, DC

Purity and Stability of an Electrolytically-Generated Hypochlorous Acid Solution

Briotech with the University of Washington

Paper presented August 2017

2. ***Hypochlorous Acid: Harnessing an Innate Response***

<https://infectioncontrol.tips/2017/10/06/hypochlorous-innate-response/>

Published online, October 6, 2017

3. **2017 (GHTC) IEEE Global Humanitarian Technology Conference** | San Jose, CA

Stabilized Hypochlorous Acid Disinfection for Highly Vulnerable Populations: Brio HOCl Wound Disinfection and Biofilm Eradication

Primary research article

<https://ieeexplore.ieee.org/document/8239259>

Published online, December 2017

4. **By Special Invitation, Briotech Presents at the US FDA HQ** | Silver Spring, MD

High Level Inactivation of Prions and Other Infectious Pathogens with Novel Preparations of Hypohalous Acids: BrioHOCl

Seminar presented February 6, 2018

5. **18th International Congress on Infectious Disease** | Buenos Aires, Argentina

Inactivation of Prions and Amyloid Seeds with Hypochlorous Acid

Three papers presented March 1-4, 2018

6. **2018 CJD Foundation Family Conference** | Washington, DC

Investigation of Prions Inactivation by Reactive Oxygen Species in Vivo

Primary research by Allison Kraus, PhD, NIH, Presented July 13-15, 2018

7. **2019 Annual Conference** | London, England

Hypochlorous Acid (HOCl) and its potential applications in international dermatology

Briefing paper presented September 11, 2019

8. **2019 ICPIC Conference** | Geneva, Switzerland

Inactivation of Scrapie Prions and Antimicrobial Properties of Hypochlorous and Hypobromous Acids

Paper P487 presented September 13, 2019

9. **2019 Greater New York Dental Conference** | New York, NY
Efficacy Testing on HOCl as a Disinfectant for High-Risk HPV
Paper P483 presented September 13, 2019

10. Dennler-Church TE *et al*, *Modification of Major Contributors Responsible for Latrine Malodor on Exposure to Hypochlorous Acid: The Potential for Simultaneously Impacting Odor and Infection Hazards to Encourage Latrine Use*. **The American Journal of Tropical Medicine and Hygiene**, (2020). <https://www.ajtmh.org/content/journals/10.4269/ajtmh.20-0553>, 15 October 2020.

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Appendix 1: Case Reports

Case 1

65yo male
DM II
Presented to the ER
presumed septic shock
Blood cultures later grew
Klebsiella sp and
Pseudomonas sp.
Phlegmon in left inner thigh
unclear etiology
Initial excision 01 October 2020
This photograph 5 days after excision:
Iodophor gauze to wound edges.



65yo male
DM II
Presented in sepsis
Two weeks of topical BrioHCl



65yo male
DM II
Left thigh phlegmon
Day 30 of BrioHCl tid



Case 2

Infected diabetic foot

Widespread necrosis

Topical BrioHOCl spray

First application 15 October 2020



Day 1 of Brio tid

Infected diabetic foot

Widespread necrosis

Topical BrioHOCl spray



Day 27 of Brio tid

Case 3 (used with permission)

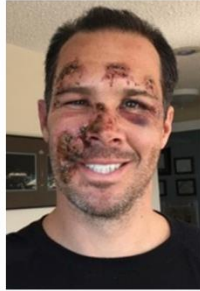
Traumatic Abrasion Wound Resolution

Age: 37 Sex: Male

Treatment: Sole use of BrioHOCl spray applied multiple times daily



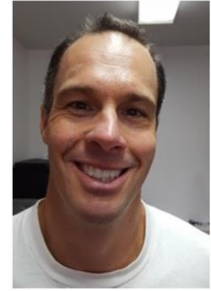
Day of Injury



Day 2



Day 11



8 Months After

Case 4

Deep Laceration Wound Resolution

Age: 52 Sex: Male

Treatment: BrioHOCl Topical Skin Spray and light dressing – 5 daily applications, no sutures



Day of Injury



Day 3



Day 5

Case 5

Serious Full-Thickness Burn Resolution

Age: 50 Sex: Female

Treatment: BrioHOCl Topical Skin Spray using 4-5 daily applications



Day 6



Day 7



Day 9



2 Months After

Case 6 (used with permission)

Chronic cystic acne since age 4

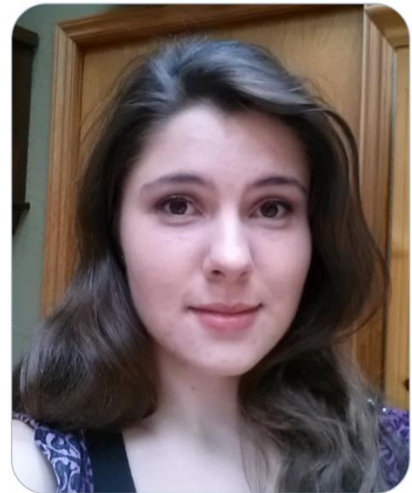


Reproduced with permission

- at inception of Brio HOCl therapy -



No
cosmetics



- one month later after TID application -

Case 7 (used with permission)

3yo boy fell into a cooking pot near the Ebola Clinic in Bukavu, DRC.

March 2019

3yo boy
Boiling water scald
Clinic presentation
NE of Kinshasa, DRC

BrioHOCl as sole therapy.

(Only care available – was
not a study or a choice)

Used with permission.



March 2019

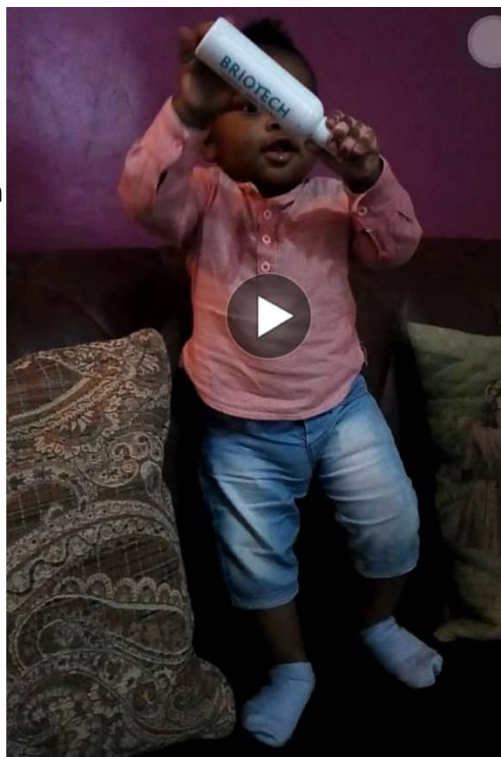
3yo boy
Boiling water scald

96 hours after presentation.

BrioHOCl was only therapy.



Still from video
showing full function



Staff at the DRC Clinic in Bukavu, DRC expressing thanks for the HOCl burn care.

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