**Medical Surfaces ATP Study:**

**Hygienic Surface Washing Comparing**

**Germicidal Wipes Alone vs Germicidal Wipes + Ozone Water Rinse**

**And Soapy Water vs Soapy Water + Ozone Water Rinse**

**An Evaluation of Adenosine Triphosphate (ATP) Reduction on Medical Surfaces, as a Measure of Biologic Material Reduction.**

**Alvaro Liceaga, M.D.; Elvin Mercado, C.S.T.; Mary Narcaroti R.N.MSHA; Lisa Liceaga B.S.**

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**Study:** **Medical Surfaces ATP Study: Hygienic Surface Washing Comparing Germicidal Wipes Alone vs Germicidal Wipes + Ozone Water Rinse and Soapy Water vs Soapy Water + Ozone Water Rinse. An Evaluation of Adenosine Triphosphate (ATP) Reduction on Medical Surfaces, as a Measure of Biologic Material Reduction.**

**Introduction**

Clean Medical Surfaces are important in reducing cross contamination and risk of nosocomial infection. When clean hands touch dirty surfaces in a medical office or suite, microorganisms are spread from that point onward. It is proposed that the addition of ozonated water to a surface wash regimen can markedly reduce or eliminate pathogens on surfaces.

It is postulated that hygienic hands are the single most important factor in preventing the spread of pathogens and in reducing the incidence of healthcare associated nosocomial infections. In *Hand hygiene skin ATP study: Hand washing with tap water and soap vs. ozonated water and soap vs. antiseptic and ozonated water vs. antiseptic and tap water. A Unique Comparison in Regard to Adenosine Triphosphate (ATP) Reduction in Skin as a Measurement of Biologic Material Reduction,* Liceaga et al., 2016 (1), found that the addition of ozonated water to a supervised handwashing regimen was highly effective in ATP reduction, which is a measure of biologic material reduction. Handwashing with soap and rinsing with ozonated water resulted in an over-all 97.3% reduction in ATP levels that was comparable to the use of Chlorhexidine solution at 97.5% reduction.

Of great concern are the solid surfaces that healthcare workers and patients touch in a medical suite. Curtis J. Donskey MD’s research article in 2013, reported that a growing body of evidence has accumulated, suggesting that improvements in environmental disinfection may prevent transmission of pathogens and reduce health care-associated infections. Contaminated environmental surfaces provide an important potential source for transmission of many health care-associated pathogens79.

Contact with unclean medical or surgical surfaces is a common mode of transmitting pathogens. Hospitals, operating rooms, medical offices, specialty clinics, therapy centers, and outpatient surgery centers are in need of an effective surface cleaning regimen. Additionally, it has been found that antibiotic-resistant superbug bacteria are growing in hospital sinks and drains and can splash out onto counters, causing undesired spread.

Soap and Water physically removes particulates, soil and organic material and reduces microbial contamination on surfaces. Unfortunately, water alone cannot remove all contaminants, such as oils, fats and microorganisms, thus requiring the use of soaps or detergents to dislodge and dissolve those materials.

Standard washing solutions and techniques are described by various governmental agencies. Despite best cleaning efforts, washing is only partially effective in this regard.

A number of sanitizing chemicals have become commonplace in the medical setting:

* Isopropyl alcohol solution typically contains a 70% solution of isopropyl alcohol in water used as a sanitizer to nearly eliminate germs.
* Germicidal wipes are very commonplace in a medical setting. We chose Sani-Cloth® Germicidal Wipes since they are in widespread use. They have specific recommendations for use, issued by the manufacturer.
* Chlorinated water is a common sanitizer, but some microorganisms such as E. coli and Giardia can build resistance to chlorine over time. Chlorinated water creates chloramines and trihalomethanes (THMs), which can irritate eyes, throat, lungs, and skin. Chloroform compound, a carcinogen, is found in THMs.
* Sanitizing alcohol based gels containing 70% ethanol are in widespread use, but have the downside of gel buildup that reduces effectiveness and alcohol does not effectively kill all pathogens.
* Antibacterial soaps had been in widespread use. Additives like Triclosan 0.375% or Chloroxylenol 0.95% (PCMX) and 17 other chemicals were banned in 2016 by the FDA(3). Manufacturers have replaced Triclosan with one of three other chemicals: Chloroxylenol (PCMX) Chloride, Benzalkonium Chloride, and or Benzethonium.
* Betadine (Povidone-iodine) has long demonstrated antibacterial effectiveness, but is used mainly for procedural applications or surgical prep and not for surface washing.
* Chlorhexidine (Hibiclens) is an antibacterial used as an antiseptic and for many medical and dental applications. Surgical Scrub Hibiclens is used mainly for procedural applications or surgical prep and not for surface washing.
* Hydrogen peroxide (H2O2) can be used for the sterilization of surfaces, such as surgical tools and for wound disinfection. H2O2 has broad-spectrum properties against bacteria, viruses, yeasts, and spores, but can be harmful to skin tissue. H2O2 is used mainly for procedural applications and generally not for surface washing.
* Enzymatic solution is a biodegradable, non-toxic cleaning agent that contains two proteolytic enzymes and is used for effective medical instrument cleaning. It removes any trace of blood, tissue and other protein rich body fluids that can be found on surgical instruments. Enzymatic solution is only used on instruments.
* Ozonated water (aqueous ozone): It is proposed that the addition of ozonated water to a best-performed wash regimen can substantially and markedly reduce residual contaminants on surfaces. Unfortunately, there are only a few studies regarding surface sanitation with ozonated water. More are needed.

**AGENTS USED FOR THE PROPOSED STUDY:**

**SANI-CLOTH®** **Germicidal Disposable Wipes.**  We chose Sani-Cloth Germicidal Wipes® since they are in widespread use. They are commonly used in many medical practices and are described as: A Quaternary chemical, for the disinfection of sensitive hard, non-porous surfaces and equipment. They are bactericidal, fungicidal and virucidal. Sani-Cloth®HB Germicidal Disposable Wipes kill HBV, Influenza A/Hong Kong, Acinetobacter, Aspergillus Niger and Trichophyton mentagrophytes plus over 100 microorganisms in 10 minutes. They are designed for daily use in fast-paced environments that require the shortest contact times and broadest coverage of microorganisms.

Sani-Cloth® reportedly kills: Acinetobacter Baumannii; Klebsiella Pneumoniae; Candida Albicans; ESBL Producing E. Coli; Influenza A; MRSA; VRE; HIV; HBV; HCV. Chemical Composition: Isopropanol 30-60%; Benzyl-C-12-18-alkyldimethyl ammonium chlorides 0.1-1 %; Quaternary ammonium compounds, C12-18-alkyl [(ethyl phenyl) methyl] dimethyl, 0.1-1 %.

**BACTERICIDAL SOAP:** McKesson Pro Tech RTU Disinfectant Surface Cleaner®; Chemical name: Diethylene Glycol Monobutyl Ether; MFR #: 53-28561, 53-28564. Product Description: A ready-to-use disinfectant cleaner formulated with cationic & nonionic surfactants, chelating agents, builders, and glycol ether.

**AQUEOUS OZONE**: Ozon**e,** from the Greek word “Ozein” which means “to smell”, has a very characteristic odor and is a powerful oxidant. Originally used for water decontamination, ozone is a common disinfectant in industrial and food applications, e.g. water treatment, food processing, and sanitation of the processing facility.

In the food industry, aqueous ozone is sprayed on equipment, walls, floors, drains, tubs, and tables (78). It is also commonly used as a disinfectant on fruits, vegetables, poultry and seafood. On surfaces, aqueous ozone is used in a two-step ozone sanitation process where surfaces are cleaned and biofilms are removed with a hot water or cleaning step, then aqueous ozone is used to sanitize the surface destroying bacteria, viruses, fungi, and spores. No rinsing steps are necessary since the ozone leaves no residual on surfaces. This industry reportedly saves cleaning time, chemical use and water usage costs without the harmful effects on metal or wooden equipment.

In food processing, keeping food products pathogen free and reducing the potential for cross-contamination of potentially harmful or deadly pathogens is of great importance80. The U.S Center for Disease Control (CDC) estimated that each year 16% of Americans get sick from food borne diseases. Currently, food processing plants use aqueous ozone for antimicrobial intervention steps directly on the surface of food products.

**Ozonated water’s potential use for medical surface sanitation.**

Ozonated water has been proposed as an alternative disinfection agent to prevent deformation or other damage during conventional autoclave disinfection and is approved by the US Center for Disease Control for the rapid disinfection of medical surfaces and equipment *(CDC, 2008) (73).*

Ozone is produced using an ozone generating electrical device, by an ozone generator located under the faucet. The gas is dissolved into the water via a venture-type mixing system so that the ozone concentration at the tap outlet remains constant for a given flow rate. Typical industrial tap ozone gas concentrations can range from 0.5 to 4 ppm. The faucet used produces a range of ozone dissolved in water at 0.5 to 1.4 ppm. It is believed that better contact time at lower concentration produces greater effectiveness and less overspill concerns in the atmosphere.

In water, ozonated water is produced when gaseous oxygen (O2) molecules are dissociated by an electrical charge into nascent oxygen atoms (O-) which then subsequently collide with oxygen molecules to form the unstable ozone gas molecule (O3) which has very high oxidizing capacity. Water, when “ozonated” (O3-H20), produces free radical oxygen molecules that react with the microbes and effectively kill them via oxidation. Ozone’s antiseptic properties arise from its ability to denigrate cell walls and in some cases cell RNA as well as oxidize organic and non-organic compounds.

Ozonated water can effectively kill all microbes and pathogens, including bacteria, viruses, molds and spores. Ozone is fast reacting and is nonselective to all microorganisms. According to the US EPA Office of Water, ozone is one of the most potent and effective germicides when used in water treatment and is effective against bacteria, viruses, mold and protozoan cysts *(US EPA, 1999).*

The University of Dundee evaluated the antimicrobial efficiency of ozone water from an ozone generating faucet (21). They found that ozonated water is effectively used as a surface sanitizer in food preparation, cooking and kitchen surfaces. It was shown that ozone has strong antimicrobial properties and is at least 100 times stronger and reacts 3000 times faster than chlorine as a disinfectant. Although chlorine use is known to react with organisms (eg, bacteria, flesh), it can form highly toxic THMs (tri-halothanes) that are carcinogenic. By comparison, ozone leaves no trace of residual products upon oxidative reaction. In fact, in approximately twenty minutes, the ozonated water will break down into plain pure water and oxygen gas. The investigators at the University of Dundee concluded that ozonated water can inactivate bacteria on solid surfaces by over 99.8% within 1 minute. The investigators further believed that the use of ozone water may reduce the use of other potentially toxic chemical disinfectant use by up to 50%.

**Sani-Cloth® Disinfecting Wipes vs. Ozonated Water**

In this study, we will evaluate whether the bioload present on the surfaces (measured by reading the ATP of viral, bacterial and/or other organisms), is lowered by the addition of ozone water as compared with Sani-Cloth germicidal wipes.

Since ozonated water (O3-H20) is known to possess antimicrobial properties, we proposed this study in our series of tests to compare the effectiveness of ozonated water (O3-H20) by measuring ATP on a variety of medical surfaces. Ozonated water is generated through a Lenova Faucet System. Sani-Cloth germicidal wipes were chosen because they are in common use in the medical environment.

**Materials:**

**OZONE FAUCET**

The aqueous ozone generator used in this study is the Sanitas by Lenova Ozone Faucet. It is used in medical, dental and commercial applications for surface disinfection. The faucet system consists of a stainless steel or chrome faucet connected to both typical water supply line and an AC outlet. The ozone is produced through a closed, self-contained system that produces ozonated water by a patented ozone gas generator and valve system (that insures safe constant ozone concentration), and a Venturi mixing tube, designed to attach to a typical plumbing system with normal water pressure. The valve system allows the faucet to produce a consistent flow rate of about 1.2 gallons per minute (same as any common faucet) with an ozone concentration between 0.8 and 1.4 ppm. This concentration is considered safe to use and is very effective as an antimicrobial that can reportedly kill most pathogens in 30 seconds to 2 minutes of contact time.

 

**HYGIENA**

ATP (adenosine triphosphate) is an energy molecule found in all living cells that allows cellular metabolism to take place. All organic matter contains ATP, including skin, blood, mucus, saliva, viruses, fungi, molds, spores and bacteria. Hygiena’s SystemSURE Plus ATP Cleaning Verification System is a common commercial tool used to monitor and improve the cleanliness levels of surfaces in healthcare facilities. It uses bioluminescence technology to identify and measure ATP. The Hygiena’s SystemSURE device includes UltraSnap ATP Swabs that contain an enzyme called Luciferase which produces a bioluminescence (light-producing) reaction when it comes into contact with ATP. A Luminometer, a handheld, light-reading unit, is used to measure low levels of ATP collected with a swab. Contaminations can be detected in approximately 15 seconds. Results are expressed numerically on the SystemSURE Plus screen as Relative Light Units. (RLU).

In healthcare facilities, organic matters in bodily fluids such as blood and sputum, or on surfaces that patients and staff touch, contain a variety of microorganisms and can become a point of cross-contamination. Cross-contamination between staff and patients may lead to the spread of infections if surfaces and hands, are not properly cleaned or disinfected. Therefore, this study examines the detection of ATP on several surfaces after cleaning. The persistence of ATP on a surface may be an indication of incomplete or improper cleaning technique.

**O3 Concentration:** Standard O3 content, pH and water temperature conditions were met during testing. We used an Ozonesolutions.com Monitoring Device which measures ozone content in the water (up to 5.0 PPM). Testing in this study observed the content of ozone in the water at average of 1.18 ppm.

**Methods and Procedures**

All testing was conducted with sterile gloves.

**Control test**: Six representative medical surfaces were selected. The patient lobby counters, front office desks, pre-operative counters, patient gurneys, nursing station desk and the dirty room counter (Sterile Processing Department). All surfaces were tested after a regular use working day. Before initial cleaning, the surfaces were swabbed using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The control numbers were recorded.

**Sani-Cloth® Germicidal Disposable Cloth Using One O3 Water Rinse:**

First, the surfaces were wiped with a Sani-Cloth Germicidal Disposable Cloth using a circular cleaning technique and the area left visibly wet following the manufacturer’s instructions. The area was then allowed to dry. Once dry, we swabbed the area using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The result numbers were recorded.

Next, we introduced the ozonated water. The same area was cleaned with a sterile towel soaked enough to rinse once with ozonated water. The surface was then allowed to dry. One dry, we swabbed the same area using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The result numbers were recorded.

**Sani-Cloth® Germicidal Disposable Cloth Using Two O3 Water Rinses:**

The following week, the surfaces were wiped with Sani-Cloth Germicidal disposable cloth using a circular cleaning technique and the area left visibly wet following the manufacturer’s instructions. The area was then allowed to dry. Once dry, we swabbed the area using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The result numbers were recorded.

Next, we introduced the ozonated water. The same area was cleaned with a sterile towel soaked enough to rinse once with ozonated water and the surface was then allowed to dry. Each surface was then rinsed a second time with ozonated water, where the same towel was then washed in fresh running ozonated water and used it again to wipe the area for the second time. Once the surface is dry, we swabbed the area using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The result numbers were recorded.

**Soapy Water Test and Ozone Using One O3 Water Rinse:**

**Control test**: A week later, the test was repeated on the same surfaces. The six representative medical surfaces were selected (patient lobby counters, front office desks, pre-operative counters, patient gurneys, nursing station desk and the dirty room counter). All surfaces were tested after a regular use working day. Before initial cleaning, the surfaces were swabbed using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The control numbers were recorded.

Each surface was washed with soapy tap water and rinsed once with ozonated water. Using a circular cleaning technique, the surfaces were cleaned with sterile towel soaked in soap and tap water. The surface was left visibly wet and allowed to air dry. Once the surface is dry, we swabbed the area using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The result numbers were recorded.

**Soapy Water Test and Ozone Water Using Two O3 water Rinses:**

**Control test**: A week later, the test was repeated on the same surfaces. The six representative medical surfaces were selected (patient lobby counters, front office desks, pre-operative counters, patient gurneys, nursing station desk and the SPD dirty room counter). All surfaces were tested after a regular use working day. Before initial cleaning, the surfaces were swabbed using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The control numbers were recorded.

**Two Ozonated Water Rinses:** Each surface was washed with soapy tap water and rinsed with ozonated water. Using a circular cleaning technique, the surfaces were cleaned with sterile towel soaked in soap and tap water. The surface was left visibly wet and allowed to air dry. Each surface was then rinsed a second time with ozonated water. The same towel was then washed in fresh running ozonated water and used it again to wipe the area for the second time. Once the surface is dry, we swabbed the area using the UltraSnap ATP detection device and measured with the SystemSURE Plus Luminometer. The result numbers were recorded.

**Raw Data:**



**Percent Reduction Rate Results and Observation:**

**Sani-Cloth® = 58.43%**

**Ozone Water Rinse x 1 = 85.25%**

The surfaces that were cleaned with only Sani-Cloth resulted in average of **58.43%** reduction in ATP levels.

The surfaces that were cleaned with Sani-Cloth and then once rinsed once with ozonated Water resulted in an average of **85.25%** reduction in ATP levels.

**Percent Reduction Rate Results and Observation:**

**Soapy Tap Water Only = 73.20%**

**Ozone Water Rinse x 1 = 91.30%**

The surfaces that were cleaned with just using soapy tap water resulted in average of **73.20%** reduction in ATP levels.

The surfaces that were cleaned with soapy tap water and then rinsed once with ozonated water resulted in an average of **91.30%** reduction in ATP levels.

**Percent Reduction Rate Results and Observation:**

**Sani-Cloth® Only = 61.5%**

**Swipe with Ozone Water x 2 = 91.5%.** *(Using improved cleaning techniques with 2 swipes)*

The surfaces that were cleaned with just using just Sani-Cloth resulted in average of **61.5%** reduction in ATP levels.

The surfaces that were cleaned with Sani-Cloth and then rinsed twice with ozonated water resulted in an average of **91.5%** reduction in ATP levels.

**Percent Reduction Rate Result and Observation:**

Soapy Tap Water = 72.50%.

The surfaces that were cleaned with just using soapy tap water resulted in average of 72.5% reduction in ATP levels.

Swipe with Ozonated Water x 2 = 94.3%. *(Using improved cleaning techniques with 2 swipes)*

The surfaces that were cleaned using improved cleaning techniques with soapy tap water and then rinsed twice with ozonated water resulted in an average of 94.8% reduction in ATP levels.

**LEGEND**

**Discussion**

Clean hygienic surfaces are an important factor in preventing the spread of pathogens and in reducing the incidence of healthcare associated nosocomial infections, especially with the growing risk of infection with resistant pathogens. This study helps confirm that ozonated water possesses excellent antimicrobial properties and should have a place in effective surface hygiene protocols.

In this study with a standard application method, the surfaces tested with Sani-Cloth® alone had just a 58.43% average reduction rate in ATP levels. The surface tested with Sani-Cloth® and ozone rinse x 1 had an average 85.25% reduction rate in ATP levels. The soapy water test had an average reduction rate of 73.20% in ATP levels. The soap and ozonated water x 1 test had an average reduction rate of 91.30% in ATP levels. The soap and ozonated water rinse x 2 test had an average reduction rate of 94.3% in ATP levels.

The above shows that routine surface washing in patient care is effective in removing organic material and microbial contamination acquired by contact with patients or the environment. It is known that unfiltered tap water may contain a variety of microorganisms and pathogens and can be a source of nosocomial infections. Even if hygiene practices are in place, a plausible route for transmitting these organisms from water to patient could be through the use of contaminated tap water.

Surfaces that are cleaned with tap water will be re-contaminated by rinsing with unfiltered tap water. A great additional benefit of utilizing ozonated water is that, it in effect, ozone disinfects unfiltered tap water. Thus, the surfaces that are cleaned with ozonated water will not be re-contaminated.

**Surface Biofilm**: A Medlinesearch from 1966 to 2001, cited below, found 43 outbreaks, of which 69% could be linked by epidemiological and molecular evidence to a surface biofilm. The aforementioned routines show that disruption of the surface biofilm is most important to allow the ozone molecule to be maximally effective. The use of soapy water or use of Sani-Cloth® Germicidal wipes may help to achieve this. Although in this study, Sani-Cloth alone had just a 58.43% average reduction rate in ATP levels, whereas the soapy water test had an average reduction rate of 73.20%.

**Ozonated Water:** We found that the addition of 0.8 to 1.5 ppm of ozone dissolved in water, substantially reduced levels of undesirable organisms on surfaces (up to 94%). The results of this study confirm that there is substantial ATP reduction on the surface that was further reduced with the addition of ozonated water after surface washing. It is suggested that regular use of ozonated water in a surface sanitation program may assist in the reduction of the biofilm over time.

Additional testing is recommended to confirm the above hypothesis, perhaps including bacterial, viral and fungal culture testing.

**Summary:**

The addition of ozonated water to the surface washing regimen is recommended by the authors, as it has proven to be of value in reducing ATP levels on the medical equipment surfaces following this preliminary study. Further studies are needed to issue additional recommendations.

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