# An investigation of electro-chemical activation systems and solutions and alternative procedures for enhanced cruise ship and facilities hygiene and sanitization.

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## *Abstract*

An alternative method and means for surface cleaning and sanitizing was investigated on the Golden Princess cruise ship. Existing cleaning and sanitizing procedures use chemical concentrates that are stocked on board. A sixty-day at-sea trial beginning 1 October, 2013 investigated the attributes of electro-chemically activated (ECA) cleaning and sanitizing solutions that were generated on-board by a new fully automatic electro-chemical activation system (ECAS) by Clarentis® Technologies. Our investigation examined solutions efficacy, impacts on operations, comparative risks to crew, guests, equipment, and furnishings and estimated costs as compared to current public health and house keeping procedures. Results demonstrated that the anolyte sanitizer product generated on site by the device (branded elsewhere as Ultra-Lyte®) reduced soil loads significantly and when used as a two-step "ESOP" process, it demonstrated superior reductions of HPC.

## *Introduction*

Outbreaks in cruise settings pose a significant economic impact resulting from the costs to disinfect areas to prevent the propagation and recurrence of infection, the potential delay or cancellation of cruises, loss of productivity associated with ill employees, deployment of resources to control and investigate an outbreak, and the hospitalization of affected individuals. Norovirus outbreaks are particularly difficult to control due to the viruses' having high attack rates, environmental stability, and a human infectious dose estimated to be as low as 10 virions (Park, 2007).

Since 2007, Princess Cruises has used an accelerated hydrogen peroxide product known as Virox™ for sanitation and disinfection for surfaces. While Virox™ has shown to be successful in reducing viral and bacteriologic loads in vitro and during operation, its prolonged use has been associated with corrosivity of fabrics, brass and other metal finishing's and presumptively associated with cases of contact dermatitis on hands and other exposed skin surfaces among crew members. A combination of the above factors as well as transport and logistics concerns led the Public Health Department to conduct a comprehensive review of alternative solutions. Pre-selected criteria included the following:

Ease of implementation with current operation,

Reduction in amount of damaged surfaces

Onboard production and lastly,

Cost

Of the several solutions evaluated, the one, which appeared to include the considerations above, was an electrochemically-activated solution, commonly referred to as ECAS. Numerous studies have found ECAS to be highly efficacious, as both a novel environmental decontaminant and a topical treatment agent (with low accompanying toxicity). In addition to efficacy, most ECAS can be produced on site and the equipment requires minimal maintenance for continued use. A literature review of ECAS shows they have been placed in hospitals, operating clinics, major food production and processing plants, in dental lines for use of biofilm removal, etc. Electrochemically activated solutions are produced by electrochemical unipolar action. This reaction produces two solutions - one referred to generically as anolyte contains a variety of oxidants, including hypochlorous acid, free chlorine and free radicals, known to possess antimicrobial and antiviral properties (Thorn, 2011) and another generically referred to as catholyte containing an alkaline solution with surfactant properties. ECAS anolyte has been demonstrated to have broad-spectrum antimicrobial and antiviral activity, and also have the potential to be widely adopted within the cruise industry due to low-cost raw material requirements and ease of production (Tagawa, 2000; Morita, 2000; Park G. B., 2007; Kitano, 2003; Thorn, 2011).

## ***Methods and Means***

In September 2013, Princess Cruises was approached by Johnson Diversified Products, Inc. (JDP) to conduct an evaluation of Ultra-Lyte®, an EPA registered ECAS product proven effective at reducing targeted key pathogens, removing biofilm and capable of being produced onboard by using water, salt, and small amounts of electricity. Princess Cruises agreed to conduct a trial aboard the Golden Princess for a 12-week period to evaluate this product against Virox™. JDP provided 2 Clarentis Technologies, LLC, model UL-75a electro-chemical activation system (ECAS) one of which was pre-installed on a mobile modular base.

The system modules were secured in vessels chemical locker on 30 September, 2013 on Gala Deck 4 aboard the Golden Princess. A plug-n-play system, once in place it was connected to ships water and to mains electric (either 120V or 220V) and the system was operational within an hours time.

### *Preparation of solutions*

The Clarentis® UL-75a (mini) has an integral Schneider Electric PLC, allowing user-defined anolyte "recipes" automatically generating from dilute brine a highly charged ionically bonded species of hypochlorous acid. The ECAS device was commissioned to generate an anolyte with a pH of 6.5 at a free available chlorine (FAC) concentration in a range from 180-200ppm based on a peer reviewed publication Mr. Johnson presented whose principal investigator and corresponding author was Professor Sagar Goyal, a virologist and technical consultant to CDC. Clarentis Technologies brands their HOCl anolyte product as Ultra-Lyte® when generated solutions are packaged and distributed or sold. A surfactant catholyte with a reduced surface tension, high pH and excellent residue-free cleaning properties was generated simultaneously. Clarentis® brands their catholyte cleaning solution product as Zero-Lyte™.

JDP also provided Princess Cruises with a Hygiena SystemSure™ Plus II ATP detector and UltraSnap™ surface test swabs for the trial.

***Background***

It is unarguable that the cruise industry has become much better at controlling norovirus outbreaks since they became an issue in 2000. The disease control response measures are more aligned with best public health measures recommended by a host of national and international regulatory agencies. The number of outbreaks associated with environmental transmission highlights a continued need for the evaluation and redesign of effective cleaning and disinfection procedures and processes; and successful trialing of biocidal agents to effectively reduce bio-burdens on surfaces. Table 1 includes a summary of outbreaks aboard Princess Cruises vessels since 2007.

***TABLE 1. U.S.-Based PCL Norovirus Outbreaks, 2007-2013*****

| Princess Norovirus Outbreaks1, 2007-2013 | | | | |
|---|---|---|---|---|
| | Passengers | | Crew | |
| Vessel (Year) | Number Ill | Percent (%) Ill | Number Ill | Percent (%) Ill |
| RU (2013) | 266 | 8.50 | 10 | 0.84 |
| EP (2012) | 189 | 5.84 | 31 | 2.61 |
| RU (2012) | 149 | 5.02 | 14 | 1.19 |
| DP (2012) | 114 | 6.41 | 11 | 1.29 |
| SP (2012) | 201 | 10.48 | 15 | 1.79 |
| RU (2012) | 129 | 4.10 | 9 | 0.76 |

**Cruise ship outbreak updates are posted when they meet the following criteria:

- Fall within the purview of the Centers for Diseases Control Vessel Sanitation Program,
- Includes voyages 3-21 days in duration and carrying ≥ 100 passengers

| | | | | | |
|---|---|---|---|---|---|
| KP (2012) | 288 | 9.36 | 75 | 6.37 | 89 90 |
| KP (2012) | 364 | 11.73 | 32 | 2.74 | 91 92 |
| CP (2011) | 144 | 6.77 | 8 | 0.95 | 93 |
| CP (2011) | 128 | 6.23 | 13 | 1.55 | 94 95 |
| CO (2011) | 64 | 3.22 | 3 | 0.57 | 96 |
| CO (2009) | 252 | 12.6 | 19 | 2.1 | 97 98 |
| CB (2008) | 172 | 5.58 | 13 | 1.12 | 99 100 |
| EP (2007) | 156 | 4.86 | 22 | 1.82 | 101 |
| IP (2007) | 179 | 9.25 | 37 | 4.04 | 102 103 |
| TOTAL | 2,795 | 7.33 | 312 | 1.98 | |

105

106 ***The objectives of this evaluation study were to:***

107 1. Provide a comprehensive overview of the scientific evidence for the mode of action,
108 antimicrobial spectrum and potential cruise industry-related applications for alternate
109 methods using electrochemically activated solutions generated by advanced
110 automated ECA systems;

111 2. Utilize two biological measures to evaluate the in vivo efficacy of Ultra-Lyte® in
112 comparison to Virox™;

113 3. During the trial period, compare rates of AGE illness onboard the Golden Princess to
114 Princess vessels with a North American itinerary and;

115 4. Establish an index of corrosivity and compare Virox™ against Ultra-Lyte® using
116 pre-selected material.

117

118 ***Literature Review***

119 The use of electrolysis for disinfection has been employed for over 100 years (Nakagawara,
120 1998), although it was not until the 1970s that the physicochemical properties of ECAS were
121 extensively researched (Prilutsky, 1997). ECAS have since found numerous biocidal
122 applications: potable water disinfection (Kraft, 2008) and within the food industry (Huang,
123 2008). This is largely due to their biocidal properties, use of relatively inexpensive raw materials
124 and ease of production. The objective of the literature review was to determine the efficacy of
125 ECAS against specific microbial targets. Aside from efficacy, the use of ECAS in situ must
126 satisfy other requirements such as low human toxicity and low potential to damage treated
127 materials.

128 A large body of scientific evidence has demonstrated the virucidal activity of ECAS against a
129 broad range targets (Tagawa, 2000; Morita, 2000; Park G. B., 2007; Kitano, 2003; Thorn, 2011).
130 Standard methodologies expose virus particles in suspension to ECAS in the presence/absence of
131 organic loading, whereby ECAS reduces the number of viable virus particles as measured by
132 cytopathic effects of the target virions in subsequently infected cell lines. The ECAS treatment of
133 the Norovirus surrogate bacteriophage MS2 was shown to significantly reduce infectivity (Park
134 G. B., 2007). Table 2 summarizes a literature review on ECAS solutions for surface inactivation
135 of Norovirus.

136

137 ***TABLE 2. Inactivation kinetics of norovirus by ECAS.*** Reduction rates are expressed as
138 log10 colony-forming units (CFU) ml-1 reduction per minute from the viable count and time
139 data points provided within the literature, and must be taken as the lowest estimates.

| Study | Inactivation Kinetics of Norovirus by ECAS | | |
|---|---|---|---|
| | Reduction | Contact Time | Dose |
| (Gwy-Am, 2008) | 5log10 | 20(s) | 1 mg/L |
| (Park G. B., 2007) | ≥3log10 | 20(s) | 20-200 ppm |
| **(Goyal, 2010)** | **5log10** | **1(min)** | **150 ppm** |
| (Miller, 2006) | ≥4.93log10 | 10 (min) | |
| (Kingsley, 2013) | 4.14log10 | 1(min) | 189ppm |

Peer reviewed studies have found that diluted solutions of ECAS, containing from 20-200 mg/liter of free chlorine, are effective for disinfection of surfaces contaminated with norovirus. Furthermore, norovirus is not highly resistant to free chlorine disinfection (Gwy-Am, 2008).

Numerous bacterial species have also been shown to be susceptible to ECAS treatment during in vitro testing. The data summarized in Appendix F is representative of the spectrum of its bactericidal activity. ECASs clearly have a broad spectrum biocidal activity, including clinically relevant pathogens after short exposure times, comparable to regularly used disinfectants, including sodium hypochlorite, chlorhexidine gluconate, glutaraldehyde, and benzalkonium chloride.

Bacterial microorganisms are known to form resistant biofilm structures (Costerton, 1999), which are thought to have evolved as a survival strategy (Jefferson, 2004). Although these structural communities are undoubtedly ubiquitous in nature, few experimental studies have been performed to specifically investigate the sensitivity of these communities to ECAS. The effective removal of mature Pseudomonas aeruginosa biofilms from the surface of glass and stainless steel after treatment with ECAS has been observed in vitro (Thantsha, 2006). In addition, removal of the extracellular matrix of both Escherichia coli and sulphate-reducing bacterial biofilms has been observed after treatment with ECAS. Collectively, the literature supports the potential use of ECAS against biofilms, but further research is required in this area to characterize appropriate treatment regimens.

ECAS is a broad-spectrum, non-selective biocide, and has been shown to also effectively inactivate certain pathogenic eukaryotes (refer to Appendix F). **Of particular note is its efficacy against Cryptosporidium parvum, a waterborne pathogen that has previously been shown to be resistant to standard chlorine treatment (Lisle, 1995).** Although few eukaryotic species have been tested for their sensitivity to ECAS, it is evident from the literature that it has significant broad-spectrum antifungal properties (Buck, 2002).

The ability of ECAS to inactivate microbial toxins has also been investigated. Significant inactivation was observed when staphylococcal enterotoxin-A (SEA), a heat-stable and treatment resistant toxin, was treated with ECAS (Suzuki T. I., 2002). The ability of ECAS to inactivate fungal toxins has also been researched using the aflatoxin of Aspergillus parasiticus and a significant reduction in the mutagenic potential of this aflatoxin was measured using a conventional Ames test (Suzuki, 2002). **The literature supports the ability of ECAS to inactivate microbial toxins, indicating its efficacy not only at killing whole microorganisms, but also deactivating or degrading their virulence factors.**

The potential for biocides to cause material corrosion must also be investigated before being widely used to disinfect inanimate surfaces. Few empirical studies have been performed to investigate this property. One study found no observable corrosion problems after 3 years of ECAS usage within a clinical setting (Tanaka N. F., 1999). A more recent study has shown that acidic ECAS had no adverse effect on stainless steel surfaces (after 8 days of contact), but significant corrosion was seen for carbon steel, and, to a lesser extent, on copper and aluminum

surfaces (Ayebah, 2005). This observation was likely to be due to the known susceptibility of these materials to oxidizing agents. This study also showed how corrosion could be limited by using neutralized ECAS, highlighting the importance of testing the corrosive nature of specific ECAS within the situation they are to be applied.

***Methods***

An evaluation study to compare the use of Ultra-Lyte® to Virox™ was conducted aboard the Golden Princess from 30 September – 1 December 2013.

***Quantitative Methods***

Two quantitative measurements were used to evaluate the efficacy of Ultra-Lyte® in comparison to Virox™ as well as the procedures of using a detergent prior to disinfection compared to the current practice:

Heterotrophic Plate Count (HPC) for measuring biologic activity on a surface,

Adenosine triphosphate (ATP) counts to measure soil load on a surface and use a proxy indicator for viral load, particularly norovirus.

***Sampling***

Sampling for HPC was carried out by EM Laboratory P&K - an independent third party laboratory. Forty-eight (48) samples were collected during each shipboard visits:

-16 samples pre- and post- application of Virox™;

-16 samples pre- and post- application of Ultra-Lyte®;

-16 samples pre- and post- application of a 2 step process of initially applied Zero-Lyte™ surfactant cleaner followed by Ultra-Lyte® sanitizer disinfection.

A total of 96 HPC samples were collected during the 2 shipboard visits for evaluation. A detailed description on methodology can be found in the appendix.

Sampling for ATP was conducted through use of a Hygiena SystemSURE Plus™ Luminometer and Ultrasnap™ ATP Test. Detailed sampling procedures can be found in the appendix. The test surface selection was based on a qualitative assessment for areas considered to be high frequency touch points. Surfaces samples included: buffet tables and chair arm rests, mid-ship stair case hand railings, and door handles leading to the deck 7 promenade. The dates of the tests were pre-determined based on turnaround ports and disembarkation times.

Additionally, rates of AGE illness onboard the Golden Princess were compared with the following Princess vessels with a North America itinerary: Coral Princess, Grand Princess, Island Princess, Sapphire Princess, and Star Princess. Due to the variation of cruise length, the rate of AGE illness for the first 4 days of each voyage was used to compare with the rates found onboard the Golden Princess during the duration of the study.

### *Qualitative Methods*

A qualitative evaluation was also conducted for observation of damage and discoloration. Test surfaces were deliberately exposed to the solutions under study. Based on recent feedback provided to the Public Health Department from Hotel Operations, Furnishings and Interior Design, surfaces were selected based on the frequency of damage sustained throughout the fleet, cost of replacement and information gathered from the ships. Based on that, the surfaces selected for testing included:

carpet swatches,

laminate covered railings,

wall laminate, and

stainless steel plates.

In addition to the above criteria, these surfaces were considered to be frequently touched and, therefore, had higher exposure to sanitizing products.

The proposed design included a 3-system exposure of Virox™, Ultra-Lyte®, and no application (for control comparison purposes). The onboard Accommodations Department was instructed to daily apply each sanitizer to each surface. Corrosivity tracking included routine subjective observation. The onboard departmental personnel utilizing Ultra-Lyte® were surveyed which included Accommodation and F&B departments, and the Technical department who maintained the equipment. This feedback is contained in the 'Results' section of this document.

### *Results*

Due to the influence of outliers, and the distribution of data being skewed, median values were preferred to mean calculations. Hartley's Fmax test was used to confirm that the dataset lacked homogeneity of variance. As a result of the large variations within the dataset, statistical analysis was limited to descriptive statistics and non-parametric tests, including Fisher's Exact.

***TABLE 3. Median summary of ATP Testing on Virox™***

| Virox™ | | | | |
|---|---|---|---|---|
| Surfaces (n=124) | Pre-Treatment (RLU) | Post-Treatment (RLU) | Median Change (RLU) | Percent (%) Change |
| Tables | 340.5 | 50.0 | -238.0 | -85.3 |
| Chairs | 493.5 | 125.5 | -395.0 | -74.6 |
| Doorhandles | 579.5 | 121.5 | -432.0 | -79.0 |
| Railings | 276.0 | 30.5 | -232.0 | -88.9 |
| Total | 366.0 | 64.5 | -292.0 | -82.4 |

Table 3 shows a median reduction of 82.4% (n=124) in environmental soil load for surfaces exposed to Virox at a dilution of 1:128. This was calculated through use of relative light units (RLU) of adenosine triphosphate (ATP). The results summarize the outcomes of six replicate experiments using four comparable sample surfaces (matching based on area). These surfaces included buffet tables, chairs, door handles, and railings. Of those, railings experienced the largest decrease in soil load (88.9%), followed by tables and chairs, with door handles showing the smallest reduction at 74.6%. Overall, pre-treatment RLU measurements for Virox solution was 366.0 RLU and post-treatment measures were 64.5, representing an overall decrease of 82.4% for Virox solution.

***TABLE 4. Median summary of ATP Testing on Ultra-Lyte®***

| Ultra-Lyte™ | | | | |
|---|---|---|---|---|
| Surfaces (n=120) | Pre-Treatment (RLU) | Post-Treatment (RLU) | Median Change (RLU) | Percent (%) Change |
| Tables | 197.5 | 29.0 | -159.0 | -85.3 |
| Chairs | 403.0 | 114.0 | -335.5 | -71.7 |
| Doorhandles | 424.0 | 22.5 | -380.0 | -94.7 |
| Railings | 261.0 | 17.0 | -249.5 | -93.5 |
| Total | 300.5 | 30.0 | -259.5 | -90.0 |

In comparison, Table 4 shows that exposure of test surfaces to Ultra-Lyte® at a dilution of 180 ppm. Consistent with Virox™, Ultra-Lyte® had an increased efficacy in reducing soil load on railings (93.5 %); however, door handles showed the largest decrease (94.7 percent) in soil load. The results for buffet tables (85.3%) and chairs (71.7%) showed a comparable variability similar to findings with Virox™. Overall, pre-treatment measurement for RLUs was 300.5 with post-treatment RLU median levels recorded at 30, representing an overall decrease of 90% (n=120) for the Ultra-Lyte® solution.

***TABLE 5. Median summary of ATP testing on two step process***

| Two-Step (Clean & Sanitize) | | | | |
|---|---|---|---|---|
| Surfaces (n=54) | Pre-Treatment (RLU) | Post-Treatment (RLU) | Median Change (RLU) | Percent (%) Change |
| Tables | 107.0 | 5.5 | -92.0 | -94.9 |
| Chairs | 466.0 | 25.5 | -434.5 | -94.5 |
| Railings | 276.5 | 5.0 | -272.5 | -98.2 |
| Total | 252.5 | 9.5 | -239.0 | -96.2 |

The final ATP test examined Ultra-Lyte® with both a clean and a sanitize step (with catholyte used as

the detergent, and anolyte as the sanitizer). Table 5 includes the results of this two-step process. In comparison to the other methods used, the 'clean + sanitize' two-step method showed a decrease from 252.5 RLU to 9.5 RLU, representing an overall reduction of 96.2%. The ECA two step procedure consistently achieved a > 94% decrease in soil load among each of the test surfaces. Table 7 summarizes Tables 4-6 by solution.

***TABLE 6. Fisher's Exact test summary***

| Benchmark | Solution | | Row Totals |
|---|---|---|---|
| | Ultra-Lyte™ | Virox™ | |
| ≤85 RLU | 93 | 69 | 162 |
| >85 RLU | 27 | 55 | 82 |
| **Column Totals** | 120 | 124 | 244 |
| 1-Tail: 0.0002; 2-Tail: 0.0004 | | | |

With guidance from Hygiena, the manufacturer of the luminometer, a pre-determined benchmark level for acceptable ATP counts post-sanitization was set at 85 RLU. Post-treatment with Ultra-Lyte® yielded 93 (n=120) samples that achieved an RLU ≤85, compared to 69 (n=124) Virox™ treated surfaces. Fisher's Exact was used to test the null hypothesis that the probability of achieving the benchmark level of ≤85 RLU is the same whether you treat a surface with Ultra-Lyte® or Virox™. Table 6 summarizes the results of the Fisher's Exact analysis. Results of both a one-tailed and two-tailed analysis included statistically significant p-values ($p<.05$).

Results from HPC sampling (refer to Table 7) found that the two-step procedure reduced biological activity by 98.9% (n=11). In comparison, Virox™ decreased HPC by 77% (n=9), whereas Ultra-Lyte® was observed at an average decrease of 67.9%.

***TABLE 7. Summary of HPC surface sampling***

| Virox vs. ECA vs. Two Step CFU Surface Sampling | | | | | | |
|---|---|---|---|---|---|---|
| Date: 11 November 2013 | | | | | | |
| Samplers: EMLab P&K | | | | | | |
| | Virox | | ECA | | ECA 2 Step | |
| Surfaces | Number of Samples | Percent (%) Change | Number of Samples | Percent (%) Change | Number of Samples | Percent (%) Change |
| HC Tables | 3 | -66.7 | 4 | -54.2 | 5 | -97.6 |
| HC Chairs arm rest | 6 | -82.2 | 7 | -75.8 | 6 | -99.9 |
| **Total** | 9 | -77.0 | 11 | -67.9 | 11 | -98.9 |

Table 8 provides an overview of the AGE illness tracking during the trial period by ship after a total of 18 voyages during the trial period, 31 (0.07%) passengers and 12 crew (0.06%) aboard Golden Princess. In comparison, the Coral Princess reported 11 (0.12%) passenger and 5 (0.11%) crew in five voyages; the Grand Princess reported 24 (0.19%) passenger and 5 (0.09%) crew in five voyages; the Sapphire Princess reported 21 passengers (0.15%) and 7 crew (0.13%) in five voyages; and the Star Princess reported 10 (0.10%) passengers and 4 (0.09%) crew AGE illnesses in four voyages. The highest number of cases among passengers were reported on the Island Princess, with 31 (0.41%) AGE illnesses among passengers over four voyages.

***TABLE 8. Summary of HPC surface sampling***

**AGE ILLNESS COMPARISON BY SHIP**

| | PAX | | | CREW | | |
|---|---|---|---|---|---|---|
| Ship (Voyages) | Total Number PAX | Total Number of PAX ILL | Percent (%) PAX ILL | Average Number of Crew | Total Number of Crew ILL | Percent (%) CREW ILL |
| Golden (18) | 45795 | 31 | 0.07 | 1087 | 12 | 0.06 |
| Coral (5) | 9068 | 11 | 0.12 | 879 | 5 | 0.11 |
| Grand (5) | 12935 | 24 | 0.19 | 1079 | 5 | 0.09 |
| Island (4) | 7566 | 31 | 0.41 | 880 | 6 | 0.17 |
| Sapphire (5) | 13783 | 21 | 0.15 | 1070 | 7 | 0.13 |
| Star (4) | 10095 | 10 | 0.10 | 1064 | 4 | 0.09 |

Results from the surface corrosion tests were inconclusive. There were no discernible differences in appearance on the laminated wooden panels and stainless steel plates, while the carpet swatch treated with Ultra-Lyte® appeared to be slightly more faded that the swatch treated with Virox™. The treated wooden railing samples exhibited a darker appearance in comparison to the untreated sample, with the Virox™ treated sample showing a more appreciable darkening of the laminated wood.

**Qualitative feedback** from surveyed users of Ultra-Lyte®, including Accommodation and Food & Beverage staff, was **largely positive**. Both Accommodation and F&B departments reported that Ultra-Lyte® **did not yield any adverse reactions** on the skin of the users. Furthermore, Accommodation staff reported that exposure to Ultra-Lyte® **does not cause respiratory irritation (coughing) when applied in enclosed environments,** including staterooms and restrooms. Accommodation staff preferred the odor of the Ultra-Lyte®. In contrast, historical comments were received from both crew and passengers that **Virox™ left behind a sour odor**. Lastly, both Accommodation and F&B departments reported that **Ultra-Lyte® was easier to use and improved operation, as it only required a one-minute contact time**, whereas Virox™ required a five-minute contact time. As reported from the Technical Department there was **no impact to the wastewater treatment system** and maintenance of the equipment is reasonable and achievable.

***Discussion***

The results of the ATP testing demonstrated highest reduction in soil load with use of a two-step process using Ultra-Lyte® in combination with a surfactant followed by use of Ultra-Lyte® alone and Virox™ respectively. The HPC test yielded different results, suggesting that the 2 step process yielded the greatest reduction in biological activity followed by Virox™ and Ultra-Lyte® alone respectively. Due to lack of statistical power, further research is required to validate these findings. However, if ATP is an appropriate proxy for biological activity, the results of this study are commensurate with the results of the literature review.

The null hypothesis for the Fisher's Exact test was that the probability of achieving the benchmark level of ≤85 RLU is the same whether you treat a surface with Ultra-Lyte® or Virox™. **The results included a highly statistically significant difference (13.0545, p-va <.05) indicating a marked difference between Virox™ and Ultra-Lyte® in the reduction of environmental soil load against the benchmark level of 85 RLU.**

TABLE 7. Summary statistics by solution

| Summary Statistics | | | | |
|---|---|---|---|---|
| Solution | Pre-Treatment (RLU) | Post-Treatment (RLU) | Median Change (RLU) | Percent (%) Change |
| Virox™ | 366.0 | 64.5 | -292.0 | -82.4 |
| Ultra-Lyte™ | 300.5 | 30.0 | -259.5 | -90.0 |
| Two-Step | 252.5 | 9.5 | -239.0 | -96.2 |

Consistent with the ATP test, higher HPC counts were observed among arm-chairs as opposed to tables; however, across all solutions chairs achieved higher average rates of decrease in HPC counts post-treatment. **HPC testing showed that the two-step procedure decontaminating first with catholyte (Zero-Lyte™) in a bucket with a towel, and sanitizing second with anolyte (Ultra-Lyte®) applied as a spray yielded the greatest reduction in HPC post treatment (-98.9%).** Pretreatment samples (n=17) that were below the limit of detection by laboratory analysis were removed from the study. Therefore, due to low sample size (n=31), no inferences were made in relation to the efficacy of any intervention.

During the trial period, attack rates for all AGE comparison vessels were at expected baseline levels. This may be attributable to the relatively short length of the study or that AGE observation was limited to the first four days of each cruise. This may have affected the study, as it only allowed for a brief exposure period and may not have captured the actual rate of AGE for those specific voyages.

The inconclusive results of the corrosivity test may also be attributable to the relatively short length of the study. Historical information received by the onboard operations suggests that

prolonged use of Virox™, particularly at high concentrations, was damaging to the surfaces onboard the vessel. The 60 day trial may not have been adequate to mimic the typical onboard exposure.

A number of the advantages and disadvantages of ECAS as applied to its potential use within a shipboard setting were discovered during this study and are listed in Table 10. Although there is an initial expenditure on the electrolytic cell system, once installed, the production of active solutions is inexpensive due to the relative abundance of raw materials ($H^2O$ and NaCl). Due to on-site production and low operator skill requirements, high ECAS production rates can be achieved, negating the need for the transport or storage of biocidal chemicals. The broad-spectrum antimicrobial activity of ECAS enables high-level disinfection as defined by the Centers for Disease Control and Prevention (CDC) (Rutala, 2008). These devices comply with the requirements of 40 CFR 152.500 and 40 CFR 156.10. They are exempt form FIFRA Chapter 3 label registration requirements as these ECAS solutions are produced in what EPA classifies as an "on-site pesticidal device". Note that the spray containers were nonetheless labeled with the active ingredient and the other label requirements pursuant to 40 CFR 156.10, as were the concentrate reservoirs attached to the Clarentis Technologies ECAS device.

TABLE 10. Generalized Advantages and Disadvantages of shipboard utilization of ECAS

| Advantages | Disadvantages |
|---|---|
| **Higher reduction of soil load** | Initial expenditure |
| **Broad-spectrum antimicrobial activity** | Unit service and maintenance |
| **Rapid disinfection time (1 min.)** | |
| **Inexpensive raw materials** | |
| **Ease of verifying concentration** | |
| **Only one concentration required** | |
| **On-site generation** | |
| **Requires minimal operator skill** | |
| **Limited toxicity** | |
| **Environmentally compatible** | |

An additional discovery of the study was that buffet chair arm-rests, according to ATP sampling, exhibited the highest level of environmental soil among the four test surfaces. Additionally, chair arm-rests proved to be the most difficult to clean as demonstrated by the significantly lower rate of reduction in soil load. The study also demonstrated that a two-step process effectively removed soil load, including on difficult to clean surfaces such as chair arm-rests. This outcome is suggestive of an operational gap where the arm-rests on chairs are not cleaned as frequently as tables, and how this gap can be closed to assist in the prevention and control of infectious disease outbreaks.

The effective use of disinfectant/sanitizing solutions within the cruise industry almost certainly provides widespread protection to both passengers and crew members against possible contamination with potentially pathogenic organisms. **ECAS have been studied for many years**

**and have been found to be highly efficacious biocidal agents**, with increasing reports of their effectiveness in real-world applications; however, they are still not in widespread use, particularly within the cruise industry. The paucity of wide-ranging clinical trials is likely to be a contributing factor, but **recent studies do recognize the potential of ECAS for disinfection and sanitization in healthcare facilities (Rutala, 2008).**

### *Limitations*

The results of this study clearly demonstrated a decrease in soil load and HPC with the application of Ultra-Lyte®, particularly when applied in a clean and sanitize process. A limitation of this evaluation study included external validity and reliability. Due to the lack of homogeneity of variance, the ATP data collected cannot be considered a normal distribution, therefore limiting study generalizability. In defense of the study, however, a surfeit of empirical evidence exists in support of the broad spectrum biocidal efficacy of ECAS.

This study also utilized inappropriate comparison methodology, using bacteria as HPC as a baseline for viruses as an outcome. Absent inoculation of the test vessel with norovirus, this was an acknowledged limitation of a novel in vivo experiment. As noted previously, and identified within the literature review of this paper, this limitation is strengthened through the significant depth of in vitro empirical evidence available concerning the effectiveness of ECAS as a virucidal disinfectant.

A further limitation of the study pertains to the lack of pre-trial sampling data on Hygiena's SystemSURE Plus™ Luminometer and Ultrasnap™ ATP Test. This paucity of data limits the ability to align Hygiena's luminometer against previous results; therefore test-retest reliability for the assessment tool cannot be confirmed. Furthermore, lack of operator consistency may also have impacted study results, as no single individual was designated as primary luminometer sampler. As a result, no a priori controls or baseline measurements were established. One final consideration was the study design. Although protocols were altered at the midpoint, the study was not initially adaptable by design.

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***APPENDIX***

## *APPENDIX A*

### *FIGURE 1. Virox™ vs. Ultra-Lyte® Surface Sampling, 10-Oct-2013*

**Virox™ vs. Ultra-Lyte™ ATP Surface Sampling**

Date: 10-Oct-2013
Samplers: James Leonard, Dennis Peyton, Jimmy Young

| | | Virox™ | | | Ultra-Lyte™ | | |
|---|---|---|---|---|---|---|---|
| Surfaces | Sample Number | Before (RLU) | After (RLU) | Percent (%) Change | Before (RLU) | After (RLU) | Percent (%) Change |
| Tables | 1 | 136 | 47 | -65.4 | 568 | 44 | -92.3 |
| | 2 | 1811 | 266 | -85.3 | 963 | 26 | -97.3 |
| | 3 | 5561 | 1405 | -74.7 | 591 | 28 | -95.3 |
| | 4 | 1123 | 187 | -83.3 | 5175 | 721 | -86.1 |
| | 5 | 712 | 106 | -85.1 | 1351 | 113 | -91.6 |
| | 6 | 594 | 65 | -89.1 | 220 | 32 | -85.5 |
| Chairs | 1 | 1075 | 126 | -88.3 | 1238 | 109 | -91.2 |
| | 2 | 706 | 286 | -59.5 | 1657 | 136 | -91.8 |
| | 3 | 1432 | 381 | -73.4 | 1247 | 202 | -83.8 |
| | 4 | 570 | 260 | -54.4 | 4463 | 184 | -95.9 |
| | 5 | 1047 | 260 | -75.2 | 534 | 177 | -66.9 |
| | 6 | 592 | 170 | -71.3 | 927 | 416 | -55.1 |
| Doorhandles | 1 | 471 | 89 | -81.1 | 145 | 3 | -97.9 |
| | 2 | 800 | 143 | -82.1 | 280 | 16 | -94.3 |
| | 3 | 512 | 152 | -70.3 | 198 | 40 | -79.8 |
| Railings | 1 | 176 | 19 | -89.2 | 114 | 17 | -85.1 |
| | 2 | 68 | 35 | -48.5 | 174 | 19 | -89.1 |
| | 3 | 239 | 39 | -83.7 | 404 | 34 | -91.6 |
| | 4 | 320 | 32 | -90.0 | 661 | 21 | -96.8 |
| | 5 | 190 | 21 | -88.9 | 104 | 20 | -80.8 |
| | 6 | 283 | 52 | -81.6 | 136 | 61 | -55.1 |

*FIGURE 2. Virox™ vs. Ultra-Lyte® Surface Sampling, 21-Oct-2013*

**Virox™ vs. Ultra-Lyte™ ATP Surface Sampling**

Date: 21-Oct-2013

Samplers: Treavor Johnson, RaeAnne Lanfear, James Leonard, Jimmy Young

| | | Virox™ | | | Ultra-Lyte™ | | |
|---|---|---|---|---|---|---|---|
| Surfaces | Sample Number | Before (RLU) | After (RLU) | Percent (%) Change | Before (RLU) | After (RLU) | Percent (%) Change |
| Tables | 1 | 66 | 44 | -33.3 | 431 | 36 | -91.6 |
| | 2 | 426 | 76 | -82.2 | 245 | 23 | -90.6 |
| | 3 | 915 | 258 | -71.8 | 1040 | 138 | -86.7 |
| | 4 | 487 | 149 | -69.4 | 166 | 19 | -88.6 |
| | 5 | 170 | 82 | -51.8 | 11 | 43 | 290.9 |
| | 6 | 1840 | 213 | -88.4 | 526 | 80 | -84.8 |
| Chairs | 1 | 391 | 152 | -61.1 | 272 | 152 | -44.1 |
| | 2 | 961 | 169 | -82.4 | 547 | 105 | -80.8 |
| | 3 | 221 | 143 | -35.3 | 135 | 134 | -0.7 |
| | 4 | 2315 | 556 | -76.0 | 1996 | 193 | -90.3 |
| | 5 | 826 | 191 | -76.9 | 378 | 131 | -65.3 |
| | 6 | 505 | 232 | -54.1 | 1004 | 119 | -88.1 |
| Doorhandles | 1 | 485 | 190 | -60.8 | 219 | 15 | -93.2 |
| | 2 | 1425 | 142 | -90.0 | 492 | 23 | -95.3 |
| | 3 | 1993 | 108 | -94.6 | 1137 | 131 | -88.5 |
| Railings | 1 | 109 | 20 | -81.7 | 378 | 17 | -95.5 |
| | 2 | 377 | 20 | -94.7 | 256 | 13 | -94.9 |
| | 3 | 411 | 24 | -94.2 | 331 | 16 | -95.2 |
| | 4 | 433 | 66 | -84.8 | 121 | 22 | -81.8 |
| | 5 | 139 | 17 | -87.8 | 396 | 53 | -86.6 |
| | 6 | 348 | 21 | -94.0 | 335 | 10 | -97.0 |

*FIGURE 3. Virox™ vs. Ultra-Lyte® Surface Sampling, 28-Oct-2013*

**Virox™ vs. Ultra-Lyte™ ATP Surface Sampling**

Date: 28-Oct-13

Samplers: James Leonard, Yvonne Naimey, Dennis Peyton, Jimmy Young

| | | Virox™ | | | Ultra-Lyte™ | | |
|---|---|---|---|---|---|---|---|
| Surfaces | Sample Number | Before (RLU) | After (RLU) | Percent (%) Change | Before (RLU) | After (RLU) | Percent (%) Change |
| Tables | 1 | 292 | 46 | -84.2 | 298 | 23 | -92.3 |
| | 2 | 1023 | 266 | -74.0 | 776 | 126 | -83.8 |
| | 3 | 340 | 270 | -20.6 | 355 | 136 | -61.7 |
| | 4 | 455 | 225 | -50.5 | 185 | 50 | -73.0 |
| | 5 | 125 | 18 | -85.6 | 45 | 60 | 33.3 |
| | 6 | 1064 | 264 | -75.2 | 245 | 47 | -80.8 |
| Chairs | 1 | 1005 | 131 | -87.0 | 374 | 156 | -58.3 |
| | 2 | 542 | 145 | -73.2 | 563 | 199 | -64.7 |
| | 3 | 482 | 112 | -76.8 | 62 | 80 | 29.0 |
| | 4 | 1239 | 101 | -91.8 | 200 | 87 | -56.5 |
| | 5 | 2216 | 143 | -93.5 | 312 | 454 | 45.5 |
| | 6 | 478 | 28 | -94.1 | 96 | 780 | 712.5 |
| Doorhandles | 1 | 391 | 200 | -48.8 | 576 | 3 | -99.5 |
| | 2 | 810 | 292 | -64.0 | 341 | 18 | -94.7 |
| | 3 | 1301 | 125 | -90.4 | 1360 | 57 | -95.8 |
| Railings | 1 | 315 | 55 | -82.5 | 178 | 20 | -88.8 |
| | 2 | 414 | 41 | -90.1 | 224 | 40 | -82.1 |
| | 3 | 318 | 44 | -86.2 | 122 | 1 | -99.2 |
| | 4 | 136 | 21 | -84.6 | 344 | 14 | -95.9 |
| | 5 | 269 | 36 | -86.6 | 267 | 2 | -99.3 |
| | 6 | 386 | 42 | -89.1 | 264 | 8 | -97.0 |

*FIGURE 4. Virox™ vs. Ultra-Lyte® Surface Sampling, 4-Nov-2013*

**Virox™ vs. Ultra-Lyte™ ATP Surface Sampling**

Date: Nov 4, 2013

Samplers: James Leonard, Jimmy Young, Yvonne Nairney

| | | Virox™ | | | Ultra-Lyte™ | | | Two-Step | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Surfaces | Sample Number | Before (RLU) | After (RLU) | Percent (%) Change | Before (RLU) | After (RLU) | Percent (%) Change | Before (RLU) | After (RLU) | Percent (%) Change |
| Tables | 1 | 424 | 30 | -92.9 | 79 | 28 | -64.6 | 80 | 14 | -82.5 |
| | 2 | 24 | 34 | 41.7 | 157 | 7 | -95.5 | 127 | 6 | -95.3 |
| | 3 | 17 | 43 | 152.9 | 89 | 14 | -84.3 | 54 | 8 | -85.2 |
| | 4 | 28 | 13 | -53.6 | 61 | 13 | -78.7 | 193 | 10 | -94.8 |
| | 5 | 95 | 32 | -66.3 | 199 | 45 | -77.4 | 202 | 4 | -98.0 |
| | 6 | 385 | 23 | -94.0 | 259 | 38 | -85.3 | 106 | 25 | -76.4 |
| Chairs | 1 | 186 | 131 | -29.6 | 376 | 171 | -54.5 | 496 | 47 | -90.5 |
| | 2 | 228 | 135 | -40.8 | 364 | 55 | -84.9 | 124 | 23 | -81.5 |
| | 3 | 942 | 125 | -86.7 | 1257 | 172 | -86.3 | 2236 | 13 | -99.4 |
| | 4 | 277 | 89 | -67.9 | 185 | 54 | -70.8 | 694 | 35 | -95.0 |
| | 5 | 1265 | 104 | -91.8 | 2065 | 155 | -92.5 | 299 | 3 | -99.0 |
| | 6 | 403 | 86 | -78.7 | 1132 | 272 | -76.0 | 802 | 99 | -87.7 |
| Doorhandles | 1 | 111 | 29 | -73.9 | 356 | 165 | -53.7 | N/A | N/A | N/A |
| | 2 | 81 | 19 | -76.5 | 496 | 67 | -86.5 | N/A | N/A | N/A |
| | 3 | 515 | 95 | -81.6 | 703 | 51 | -92.7 | N/A | N/A | N/A |
| Railings | 1 | 245 | 28 | -88.6 | 389 | 23 | -94.1 | 265 | 5 | -98.1 |
| | 2 | 24 | 4 | -83.3 | 303 | 2 | -99.3 | 273 | 6 | -97.8 |
| | 3 | 287 | 26 | -90.9 | 184 | 12 | -93.5 | 119 | 9 | -92.4 |
| | 4 | 456 | 38 | -91.7 | 234 | 22 | -90.6 | 194 | 1 | -99.5 |
| | 5 | 84 | 11 | -86.9 | 258 | 24 | -90.7 | 368 | 2 | -99.5 |
| | 6 | 199 | 37 | -81.4 | 156 | 4 | -97.4 | 240 | 5 | -97.9 |

*FIGURE 5. Virox™ vs. Ultra-Lyte® Surface Sampling, 10-Nov-2013*

**Virox™ vs. Ultra-Lyte™ ATP Surface Sampling**

Date: 10-Nov-13

Samplers: James Leonard, Randy Mendiola, Dennis Peyton

| | | Virox™ | | | Ultra-Lyte™ | | | Two-Step | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Surfaces | Sample Number | Before (RLU) | After (RLU) | Percent (%) Change | Before (RLU) | After (RLU) | Percent Decrease | Before (RLU) | After (RLU) | Percent (%) Change |
| Tables | 1 | 200 | 112 | -44.0 | 558 | 52 | -90.7 | 164 | 24 | -85.4 |
| | 2 | 1264 | 53 | -95.8 | 106 | 24 | -77.4 | 142 | 26 | -81.7 |
| | 3 | 107 | 39 | -63.6 | 140 | 2 | -98.6 | 82 | 15 | -81.7 |
| | 4 | 78 | 25 | -67.9 | 57 | 30 | -47.4 | 35 | 3 | -91.4 |
| | 5 | 341 | 30 | -91.2 | 49 | 21 | -57.1 | 122 | 5 | -95.9 |
| | 6 | 661 | 98 | -85.2 | 56 | 18 | -67.9 | 160 | 57 | -64.4 |
| Chairs | 1 | 246 | 37 | -85.0 | 363 | 55 | -84.8 | 219 | 31 | -85.8 |
| | 2 | 380 | 91 | -76.1 | 885 | 64 | -92.8 | 555 | 37 | -93.3 |
| | 3 | 469 | 86 | -81.7 | 428 | 96 | -77.6 | 756 | 90 | -88.1 |
| | 4 | 270 | 103 | -61.9 | 640 | 42 | -93.4 | 436 | 16 | -96.3 |
| | 5 | 475 | 82 | -82.7 | 321 | 128 | -60.1 | 716 | 17 | -97.6 |
| | 6 | 193 | 81 | -58.0 | 378 | 52 | -86.2 | 733 | 20 | -97.3 |
| Doorhandles | 1 | 1097 | 118 | -89.2 | 147 | 6 | -95.9 | N/A | N/A | N/A |
| | 2 | 1165 | 137 | -88.2 | 339 | 8 | -97.6 | N/A | N/A | N/A |
| | 3 | 841 | 79 | -90.6 | 98 | 22 | -77.6 | N/A | N/A | N/A |
| Railings | 1 | 305 | 26 | -91.5 | 199 | 16 | -92.0 | 111 | 5 | -95.5 |
| | 2 | 226 | 23 | -89.8 | 591 | 7 | -98.8 | 415 | 5 | -98.8 |
| | 3 | 125 | 30 | -76.0 | 357 | 30 | -91.6 | 773 | 10 | -98.7 |
| | 4 | 337 | 58 | -82.8 | 241 | 16 | -93.4 | 280 | 2 | -99.3 |
| | 5 | 77 | 32 | -58.4 | 191 | 24 | -87.4 | 157 | 3 | -98.1 |
| | 6 | 336 | 31 | -90.8 | 206 | 19 | -90.8 | 292 | 1 | -99.7 |

*FIGURE 6. Virox™ vs. Ultra-Lyte® Surface Sampling, 18-Nov-2013*

**Virox™ vs. Ultra-Lyte™ ATP Surface Sampling**

Date: 18-Nov-13

Samplers: James Leonard, Alberto Menjivar, Jimmy Young

| | | Virox™ | | | Ultra-Lyte™ | | | Two-Step | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Surfaces | Sample Number | Before (RLU) | After (RLU) | Percent (%) Change | Before (RLU) | After (RLU) | Percent (%) Change | Before (RLU) | After (RLU) | Percent (%) Change |
| Tables | 1 | 8 | 0 | -100.0 | 25 | 2 | -92.0 | 46 | 1 | -97.8 |
| | 2 | 57 | 18 | -68.4 | 58 | 44 | -24.1 | 46 | 2 | -95.7 |
| | 3 | 45 | 0 | -100.0 | 62 | 2 | -96.8 | 56 | 2 | -96.4 |
| | 4 | 34 | 15 | -55.9 | 92 | 2 | -97.8 | 144 | 3 | -97.9 |
| | 5 | 65 | 22 | -66.2 | 196 | 32 | -83.7 | 108 | 1 | -99.1 |
| | 6 | 103 | 47 | -54.4 | 42 | 4 | -90.5 | 66 | 2 | -97.0 |
| Chairs | 1 | 190 | 56 | -70.5 | 277 | 42 | -84.8 | 265 | 22 | -91.7 |
| | 2 | 546 | 138 | -74.7 | 168 | 84 | -50.0 | 266 | 20 | -92.5 |
| | 3 | 592 | 109 | -81.6 | 190 | 60 | -68.4 | 426 | 28 | -93.4 |
| | 4 | 461 | 64 | -86.1 | 244 | 18 | -92.6 | 300 | 18 | -94.0 |
| | 5 | 290 | 57 | -80.3 | 371 | 32 | -91.4 | 339 | 51 | -85.0 |
| | 6 | 173 | 100 | -42.2 | 278 | 72 | -74.1 | 548 | 50 | -90.9 |
| Doorhandles | 1 | 293 | 47 | -84.0 | 1838 | 70 | -96.2 | N/A | N/A | N/A |
| | 2 | 182 | 31 | -83.0 | 584 | 21 | -96.4 | N/A | N/A | N/A |
| | 3 | 644 | 200 | -68.9 | 550 | 75 | -86.4 | N/A | N/A | N/A |
| Railings | 1 | 246 | 32 | -87.0 | 272 | 4 | -98.5 | 180 | 10 | -94.4 |
| | 2 | 333 | 17 | -94.9 | 287 | 16 | -94.4 | 200 | 4 | -98.0 |
| | 3 | 224 | 18 | -92.0 | 467 | 12 | -97.4 | 401 | 2 | -99.5 |
| | 4 | 243 | 38 | -84.4 | 310 | 22 | -92.9 | 463 | 7 | -98.5 |
| | 5 | 304 | 38 | -87.5 | 278 | 15 | -94.6 | 630 | 9 | -98.6 |
| | 6 | 355 | 18 | -94.9 | 256 | 21 | -91.8 | 333 | 12 | -96.4 |

*APPENDIX B*

*FIGURE 7. Virox™ vs. Ultra-Lyte® vs. Two-Step (Clean & Sanitize) HPC Surface Sampling, 21-Oct-2013*

**Virox™ vs. Ultra-Lyte™ vs. Two-Step HPC Surface Sampling**

Date: 21 October 2013

Samplers: EMLab P&K

| | | Virox™ | | | Ultra-Lyte™ | | | Two-Step | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Surfaces | Sample Number | Before (CFU) | After (CFU) | Percent (%) Change | Before (CFU) | After (CFU) | Percent (%) Change | Before (CFU) | After (CFU) | Percent (%) Change |
| Tables | 1 | 10 | 10 | 0.0 | <10 | <10 | 0.0 | <10 | <10 | 0.0 |
| | 2 | <10 | <10 | 0.0 | 30 | 10 | -66.7 | 10 | <10 | -100.0 |
| | 3 | 65 | <10 | -100.0 | 20 | 10 | -50.0 | 120 | <10 | -100.0 |
| | 4 | <10 | <10 | 0.0 | 10 | 30 | 0.0 | <10 | <10 | 0.0 |
| Chairs | 1 | 15 | <10 | -100.0 | 70 | 10 | -85.7 | 40 | <10 | -100.0 |
| | 2 | 10 | <10 | -100.0 | 25 | 20 | -20.0 | 35 | <10 | -100.0 |
| | 3 | 25 | <10 | -100.0 | 380 | <10 | -100.0 | <10 | <10 | 0.0 |
| | 4 | <10 | 10 | 0.0 | 20 | 10 | -50.0 | 300 | <10 | -100.0 |

*FIGURE 8. Virox™ vs. Ultra-Lyte® vs. Two-Step (Clean & Sanitize) HPC Surface Sampling, 11-Nov-2013*

**Virox™ vs. Ultra-Lyte™ vs. Two-Step HPC Surface Sampling**

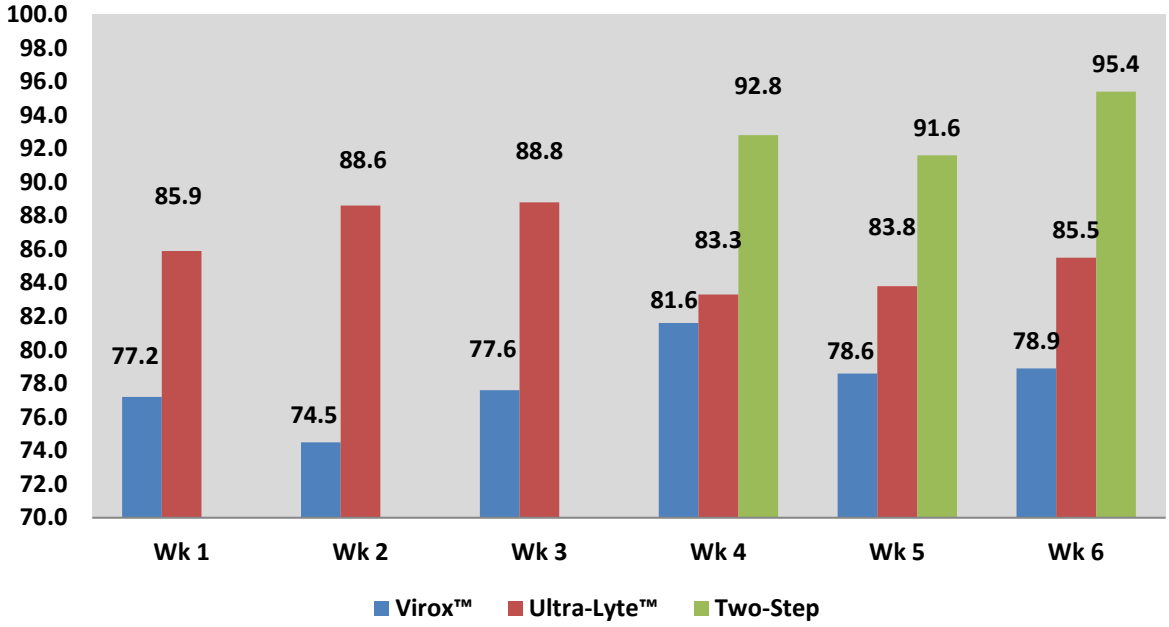
Date: 11 November 2013

Samplers: EMLab P&K

| | | Virox™ | | | Ultra-Lyte™ | | | Two-Step | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Surfaces | Sample Number | Pre (CFU) | Post (CFU) | Percent (%) Change | Pre (CFU) | Post (CFU) | Percent (%) Change | Pre (CFU) | Post (CFU) | Percent (%) Change |
| Tables | 1 | 98,000 | <10 | -100.0 | <10 | <10 | 0.0 | 170 | 20 | -88.2 |
| | 2 | <10 | <10 | 0.0 | <10 | <10 | 0.0 | 85 | <10 | -100.0 |
| | 3 | <10 | <10 | 0.0 | 30 | <10 | -100.0 | <10 | <10 | 0.0 |
| | 4 | <10 | <10 | 0.0 | <10 | <10 | 0.0 | <10 | <10 | 0.0 |
| Chairs | 1 | <10 | <10 | 0.0 | 34,000 | <10 | -100.0 | 15 | <10 | -100.0 |
| | 2 | 200 | <10 | -100.0 | 20 | <10 | -75.0 | 59,000 | 10 | -99.9 |
| | 3 | <10 | <10 | 0.0 | <10 | <10 | 0.0 | 10 | <10 | -100.0 |
| | 4 | 75 | <10 | -93.3 | 10 | <10 | -100.0 | <10 | <10 | 0.0 |

614 ***APPENDIX C***

615 CHART 1. Weekly ATP Percentage (%) Decrease, by Solution

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## *APPENDIX D*

FIGURE 9. AGE Comparison by Ship

**AGE ILLNESS COMPARISON BY SHIP**

| | | PAX | | | CREW | | |
|---|---|---|---|---|---|---|---|
| Vessel | Observation Period | Total Number PAX | Number of PAX ILL | Percent (%) PAX ILL | Total Number of Crew | Number of Crew ILL | Percent (%) CREW ILL |
| Golden Princess | 9/30-10/4 | 2627 | 1 | 0.04 | 1080 | 0 | 0.00 |
| | 10/4-10/7 | 2789 | 0 | 0.00 | 1086 | 0 | 0.00 |
| | 10/7-10/10 | 2788 | 0 | 0.00 | 1090 | 1 | 0.09 |
| | 10/10-10/14 | 2341 | 5 | 0.21 | 1081 | 2 | 0.19 |
| | 10/14-10/18 | 2698 | 5 | 0.19 | 1095 | 0 | 0.00 |
| | 10/18-10/21 | 2804 | 1 | 0.04 | 1094 | 0 | 0.00 |
| | 10/21-10/24 | 2205 | 3 | 0.14 | 1102 | 1 | 0.09 |
| | 10/24-10/28 | 2705 | 3 | 0.11 | 1098 | 1 | 0.09 |
| | 10/28-11/1 | 2632 | 2 | 0.08 | 1077 | 4 | 0.37 |
| | 11/1-11/4 | 2750 | 0 | 0.00 | 1077 | 0 | 0.00 |
| | 11/4-11/7 | 2664 | 0 | 0.00 | 1083 | 1 | 0.09 |
| | 11/7-11/11 | 2685 | 2 | 0.07 | 1091 | 0 | 0.00 |
| | 11/11-11/15 | 2692 | 3 | 0.11 | 1083 | 0 | 0.00 |
| | 11/15-11/18 | 2080 | 0 | 0.00 | 1079 | 0 | 0.00 |
| | 11/18-11/21 | 2022 | 1 | 0.05 | 1080 | 0 | 0.00 |
| | 11/21-11/24 | 1459 | 0 | 0.00 | 1078 | 0 | 0.00 |
| | 11/24-11/27 | 2911 | 2 | 0.07 | 1092 | 1 | 0.09 |
| | 11/27-12/1 | 2943 | 3 | 0.10 | 1095 | 1 | 0.09 |
| Coral Princess | 10/13-10/17 | 1754 | 1 | 0.06 | 881 | 0 | 0.00 |
| | 10/24-10/28 | 1832 | 4 | 0.22 | 878 | 1 | 0.11 |
| | 11/4-11/8 | 1921 | 3 | 0.16 | 875 | 1 | 0.11 |
| | 11/15-11/19 | 1925 | 2 | 0.10 | 894 | 2 | 0.22 |
| | 11/26-11/30 | 1636 | 1 | 0.06 | 867 | 1 | 0.12 |
| Grand Princess | 10/4-10/8 | 2587 | 4 | 0.15 | 1080 | 0 | 0.00 |
| | 10/19-10/23 | 2612 | 5 | 0.19 | 1076 | 1 | 0.09 |
| | 10/26-10/30 | 2568 | 9 | 0.35 | 1080 | 2 | 0.19 |
| | 11/10-11/14 | 2587 | 2 | 0.08 | 1084 | 0 | 0.00 |
| | 11/25-11/29 | 2581 | 4 | 0.15 | 1075 | 2 | 0.19 |
| Island Princess | 10/10-10/14 | 1878 | 7 | 0.37 | 875 | 1 | 0.11 |
| | 10/20-10/24 | 1874 | 17 | 0.91 | 883 | 3 | 0.34 |
| | 10/30-11/3 | 1873 | 3 | 0.16 | 885 | 1 | 0.11 |
| | 11/14-11/18 | 1941 | 4 | 0.21 | 875 | 1 | 0.11 |
| Sapphire Princess | 10/5-10/9 | 2726 | 0 | 0.00 | 1070 | 0 | 0.00 |
| | 10/12-10/16 | 2679 | 0 | 0.00 | 1064 | 0 | 0.00 |
| | 10/19-10/23 | 2648 | 4 | 0.15 | 1059 | 1 | 0.09 |
| | 11/16-11/20 | 2775 | 3 | 0.11 | 1071 | 1 | 0.09 |
| | 11/23-11/27 | 2955 | 14 | 0.47 | 1085 | 5 | 0.46 |
| Star Princess | 10/6-10/10 | 2487 | 7 | 0.28 | 1065 | 0 | 0.00 |
| | 10/21-10/25 | 2436 | 0 | 0.00 | 1071 | 1 | 0.09 |
| | 11/5-11/9 | 2568 | 1 | 0.04 | 1071 | 2 | 0.19 |
| | 11/20-11/24 | 2604 | 2 | 0.08 | 1048 | 1 | 0.10 |

***APPENDIX E***

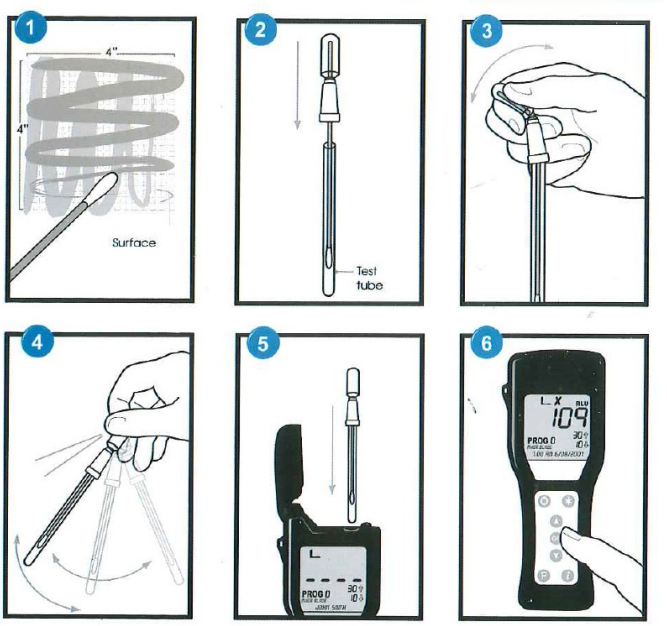
ECA TRIAL SAMPLING PROCEDURES

ATP SAMPLING

Hygiena SystemSURE Plus™ Luminometer and Ultrasnap™ ATP Test

IMPORTANT: Calibrate unit when moving to the next location where there is/might be a change in ambient temperature.

To calibrate unit: Turn off unit and turn it back on. It will take 15 seconds for the unit to calibrate.




***Sample reading procedures***:

Remove swab from test tube and swab surface. Swabbing motion should being a 4 x 4 inch square while rotating swab or a random motion that ensures a good sample collection.

Place swab back in the test tube.

Break plastic snap valve at top of swab by ending bulb. Squeeze bulb twice, pushing liquid reagent down swab shaft.

Shake test for 5 seconds.

Place test in SystemSURE Plus and close lid.

Press “OK” and reading will appear in 15 seconds. Result is the large read-out and record this number.

Surfaces to be sampled:

Horizon Court tables,

Horizon Court chair arm rests,

Mid-ship stair case railings,

Door handles on deck 7 midship leading to the promenade

***Sampling for Horizon Court tables:***

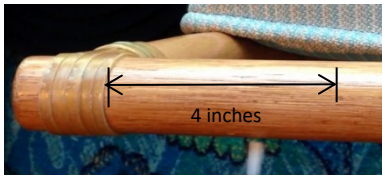
Collect sample on surface that is about 4 inches away from the edge of the table.

Collect 2 samples from one table – one for Virox, one for ECA solution. Ensure adequate separation to prevent cross contamination of sanitizers, such as on opposite side of the table.

After sample is collected, clean the surface with towel soaked with the test solution. Pass the towel over the surface 3 times to mimic normal cleaning routine.

Allow surface to dry before collecting the second sample on the same spot.

Sampling Horizon Court chair arm rests



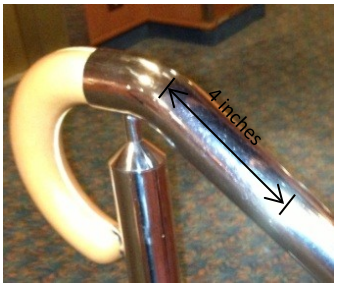

Collect sample on arm rest – see picture for specific area.

Swab surface all around the arm rest within the 4 inches length.

After sample is collected, clean the surface with towel soaked with the test solution. Pass the towel over the surface 3 times to mimic normal cleaning routine.

Allow surface to dry before collecting the second sample on the same spot.

***Sampling staircase railings***




Collect sample on railing – see picture for specific area.

Swab surface all around the railing within the 4 inches length.

After sample is collected, clean the surface with towel soaked with the test solution. Pass the towel over the surface 3 times to mimic normal cleaning routine.

Allow surface to dry before collecting the second sample on the same spot.

***Sampling door handles***

Swab all surface on the handle – outside and inside.

After sample is collected, clean the surface with towel soaked with the test solution. Pass the towel over the surface 3 times to mimic normal cleaning routine.

Allow surface to dry before collecting the second sample on the same spot.

684 ***APPENDIX F***

685 TABLE 2. Range of experimental kill rates determined for acidic (pH 2-5) and neutralized
686 (pH 5-8) electrochemically activated solution anolyte (ECAS) against aerobic, facultative and
687 anaerobic bacterial target species, bacterial spores, and eukaryotic cells, within in vitro
688 suspension tests. Kill rates (k) are expressed as log10 colony-forming units (CFU) ml-1
689 reduction per minute from the viable count and time data points provided within the literature,
690 and, therefore, must be taken as the lowest estimates. Qualitative studies are reported where no
691 quantitative data exist in the literature.

| Target Organism | Experimental kill rates (k) of ECAS (log10 CFU ml-1 reduction/minute) | |
|---|---|---|
| | Acidic ECAS | Neutralized ECAS |
| Actinobacter spp. | + | 10.0 |
| Alcaligenes faecalis | 13.6 | |
| Bacillus cereus | 2.3-5.9 | |
| Bacillus subtilis | + | 1.7 |
| Campylobacter jejuni | 44.9 | |
| Escherichia coli | 1.4-37.4 | 1.7-16.0 |
| Enterobacter aerogenes | 16.0 | 10.0 |
| Enterococcus spp. | 14.5 | 3.5-15.4 |
| Haemophils influenza | | >10.0 |
| Helicobacter pylori | + | 3.50 |
| Legionella pneumophila | | 8.0 |
| Listeria monocytogenes | 1.3-16.3 | |
| Klebsiella spp. | | 10.0 |
| Mycobacterium spp. | + | 3.5-5.1 |
| Pseudomonas aeruginosa | 14.1-37.4 | 8.0-16.0 |
| Salmonella spp. | 6.1-8.0 | 5.2-16.0 |
| Staphylococcus spp. | 3.7-37.4 | 3.9-16.0 |
| MRSA | 28.8-37.4 | 13.4 |
| MRSE | | 3.2 |

| | | |
|---|---|---|
| Streptococcus spp. | + | 3.8-5.0 |
| Bacterial spores | | |
| Bacillus anthracis | | 0.2 |
| Bacillus cereus | 1.32-6.98 | |
| Bacillus subtilis | 0.9 | 1.0-15.0 |
| Clostridium difficile | 16.3 | 2.0 |
| Clostridium perfringens | | 0.04 |
| Eukaryotes | | |
| Aspergillus spp. | 1.48 | 5.25 |
| Candida spp. | 3.5 | 3.5-16.0 |

MRSA: methicillin-resistant Staphylococcus aureus;

MRSE: methicillin-resistant Staphylococcus epidermidis

+ Qualitative study only

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