## 1.0 PREFACE

**The ZONOsanitech, LLC sanitizing cabinet, the ZONO, meets the Association of Official Analytical Chemists (AOAC) definition of sanitizing of non-food product surfaces that requires a contamination reduction of 99.9% or a three (3) log kill of bacteria.** Following the United States Environmental Protection Agency (EPA) recommended method DIS/TSS-10, Sanitizer Test for Inanimate Surfaces, (exposure time modified), testing demonstrated kill efficacy for Methicillin-resistant Staphylococcus aureus (MRSA) (skin pathogen); Staphylococcus aureus (S. aureus) (skin pathogen); Escherichia coli (E. coli) (gastrointestinal pathogen); Streptococcus pyogenes (S. pyogenes (respiratory pathogens); Shigella dysenteriae (S. dysenteriae) (gastrointestinal pathogen); Salmonella enteritidis (S. enteritidis) (gastrointestinal pathogen); and Pseudomonas aeruginosa (P. aeruginosa) (gastrointestinal pathogen).

The ZONO is a viable alternative to heat based or chemical sanitizers. The sanitizing process is compatible with porous, nonporous and semi porous surfaces: natural and synthetic surfaces, plastic, wood, ceramic, glass, paper, metal, leather, and fabric. The technology does not leave a residue, impact the integrity of the item (electronics with batteries or electrical devices) or damage the surface. After each cycle, items are ready for immediate use. The sanitizing process is safe and does not harm the environment.

## 2.0 INTRODUCTION

The objective of this study is to evaluate the efficacy of the ZONO's ozone sanitizing process to meet the AOAC definition of sanitizing non-food product surfaces following the EPA recommended method DIS/TSS-10, Sanitizer Test for Inanimate Surfaces.

## 2.1 ZONO OPERATION

The ZONO, developed by ZONOsanitech, LLC, is an environmentally friendly sanitizing process that generates ozone (O3) and humidity inside an airtight stainless steel cabinet on site using electricity, ambient air, and tap water. No heat or chemicals are used in the cabinet. Items for sanitizing are placed onto racks or suspended on hooks or rods inside the ZONO. When the magnetic lock on the door is engaged, and the sanitizing cycle is initiated, an ultra-violent light generates ozone to a designated level. Once this level is reached, the humidity is raised to a designated level, and the ozone and humidity together create a sanitizing environment. After the sanitizing is completed, the ozone is destructed into oxygen (O2) inside the airtight cabinet. There are no toxic or hazardous residues or waste products associated with the process, only ambient air and water. Since the ozone reverts to oxygen, surfaces do not require post-application rinsing to eliminate potentially harmful residues, nor do they require careful handling as is required when chemical sanitizing concentrates are used.

The ZONO is designed to monitor ozone levels inside and outside of the cabinet during each cycle, with safety stop measures in the event of the detection of ozone to the environment or unsatisfactory levels of ozone or humidity during operation.

ZONOsanitech, LLC is regulated by the U.S. EPA as a manufacturer of a pesticide control device pursuant to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)[i]. ZONOsanitech, LLC's EPA registered establishment number is 86882-GA-001. ZONOsanitech, LLC adheres to strict EPA regulations with regard to labeling, production, record keeping, and packaging and import/export requirements.

## 3.0 OZONE SCIENCE

Recognized as an organic sanitizer since the 1800s, ozone, an allotropic form of oxygen, is a molecule comprised of three oxygen atoms, whose chemical symbol is O3. Ozone is an unstable, colorless gas with a pungent characteristic odor, which occurs freely in nature. It is produced commercially by passing electrical discharges or ionizing radiation through air or oxygen. Ozone will readily combine with other atoms and alter the molecular structure of the atom, like bacteria and viruses. The gas can effectively penetrate area where access is difficult using conventional liquids and manual sanitizing procedures.

In 2002, the United States Food and Drug Administration (FDA) approved ozone for use on food contact areas and directly on food with its Generally Regarded As Safe (GRAS) designation.[1] GRAS substances are those that are intentionally added to food, which are reviewed and recognized by qualified experts, as having been adequately shown to be safe under the conditions of its intended use.[2] Following the FDA's approval for food contact, sanitizing

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1 Federal Register: June 26, 2001 (Volume 66, Number 123)

2 Electric Power Research Institute (EPRI) Generally Recognized as Safe (GRAS) declaration for ozone use in food processing in the U.S. expert panel May 1997. Food and Drug Administration Code of Federal Regulations, Title 21, Volume 3, Revised as of April 1, 2008 (21 CFR 184.1563]

non-food surfaces with ozone has become a viable alternative to traditional chemical and heat based sanitizers as ozone has a unique property of auto decomposition and will leave no toxic residues. Today, ozone is widely used as a sanitizer in the food, produce, drinking water purification, medical, beverage and water treatment industry, among others.

Ozone is a more powerful oxidizer than chlorine or peroxide based bleaches. The oxioreduction potential of ozone is 2.07 V. In comparison, the oxidoreduction potential of sodium hypochlorite (NaClO), commonly known as chlorine bleach, is 1.4 V. The oxioreduction potential of hydrogen peroxide (H2O2) is 1.8 V. In the presence of water vapor, the oxygen atom produced by the decomposition of ozone will react with a molecule of water to form hydroxyl radicals.

Ozone gas can effectively penetrate area where access is difficult using conventional liquids and manual sanitizing procedures. Unlike chemical sanitizers, ozone does not consume resources for packaging, transportation, storage, handling and recycling or disposal, and therefore does not have a large carbon footprint. In the case of the ZONO, the ozone is generated on site with the ambient air from the facility, and then the ozone reverts to oxygen. Thus, surfaces do not require post-application rinsing to eliminate potentially harmful residues, nor do they require careful handling as is required when chemical sanitizing concentrates are used.

## 3.0 MICROBIAL EFFECTIVENESS STUDY

### 3.1 OBJECTIVE

The objective of the study is to evaluate the efficacy of the ZONO to meet the Official Analytical Chemists (A.OAC) definition of sanitizing non-food product surfaces, which requires a contamination reduction of 99.9% or a three (3) log kill. To accomplish this objective, the study used seven bacteria

and a slurry (respiratory, skin and gastrointestinal pathogens), one controlled material and three variable materials that are typically found in childcare or sports facility settings, schools, homes, and businesses that are easily contaminated with bacterial pathogens. The study followed the EPA recommended method (DIS/TSS-10, Sanitizer Test for Inanimate Surfaces, which references the Official Methods of Analysis of the A.O.A.C., 12th edition (1975). Exposure time was modified to comply with the ZONO's 30 minute operating cycle. Protocol deviation is acceptable because DIS/TSS-10 is designed for liquids with an exposure time of 5 minutes or less.

## 3.2 STUDY CONCLUSIONS

The ZONO meets the AOAC requirements for sanitizing non-food product surfaces for porous, non-porous and semi porous surfaces. The sanitizing process is compatible with plastics, fabric, metal, wood, glass, and ceramics and effective on the types of materials typically found in a childcare or sports facility setting, school, home, or business that are easily contaminated with bacterial pathogens.

Sanitizing liquids and natural rubber is not recommended. The ZONO is not for sanitizing food or surfaces for service or preparation.

While not part of the study, it is important to note that the ZONO does not remove soil. Items sanitized must be cleaned according to the item's manufacturer's recommendations to remove soil.

## 3.3 OVERVIEW OF THE STUDY

Following the methodology set forth in the U.S. EPA DIS/TSS-10, Sanitizer Test for Inanimate Surfaces, this study consisted of two parts. Part One of the study consists of the direct inoculation of seven targeted bacteria, plus

slurry of three targeted bacteria, onto 1 ½ inch diameter sterile stainless steel coupons. The seven targeted bacteria and slurry are:

Methicillin-resistant Staphylococcus aureus (MRSA) (serious skin pathogen); Staphylococcus aureus (S. aureus) (serious skin pathogen); Escherichia coli (E. coli) (common gastrointestinal pathogens); Streptococcus pyogenes (S. pyogenes) common respiratory pathogens); Shigella dysenteriae (S. dysenteriae) (common gastrointestinal pathogens); Salmonella enteritidis (S. enteritidis) (common gastrointestinal pathogens); Pseudomonas aeruginosa (P. aeruginosa) (common gastrointestinal pathogens); and Slurry of E.coli, S. pyogenes and MRSA in equal amount.

These seven bacteria represent a cross-section of serious pulmonary, skin, and gastrointestinal pathogens, and represent common sources of human infection. Each has the potential to cause infection from contaminated materials in multiple settings where equipment and materials are in communal use.

Part Two of the study consists of the direct inoculation of three targeted bacteria, plus slurry of three targeted bacteria, onto porous, non-porous and semi porous materials to simulate actual contaminated materials that are used in a typical childcare setting, sports arena setting, home or business. The three bacteria, MRSA, E. Coli, S. pyogenes, and represent skin, gastrointestinal and respiratory pathogens.

## 3.4 SUMMARY OF STUDY RESULTS

The ZONOsanitech sanitizing cabinet demonstrated a four (4) to five (5) log kill on the coupons treated during Part One of the study, compared to the baseline growth on the control coupons. Part Two of the study results clearly

indicated that there was a difference of three (3) log or more among treated and non-treated materials challenged with all bacteria. The t-test tables (1-3) clearly showed highly significant differences between treated and non-treated materials in the case of each organism. Statistical evaluation of the data indicates that the results of all the treatments were highly and significantly different than the results of the non-treated materials.

### 3.5 STUDY DESCRIPTION

### 3.6 PART I - COUPON PROTOCOL CHALLENGE

**BACTERIA:**

1) E. coli (ATCC 25922)
2) MRSA (ATCC BAA-44)
3) S. pyogenes (ATCC 19615)
4) S. enteritidi (ATCC 13076)
5) P. aeruginosa (ATCC 27853)
6) S. dysenteriae (ATCC 11456)
7) S. aureus (ATCC 25923)
8) Slurry of E. coli, MRSA, S. pyogenes

**MATERIALS:**

Stainless steel coupons

**INOCULATION PREPARATION:**

The coupons were autoclaved and dried completely before inoculation to avoid any contamination.

All the organisms were subcultured on appropriate medium for 24 hours before the inoculum preparation.

Three concentrations of the inoculum to be evaluated were prepared according to the Macfarland standards. Three drops of organic load (blood) were added to the inoculum. The coupons were aseptically arranged in sterile Petri dishes with the dimple side down (if necessary). 20 µl of the inoculum was transferred aseptically on to ten sterile coupons in the Petri dishes following the EPA recommended method DIS/TSS-10, Sanitizer Test for Inanimate Surfaces. The process below was repeated for each of the inoculum concentration.

**CHALLENGE**

Five inoculated coupons were aseptically transferred to the ZONO, and a 30 minute cycle was run (specified contact time). The other set of 5 coupons as control samples (no contact time) were placed in a room at room temperature. After the specified contact time in the ZONO, five inoculated coupons were transferred to a sterile container (purple tops). Five ml of Tryptic Soy Broth (TSB) was aseptically transferred to each sterile container and the sterile container sate at room temperature for 30 minutes. Subculture the TSB using dilutions (0.1, 0.01, 0.001). Serial dilutions may be done if necessary. Incubate the TSA or Blood Agar Plates at 35° C for 48 hours. Repeat the procedure for all the inoculum concentrations in replicates of five.

**CHALLENGE PARAMETERS:**

5 inoculated materials stored at room temperature (control)

5 inoculated materials exposed in ZONO one 30 minute cycle (challenge)

Ozone level and relative humidity level confidential

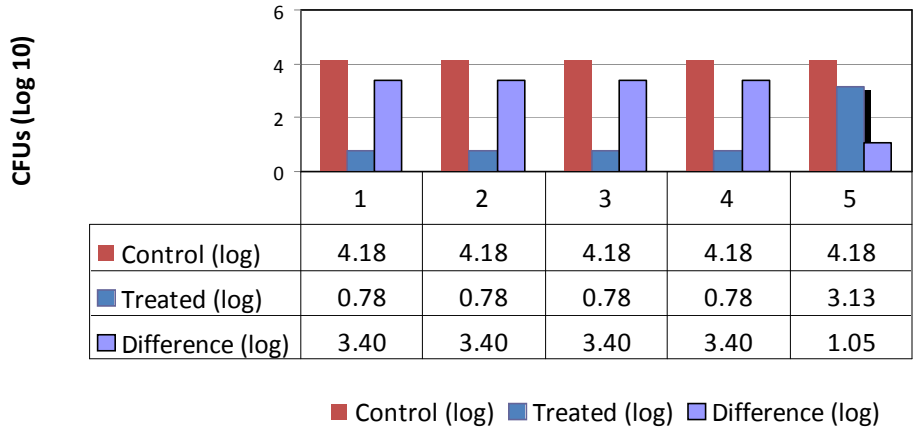
Sample Size: 1 ½ inch diameter sterile stainless steel coupon

**Concentration of inoculum:**

| | |
|---|---|
| E. coli | 10^7 & 10^8 |
| S. pyogenes | 10^7 & 10^8 |
| MRSA | 10^7 & 10^8 |
| S. enteritidi | 10^7 & 10^8 |
| P. aeruginosa | 10^7 & 10^8 |
| S. dysenteriae | 10^7 & 10^8 |
| S. aureus | 10^7 & 10^8 |
| Slurry of E. coli, MRSA, S. pyogenes | 10^7 & 10^8 |

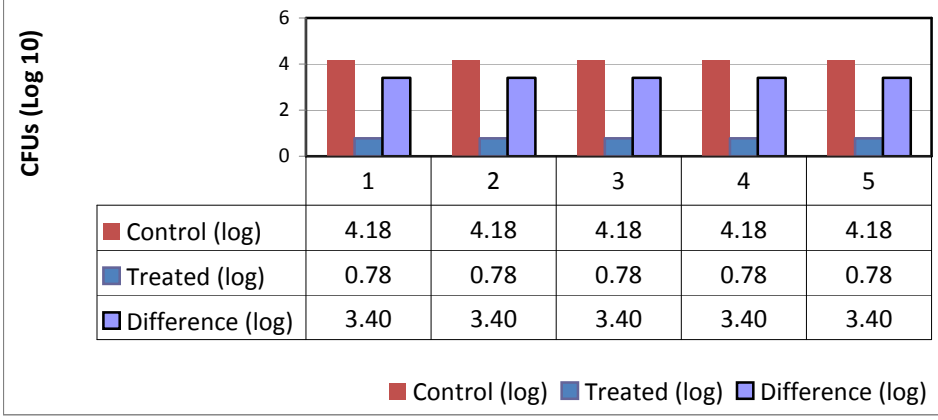
## TABLES/GRAPHS FOR PART ONE STUDY

**Fig. 1: Efficacy of ZonoSanitech on the growth of *MRSA* on challanged Coupons**



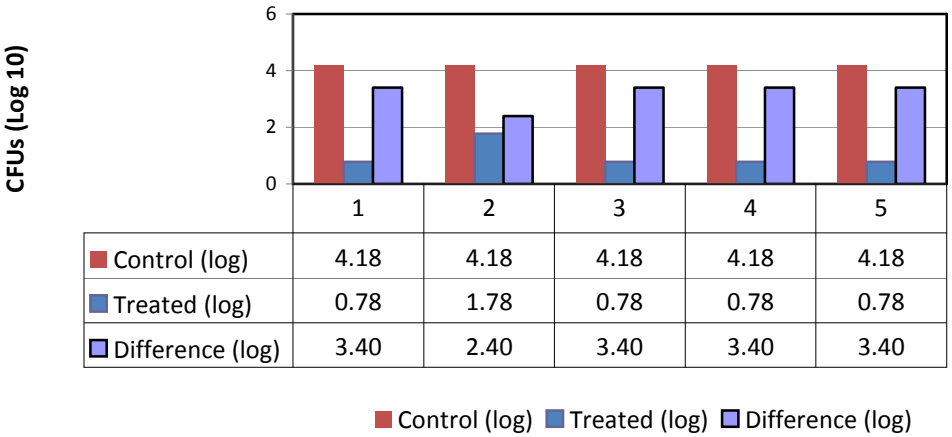

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Control (log) | 4.18 | 4.18 | 4.18 | 4.18 | 4.18 |
| Treated (log) | 0.78 | 0.78 | 0.78 | 0.78 | 3.13 |
| Difference (log) | 3.40 | 3.40 | 3.40 | 3.40 | 1.05 |

**Fig. 2: Efficacy of ZonoSanitech on the growth of *E. coli* on challanged Coupons**



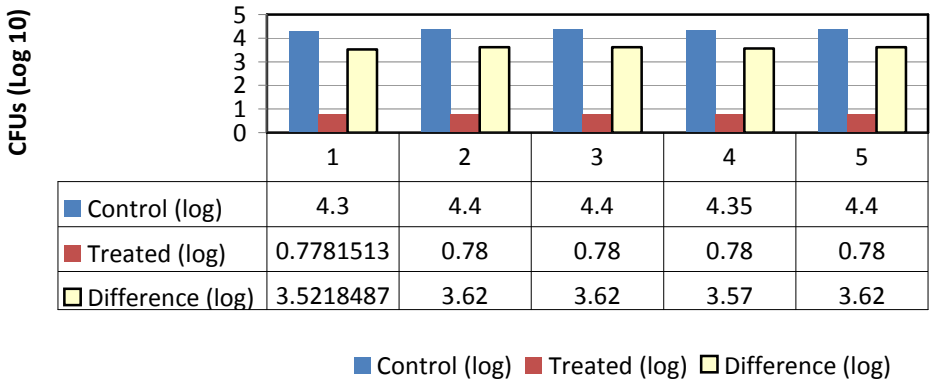

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Control (log) | 4.18 | 4.18 | 4.18 | 4.18 | 4.18 |
| Treated (log) | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| Difference (log) | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 |

**Fig. 3: Efficacy of ZonoSanitech on the growth of *S. pyogenes* on challanged Coupons**



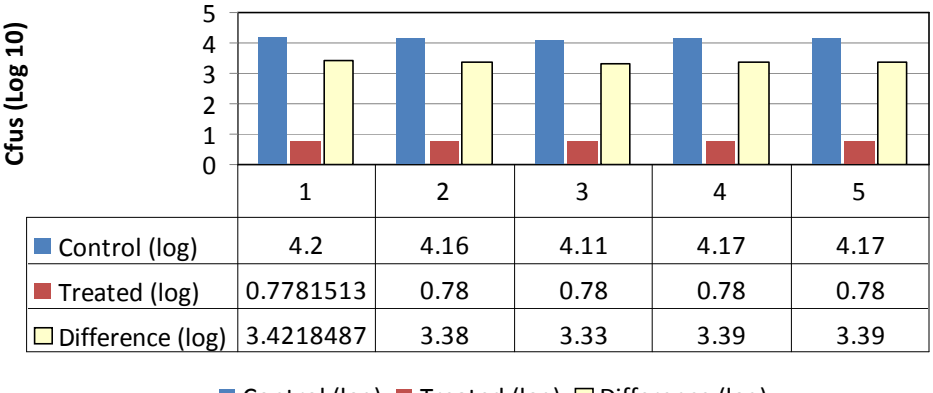

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Control (log) | 4.18 | 4.18 | 4.18 | 4.18 | 4.18 |
| Treated (log) | 0.78 | 1.78 | 0.78 | 0.78 | 0.78 |
| Difference (log) | 3.40 | 2.40 | 3.40 | 3.40 | 3.40 |

**Fig. 4: Efficacy of ZonoSanitech on the growth of *S. dysenteriae* on challanged coupons**



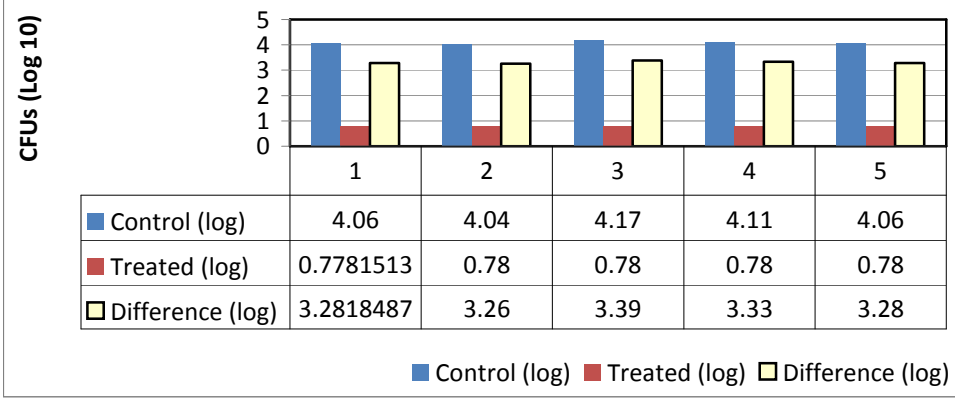

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Control (log) | 4.3 | 4.4 | 4.4 | 4.35 | 4.4 |
| Treated (log) | 0.7781513 | 0.78 | 0.78 | 0.78 | 0.78 |
| Difference (log) | 3.5218487 | 3.62 | 3.62 | 3.57 | 3.62 |

**Fig. 5: Efficacy of ZonoSanitech on the growth of *P. aeruginosa* on challanged coupons.**



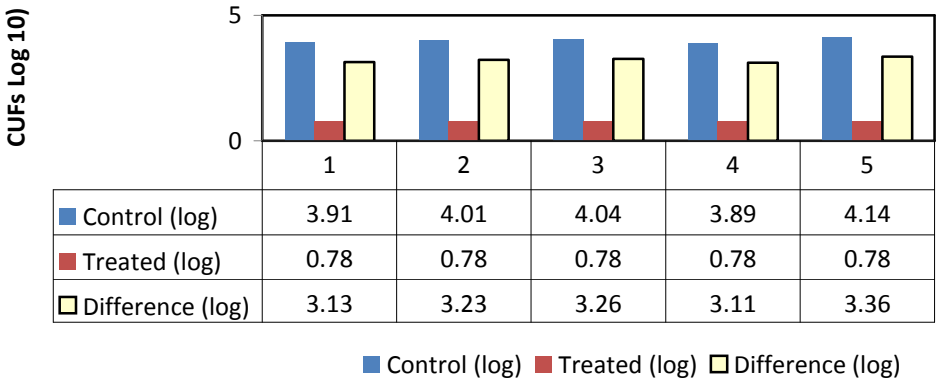

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Control (log) | 4.2 | 4.16 | 4.11 | 4.17 | 4.17 |
| Treated (log) | 0.7781513 | 0.78 | 0.78 | 0.78 | 0.78 |
| Difference (log) | 3.4218487 | 3.38 | 3.33 | 3.39 | 3.39 |

**Fig. 6:Efficacy of ZonoSanitech on the growth of *S. aureus* on challanged copupns.**




| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Control (log) | 4.06 | 4.04 | 4.17 | 4.11 | 4.06 |
| Treated (log) | 0.7781513 | 0.78 | 0.78 | 0.78 | 0.78 |
| Difference (log) | 3.2818487 | 3.26 | 3.39 | 3.33 | 3.28 |

**Fig. 7: Efficacy of ZonoSanitech on the growth of *S. enteritidis* on challanged coupons.**




| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Control (log) | 3.91 | 4.01 | 4.04 | 3.89 | 4.14 |
| Treated (log) | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| Difference (log) | 3.13 | 3.23 | 3.26 | 3.11 | 3.36 |

## 3.7 PART TWO - MATERIAL PROTOCOL CHALLENGE

BACTERIA:

1) MRSA (ATCC BAA-44)

2) E. coli (ATCC 25922)

3) S. pyogenes (ATCC 19615)

**MATERIALS 1X1 Inch:**

Porous material: Fabric made of cotton and rayon representative of a typical stuffed animal, bedding, pillows, and padding in sports equipment. The porous fabric could become contaminated with respiratory, fecal or skin bacteria, and enables the effectiveness of the penetration of the ozone to be evaluated. The fabric was autoclaved.

Semi-porous: A plastic coated cardboard book, like one found in a childcare setting, home, or library. The plastic coated cardboard material has the potential to absorb secretions much like the porous, but not as readily. The plastic coated cardboard was sterilized under ultra violet light.

Nonporous: A hard plastic, representative of a hard plastic toy that would be available in the childcare setting, computer key boards, or materials used in sports equipment. The plastic was cleaned on both sides with alcohol and sterilized under ultra violet light.

**INOCULUM PREPARATON:**

All of the organisms were subcultured for 24 hours before the inoculum preparation to avoid any contamination.

Three concentrations of the inoculum evaluated were prepared according to the Macfarland standards. Three drops of organic load (blood) were added to the inoculum. The materials were aseptically arranged in sterile Petri dishes. 50 µl of the inoculum was transferred aseptically on to ten sterile materials in the Petri dishes following the EPA recommended method DIS/TSS-10, Sanitizer Test for Inanimate Surfaces. The process below was repeated for each of the inoculum concentration.

Five of the inoculated materials were aseptically transferred to the ZONO, and a 30 minute cycle was run (specified contact time).

The five material control samples (no contact time) were placed in a room at room temperature. After the specified contact time, the five inoculated materials were transferred from the ZONO to a sterile container (purple tops). Five ml of Tryptic Soy Broth (TSB) was aseptically transferred to each sterile container, and the container sat at room temperature for 30 minutes. The TSB was subcultured using dilutions (0.1, 0.01, 0.001). Serial dilutions may be done, if necessary (Were they done). The TSA Agar Plates were incubated at 35° for 48 hours.

The procedure for all the inoculum concentration was done in replicates of five and repeated.

**Challenge Parameters: 3 materials**

5 inoculated materials stored at room temperature (control)

5 inoculated materials exposure in ZONO one 30 minute cycle (challenge)

Ozone level and relative humidity level confidential

Sample Size: 1 x 1 inch porous, semi-porous and nonporous

**Concentration of inoculum:**

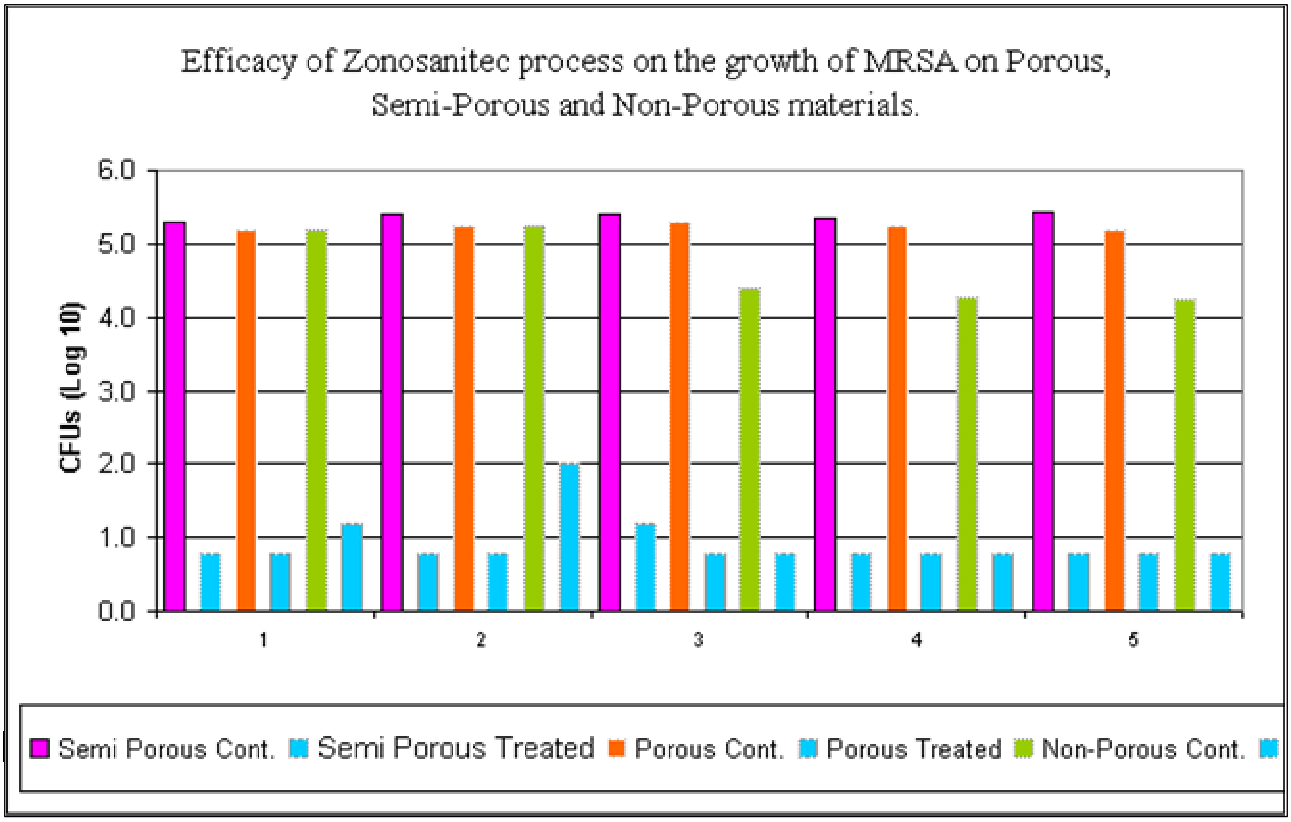
| | |
|---|---|
| E. coli | 10^7 & 10^8 |
| S. pyogenes | 10^7 & 10^8 |
| MRSA | 10^7 & 10^8 |

**STUDY RESULTS**

MRSA

All the treated materials viz. Non-Porous, Porous and Semi Porous had more than 3 log differences than the non-control counter parts, see figure 8 The means of treated porous, non-porous and semi porous materials were significantly different than the non-treated means. In the case of semi porous material the means of treated samples was 0.864 as compared to 4.38 of non-treated ones. Similarly the means of treated porous material, 0.780 was much smaller than the non-treated counterpart's of 4.23. The means of treated non-porous material, 1.11 were much less than that of non-treated, which had means of 4.66. See Table 1 below

Figure 8 and Associated Data Table



| | Semi Porous | | | Porous | | | Non Porus | | |
|---|---|---|---|---|---|---|---|---|---|
| Replicate | Control | Treated | Log Difference | Control | Treated | Log Difference | Control | Treated | Log Difference |
| 1 | 5.3 | 0.78 | 4.5 | 5.2 | 0.78 | 4.4 | 5.2 | 1.2 | 4 |
| 2 | 5.4 | 0.78 | 4.6 | 5.2 | 0.78 | 4.5 | 5.2 | 2 | 3.2 |
| 3 | 5.4 | 1.2 | 4.2 | 5.3 | 0.78 | 4.5 | 4.4 | 0.78 | 3.6 |
| 4 | 5.4 | 0.78 | 4.6 | 5.2 | 0.78 | 4.5 | 4.3 | 0.78 | 3.5 |
| 5 | 5.4 | 0.78 | 4.7 | 5.2 | 0.78 | 4.4 | 4.2 | 0.78 | 3.4 |

Table 1: T-Test for the effects of OZO Clean Technology on the growth of MRSA on Semi Porous, Porous and Non Porous materials.

| Material | Non-Treated Means | Treated Means | Std. Dev | Degree of Freedom | t- Value | Prob. |
|---|---|---|---|---|---|---|
| Semi Porous | 4.38 | 0.864 | 0.138 | 8 | 40.27 | 0.0001*** |
| Porous | 4.23 | 0.78 | 0.355 E - 01 | 8 | 154 | 0.0001*** |
| Non Porous | 3.36 | 1.111 | 0.519 | 8 | 7.77 | 0.0001*** |

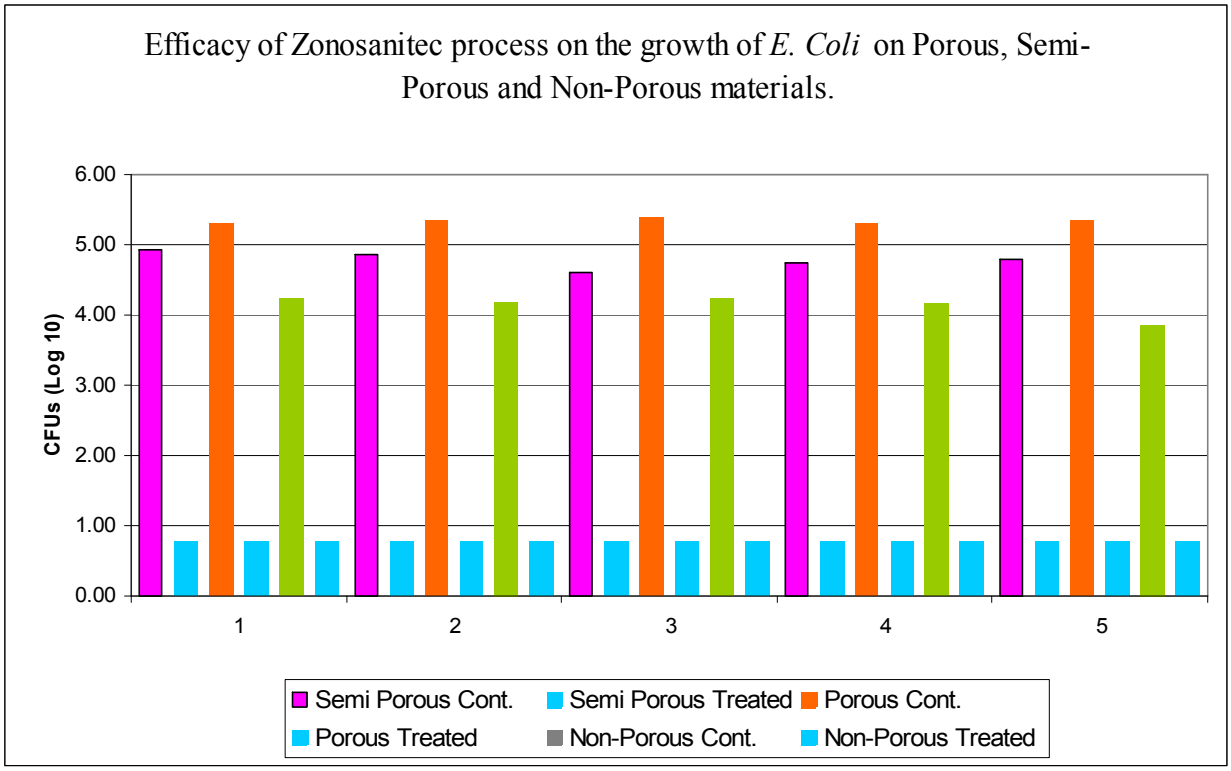
*** highly significant at $P$ <0.001 level

## E. coli

The results from the E.Coli challenge, like MRSA, showed a three (3) log difference between treated and non-treated materials as evidenced in figure 9. Table 5 depicts clear and huge differences among means of treated and non-treated materials. In this case the means of treated porous material (0.78) was much less than the non-treated (4.34) material. Similarly, the

means of treated semi porous and non-porous (0.78) materials were much lower than non-treated semi porous and non-porous materials, which were 3.79 and 3.14 respectively

Figure 9 and Associated Data Table




| | Semi Porous | | | Porous | | | Non Porus | | |
|---|---|---|---|---|---|---|---|---|---|
| Replicate | Control | Treated | Log Difference | Control | Treated | Log Difference | Control | Treated | Log Difference |
| 1 | 4.9 | 0.78 | 4.2 | 5.3 | 0.78 | 4.5 | 4.2 | 0.78 | 3.5 |
| 2 | 4.9 | 0.78 | 4.1 | 5.4 | 0.78 | 4.6 | 4.2 | 0.78 | 3.4 |
| 3 | 4.6 | 0.78 | 3.8 | 5.4 | 0.78 | 4.6 | 4.2 | 0.78 | 3.5 |
| | | | | | | | | | |
| 4 | 4.8 | 0.78 | 4 | 5.3 | 0.78 | 4.5 | 4.2 | 0.78 | 3.4 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 4.8 | 0.78 | 4 | 5.4 | 0.78 | 4.6 | 3.9 | 0.78 | 3.1 |

Table 2: T-Test for the effects of OZO Clean Technology on the growth of *E. coli* on Semi Porous, Porous and Non Porous materials.

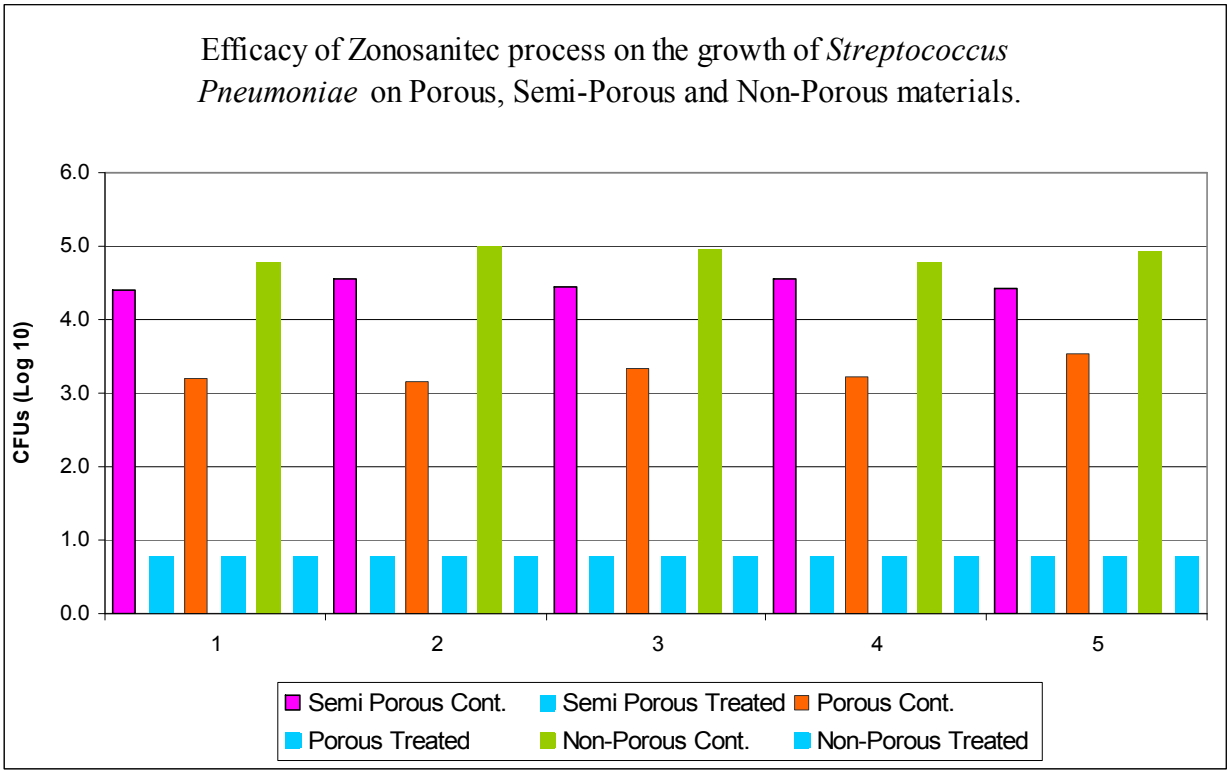
| Material | Non-Treated Means | Treated Means | Std. Dev | Degree of Freedom | t- value | Prob. |
|---|---|---|---|---|---|---|
| Semi Porous | 3.79 | 0.78 | 0.887 E-01 | 8 | 53.7 | 0.0001*** |
| Porous | 4.34 | 0.78 | 0.296 E - 01 | 8 | 190 | 0.0001*** |
| Non Porous | 3.14 | 0.78 | 0.111 | 8 | 33.4 | 0.0001*** |

*** highly significant at $P<0.001$ level

S. pyogenes

Finally, in the case of S. pyogenes, the treated semi porous, porous and non-porous materials exhibited a clear difference of log 3 or more than their non-treated counterparts (Fig 1 and associated chart). It is also evident from the table 3 where means of non-treated porous material (4.7) were slightly higher than the non-treated semi-porous (3.47) and non-porous (3.89) materials respectively. All treated materials had a mean of 0.78.

Figure 10 and Associated Data Table




| | Semi Porous | | | Porous | | | Non Porus | | |
|---|---|---|---|---|---|---|---|---|---|
| Replicate | Control | Treated | Log Difference | Control | Treated | Log Difference | Control | Treated | Log Difference |
| 1 | 4.4 | 0.78 | 3.6 | 3.2 | 0.78 | 4.7 | 4.8 | 0.78 | 4.0 |
| 2 | 4.6 | 0.78 | 3.8 | 3.2 | 0.78 | 4.7 | 5.0 | 0.78 | 4.2 |
| 3 | 4.4 | 0.78 | 3.7 | 3.3 | 0.78 | 4.7 | 4.9 | 0.78 | 4.2 |
| 4 | 4.5 | 0.78 | 3.8 | 3.2 | 0.78 | 4.7 | 4.8 | 0.78 | 4.0 |
| 5 | 4.4 | 0.78 | 3.6 | 3.5 | 0.78 | 4.7 | 4.9 | 0.78 | 4.2 |

Table 3: T-Test for the effects of OZO Clean Technology on the growth of *Strep. pyogenes* on Semi Porous, Porous and Non Porous materials

| Material | Non-Treated Means | Treated Means | Std. Dev | Degree of Freedom | t- value | Prob. |
|---|---|---|---|---|---|---|
| Semi Porous | 3.47 | 0.78 | 0.532 E-01 | 8 | 80 | 0.0001*** |
| Porous | 5.48 | 0.78 | 0.100 E-01 | 8 | 28.6 | 0.0001*** |
| Non Porous | 3.89 | 0.78 | 0.747 E-01 | 8 | 65.8 | 0.0001*** |

*** highly significant at $P<0.001$ level

## 4.0 SUMMARY OF STATISTICAL ANALYSIS

In order to perform a meaningful statistical analysis of the available data, log transformation was performed to create homogeneity in data. After transforming data, bar graphs were generated for each organism challenged to three different materials types. Similarly, an unpaired T-test was also performed for each organism tested against each material type. The graphs (1-3) and associated charts clearly indicated that there was a difference of 3 log among treated and non-treated materials challenged by MRSA, E.coli, and S. pyogenes.

The t-test tables (1-3) clearly showed highly significant differences between treated and non-treated materials in the case of each organism. It is evident from the tables (1-3), that there was a high t-values in each material type and translated into very high significant probability (P) value which was <0.001. This clearly indicates that all the treatments were statistically highly and significantly different than the non-treated materials.

## 4.5 STASTICAL DATA INTERPRETATION

Data Transformation: In each case, we transformed data to create homogeneity among data set for comparison. After treatment in the ZONO, the majority of the data points were "0" as compared to control where data points were in several thousands. To reduce the homogeneity of error, variance data was transformed using log 10 transformation by adding value of "1" to each data point (treated and non-treated) and then transformed.

## 5.0 CONCLUSION

In conclusion, the ZONO is a viable alternative to chemical or heat based sanitizers. The ZONO meets the A.O.A.C. definition of sanitizing non-food product surfaces, killing 99.9% of common bacteria as described in this report, based upon the EPA recommended method DIS/TSS-10, Sanitizer Test for Inanimate Surfaces (exposure time modified).

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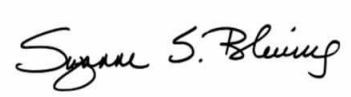
[i]Respectfully submitted,



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