

# Evaluation of greenO2 Hand Spray against 3 major bacterial pathogens vs.

common hand sanitizers (62% Alcohol)

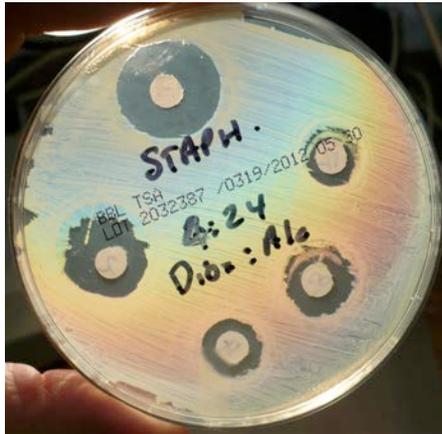
*Staphylococcus (MRSA), Ecoli & Pseudomonas Aeruginosa*

The larger the clear ring around the disc the better the kill

**HealthyHands™** by greenO2  
(Chlorine Dioxide/Alcohol Complex)

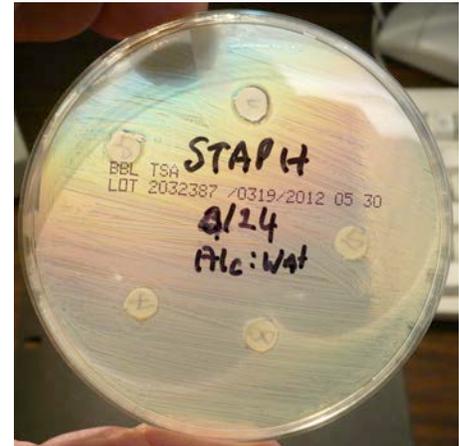
**Common Alcohol Based Hand Sanitizer**  
(62% Alcohol)

**A**

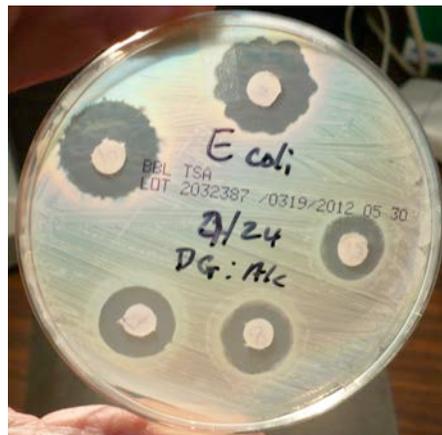


**B**

**STAPH (MRSA)**



**C**



**D**

**Escherichia  
Ecoli**

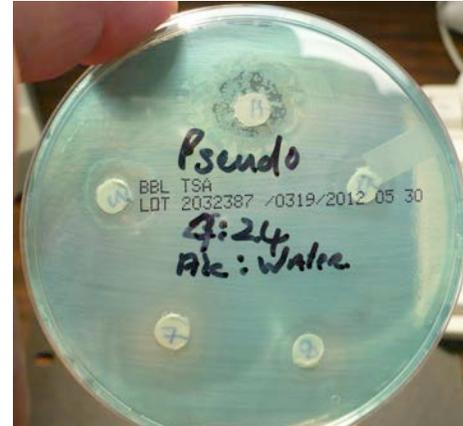


**E**



**F**

**Pseudomonas  
Aeruginosa**



# greenO2 Hand Spray against 3 Bacterial Pathogens at dilution using 62% Alcohol

*Dr Robert J. Wilkins BVSc, DVP, DACVP (April 21<sup>st</sup> 2012)*

## Method

greenO2 Hand Spray, containing concentration of 500ppm Chlorine Dioxide Complex in 62% alcohol was tested, in respective dilutions of 1) Undiluted 2) 1:2 dilution 3) 1:4 dilution 4) 1:8 dilution and 5) 1:12 dilution using 62% ethyl alcohol as diluent. Three major pathogenic bacteria, Staphylococcus aureus (MRSA), Pseudomonas aeruginosa and hemolytic Escherichia coli.

Each organisms was inoculated over the entire surface of a 9cm Trypticase Soy Agar Plate. Discs of filter paper were labeled U=undiluted, 2= 1:2 dilution, 4=1:4 dilution, 8=1:8 dilution and 12=1:12 dilution were placed equidistant onto the plate in a circle. One drop of each dilution of the greenO2 Hand Spray solution was placed on the respective disc. The plates were incubated for 18 hours at 37 degrees centigrade . The plates were photographed after incubation .

## Results

The photos above show the areas of inhibited bacterial growth around the discs demonstrating the efficacy of the bacteriocidal effects at the respective concentrations.

- Zones of inhibition are evident at all dilutions. (Photo A)
- Zones of inhibition are noted in all dilutions. (Photo C)
- Zones of inhibition are noted at all dilutions. (Photo E)

**VS.**

## Antimicrobial Effects of Dilution on 62% Ethyl Alcohol

*Robert J. Wilkins BVSc. DVP DACVP (April 25, 2012)*

**Method :** Dilutions of 62% ethyl alcohol were prepared using sterile water in respective concentrations undiluted=U, 1:2 =2 , 1:4 =4 , 1:8 =8 and 1:12 =12 .

These concentrations were tested using a standardized antiseptic testing method against 3 bacterial pathogens Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa .

Labeled discs of filter paper were placed onto inoculated plates of Trypticase Agar containing the respective pathogens. One drop of each concentration of ethyl alcohol was placed onto the respective disc. The plates were incubated, upright, for 18 hours at 37 degrees centigrade.

Each plate was photographed from the front using a contrasting background and from the back using a backlight to display the zone on inhibition of growth associated with the antiseptis.

**Results:** The results are depicted in the photographs Above.

- Note the thin rim of inhibition of growth at the undiluted and 1:2 level of dilution only (**Photo B**)
- Note the zone of inhibition in the undiluted and 1:2 concentrations only. (**Photo D**)
- Zones of inhibition of growth are noted at all dilutions with partial clearing at 1:12. (**Photo F**)

Typical sanitizers such as Purell, Clean Well and Dial have a typical log reduction of 4. Each case of log reduction is different, however, they reduce whatever the initial log was by their reduction amount. The FDA suggests a log reduction of 5 or better for hand sanitizing. greenO2 Hand Spray has a log reduction of nearly 7. Our test showed significant and nearly total kill rates within 30 seconds of use.

## PRELIMINARY TESTING OF GREENO2 HAND SPRAY

**SAMPLE:** greenO2 Hand Spray

### MATERIALS AND METHODS

Microorganisms (*Klebsiella pneumoniae* ATCC 13883) were plated on Trypticase Soy Agar (TSA) and incubated at 35-37°C for 18-24 hours. Organisms were then used to prepare a suspension in saline.

Samples were tested by adding 0.1ml of the microorganism suspension to 9.9 ml of the test solution in a sterile container and mixed. Samples were then incubated for 30 seconds. Following incubation 2.0 ml of the mixture was added to 18 ml of D/E Neutralization Broth. A 2.0 ml sample, a 1.0 ml sample and a 0.1ml sample of the D/E broth were added separately to sterile petri plates. Approximately 10 ml of liquid Trypticase Soy Agar were added to each petri plate and allowed to solidify. Plates were incubated at 35-37°C for 24-48 hours, and colony forming units were counted.

A control was run using 9.9 ml of saline instead of the active ingredients.

### RESULTS

Initial concentration of *K. pneumoniae* suspension in saline:  $4.2 \times 10^8$  CFU/ml

Sample	Challenge Inoculum (Log#cfu/ml Product)	Recovered 30 seconds (Log#cfu/ml Product) <b>Log reduction</b>
Hand Spray	$4.2 \times 10^6$ (6.62 logs)	0 (0.00) <b>&gt;6.62</b>
Control	$4.2 \times 10^6$ (6.62 logs)	$3.9 \times 10^6$ (6.56 logs)

### CONCLUSION

Testing of greenO2 hand Spray, lot 2949, indicated a log reduction of >6.62 *K. pneumoniae* in 30 seconds

### COMMENTS

Klebsiella pneumoniae was chosen for this study because it is an organism that is distributed throughout the environment and is often used as a model organism for testing. This bacteria is also found as part of the normal microbiological component of the gastrointestinal tract in the mouth and on the skin of humans. Moreover, the organism is also known to be an important human pathogen. Among other things it is known for devastating nosocomial (hospital acquired) disease including septicemia, endocarditis, pneumonia and an infections of the face. (Goldman and Green, 2009)

Klebsiella pneumoniae has become an even more serious health threat since the discovery that many strains contain Extended Spectrum Beta Lactamases (ESBL). These are enzymes which make the organism resistant to many antibiotics. This resistance has caused problems in eradicating systemic infections with currently available drugs. (Goldman and Green, 2009)

K. pneumoniae is also known to form biofilms and is a model for testing the effects of antibiotics on biofilms.

Goldman, E. and Green, L. H. CRC Practical Handbook of Microbiology 2<sup>nd</sup> Edition. CRC Press. Boca Raton. 2009

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Reviewed by:

Date 04/25/12

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