MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of 4 Patriot's Device

Test Method

Custom Device Study Based on: Modified ASTM E2315

Study Identification Number

NG16477-V1

Study Sponsor

4Patriots

2920 Berry Hill Dr., Suite 300
Nashville, TN 37204
(800)-304-4202 | 4Patriots.com

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378
Report Author: Patricia Castro, B.S.

Purpose of the Study

The purpose of this study was to determine the antimicrobial properties of the submitted test device.

Brief History of the Performing Laboratory

Microchem Laboratory is located in the greater Austin, Texas area. It is owned and operated by microbiologist Dr. Benjamin Tanner. The core of the company was founded by Dr. Tanner as Antimicrobial Test Laboratories in 2006. Antimicrobial Test Laboratories was later combined with a niche cosmetic testing lab and Microchem Laboratory, founded in 1988 by Dr. Norman Miner. The combined labs have operated under one roof as Microchem Laboratory since 2016. Microchem Laboratory is ISO 17025 accredited and offers testing in compliance with current Good Laboratory Practice (GLP) regulations as stipulated by EPA and FDA. Clients are always welcome to tour the lab, observe studies, and audit the lab's quality systems.

Study Timeline

Devices Received	Cultures Initiated	Carriers Inoculated	Carriers Treated	Enumeration Plates Evaluated	Report Delivered
		***S. enterica* ATCC 10708 and *E. coli* ATCC 8739**			
06 OCT 2020	29 OCT 2020	30 OCT 2020	30 OCT 202 0	02 NOV 2020	04 NOV 2020

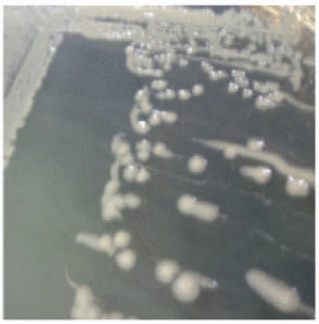
Test Device Information

Name of Test Device: Patriot Pure Aqua-Bright
Manufacturer: 4Patriots
Mode of Active: UV Light (Germicidal)

Figure 1. Patriot Pure Aqua-Bright

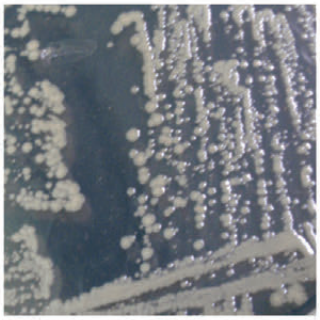
Test Microorganism Information

The test microorganism(s) selected for this test:



Salmonella enterica

This bacteria is Gram-negative, rod-shaped, facultative anaerobe. Like the closely related *Escherichia* genus, *Salmonella* are common to all parts of the world and share habitats in the digestive systems of cold and warm-blooded animals. *S. enterica* is one of the most common bacteria associated with zoonotic and foodbourne illness. Because of it's regular occurrence and pathogenicity, *S. enterica* is a common bacteria for measuring disinfectant efficacy.



Escherichia coli

This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of mammals. Although most serotypes of this microorganism are harmless there are pathogenic groups of *E. coli* such as enterohemorrhagic (EHEC), verocytotoxin producing (VTEC) and Shiga-like toxin producing (STEC) that can cause a multitude of illnesses. *E. coli* is relatively susceptible to disinfection when dried on a surface, yet it can be a challenging microorganism to mitigate in solution.

Summary of the Procedure

- Test microorganisms were prepared in liquid culture medium for bacteria.
- The suspension of test microorganism was standardized, as needed, by dilution in a buffered saline solution.
- Test and control substances were dispensed in identical volumes to sterile vessels.
- Independently, test and control substances were inoculated with each test microorganism, then mixed and incubated.
- At the conclusion of the contact time, a volume of the liquid control and test solution was harvested and chemically neutralized.
- Dilutions of the neutralized test solution were assayed using appropriate growth media to determine the surviving microorganisms at the respective contact times.
- Reductions of microorganisms were calculated by comparing microbial concentrations in the control substance to microbial concentrations in the test substance.

Criteria for Scientific Defensibility of an ASTM E2315 Study

For Microchem Laboratory to consider a Suspension Time Kill study to be scientifically defensible, the following criteria must be met:

1. The average number of viable bacteria recovered from the control samples must be $\geq 1.00 \times 10^6$ CFU/ml.
2. Ordinary consistency between replicates must be observed for the control samples.
3. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
4. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

Due to the modified nature of the study, passing criteria may be determined by the Study Sponsor prior to test initiation. If no passing criteria is established, a conclusion about the data is not provided by Microchem Laboratory, but the Study Sponsor may determine significance based on statistical interpretation or other means.

Testing Parameters

Test Substance Volume:	499.0 ml	Test Substance:	DIW
Control Substance Volume:	499.0 ml	Control Substance:	PBS
Culture Growth Media:	TSB	Culture Growth Time:	24 ± 2 hrs
Number of Replicates:	Triple	Inoculum Volume:	1.0 ml
Inoculum Concentration:	$\geq 1.0 \times 10^6$ CFU/ml	Contact Temp.:	Ambient
Contact Time:	5 min	Volume Harvested:	1.0 ml
Neutralizer (Vol.):	PBS with 0.1% Triton X-100 (9.0 ml)	Plating Media:	TSA
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	24-48 hrs

Study Notes

The device was sprayed with 1:500 bleach and rinsed with DIW twice before and in between each use.

Control Results

Neutralization Method: N/A Media Sterility: Sterile
Growth Confirmation: Confirmed, morphology on required plating media

Calculations

CFU/ml = (Average plate count) x 1:10 serial dilution factor

$$\text{Percent Reduction} = \frac{(B - A)}{B} \times 100\%$$

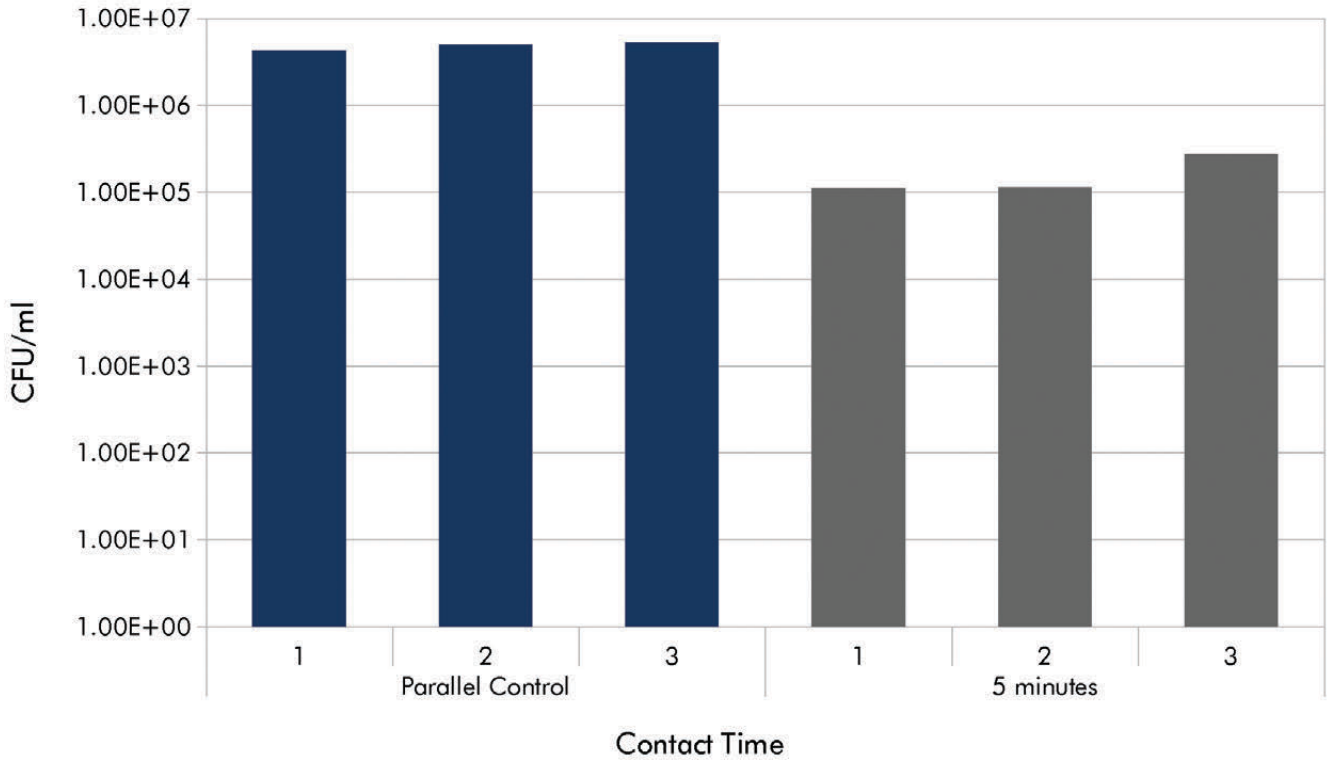
$$\text{Log}_{10}\ \text{Reduction} = \text{Log}(B/A)$$

Where:
B = Number of viable test microorganisms in the control substance after the contact time
A = Number of viable test microorganisms in the test substance after the contact time

Results of the Study – *S. enterica* ATCC 10708

Test Microorganism	Contact Time	Replicate	CFU/ml	Average CFU/ml	Average Percent Reduction Compared to Controls	Average Log_{10} Reduction Compared to Controls
S. enterica ATCC 10708	Parallel Control	1	4.25E+06	4.80E+06	N/A	N/A
		2	4.95E+06			
		3	5.20E+06			
	5 minutes	1	1.12E+05	1.67E+05	96.53%	1.46
		2	1.14E+05			
		3	2.74E+05			

Note: The limit of detection for this assay was 5.00E+01 CFU/ml. Values observed below the limit of detection are reported as <5.00E+01 CFU/ml in the table and as zero in the graph.



Results of the Study – *E. coli* ATCC 8739

Test Microorganism	Contact Time	Replicate	CFU/ml	Average CFU/ml	Average Percent Reduction Compared to Controls	Average Log_{10} Reduction Compared to Controls
E. coli ATCC 8739	Parallel Control	1	3.15E+06	3.42E+06	N/A	N/A
		2	3.55E+06			
		3	3.55E+06			
	5 minutes	1	1.80E+05	1.49E+05	95.64%	1.36
		2	1.38E+05			
		3	1.29E+05			

Note: The limit of detection for this assay was 5.00E+01 CFU/ml. Values observed below the limit of detection are reported as <5.00E+01 CFU/ml in the table and as zero in the graph.