



Final Report

An Evaluation of Antimicrobial Activity of One Test Product on Beef Primals Using a Time Kill Procedure

Test Material

AquaFew Acidic Water

Study Director

Erin S. Crowley

Performing Laboratory

Q LABORATORIES, INC.
1400 Harrison Avenue
Cincinnati, Ohio 45214

Project Identification Number

QL# 166361

Study Sponsor

Cascade Ridge Industries, LLC
PO Box 607
Quincy, Washington 98848
(Co-sponsor: Ameristar Meats)

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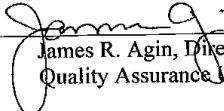
QUALITY ASSURANCE UNIT STATEMENT

Title: An Evaluation of Antimicrobial Activity of One Test Product on Beef Primals Using a Time Kill Procedure

The Quality Assurance Unit (QAU) of Q Laboratories, Inc. has inspected the study QL# 166361, in compliance with current FDA Good Laboratory Practice regulations, 21 CFR Part 58.

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below

Phase Inspected	Date of Inspection	Date Reported to Study Director	Date Reported to Management
In Process- Assay	1/8/10	1/19/10	1/19/10
In Process- Calculation of Results	1/15/10, 1/18/10	1/19/10	1/19/10
Final Report	1/19/10	1/19/10	1/19/10


James R. Agin, Director of Quality
Quality Assurance Unit, Q Laboratories, Inc. 1-19-10
Date

COMPLIANCE STATEMENT

This study meets the requirements for 21 CFR part 58 with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study.

The following analysts participated in this study:

Erin S. Crowley, Patrick M. Bird, Marianne K. Torontali, Katherine Goetz

Study Director: Q Laboratories, Inc.


Erin S. Crowley 1-19-10
Date

TEST SUMMARY

TITLE: An Evaluation of Antimicrobial Activity of One Test Product on Beef Primals Using a Time Kill Procedure

STUDY DESIGN: This study was conducted according to the procedures outlined in the signed protocol included in Appendix 1. The method used in this study was a modification of the ASTM Committee E35.15 standard test method E2315-03 “The Assessment of Antimicrobial Activity Using a Time Kill Procedure”.

TEST MATERIALS: One test product was received at Q Laboratories, Inc. on 1-7-09:

1. AquaFew Acidic Water -ORP: 900+

Two beef primals, Top Sirloin & Sirloin Butt, to be used in the evaluation of the water were received at Q Laboratories on 1-6-09 from Ameristar Meats.

SPONSOR: Cascade Ridge Industries, LLC
PO Box 607
Quincy, Washington 98848
(Co-sponsor: Ameristar Meats)

TEST CONDITIONS

Challenge Microorganisms:

Escherichia coli O157:H7 ATCC 43895
Escherichia coli O157:H7 ATCC 35150
Escherichia coli O157:H7 ATCC 51657
Klebsiella pneumoniae ATCC 10031

Active Ingredient in Test Product:

Hypochlorous Acid

Neutralizer Used:

Dilution to sub-inhibitory levels with Letheen Blanks

Contact Times

15 seconds, 30 seconds, 1 minute and 2 minutes

Contact Temperature

21 to 25°C

Media and Reagents

1. Letheen Blanks (LB)
2. Soybean-Casein Digest Media/Microbial Content Test Agar (MCT)
3. Tryptic Soy Agar (TSA)
4. Phosphate Buffer Solution (PBS)
5. Sorbitol Maconkey Agar (SMAC)
6. Tryptic Soy Agar with 5% Sheep Blood (SBA)
7. Neutralizer (Based on Sponsor's Recommendation)
8. 0.85% Saline solution
9. Gram Stain Reagents
10. Petrifilm (Aerobic Plate Count and Coliform)
11. Chlorine Standards
12. Tap Water

STUDY DATES AND FACILITIES

The analysis phase of this test was conducted at Q Laboratories, Inc in the Microbiology Research and Development laboratory, 1400 Harrison Avenue, Cincinnati, Ohio 45214, from 1/8/10 to 1/14/10. The study director signed the protocol on 1/7/10. The final report was signed on 1/19/10.

RECORDS TO BE MAINTAINED

All testing date, protocol, protocol modifications, test material records, the final report, and correspondence between Q Laboratories and the sponsor will be stored in the archives at Q Laboratories, 1400 Harrison Avenue, Cincinnati, Ohio 45214 for a period of at least seven (7) years.

RESULTS

The results of this Time Kill evaluation are presented in Tables 1 and 2. The verification of each challenge organism tested was confirmed by Gram Stain. Prior to analysis the pH of the AquaFew water was verified to be 2.39 and the concentration of Hypochlorous Acid was at 20 ppm. The results from the neutralizer effectiveness test shown in Table 3 indicated that the appropriate neutralizer was used for this test material. All sterility controls used in this assay were negative for growth.

For each challenge organism, the mean log value obtained from duplicate plates (CFU/ml) was measured against the log value of the calculated initial microbial population. The log reductions were calculated as follows:

$$\log_{10} \text{ reduction (LR)} = \log_{10} (\text{calculated initial microbial population}) - \log_{10} (\text{surviving microbial population})$$

The percent reduction was then calculated from the log reduction calculated above using the following formula:

$$\text{Percent reduction (\%)} = 100 \times (1 - 10^{-\text{LR}})$$

Calculation Note: $< 1.0 \times 10^1$ was calculated as “1” to obtain the \log_{10} reduction

RESULTS

Table 1: Results of Time-Kill for AquaFew Acidic Water ORP: 900+ on Top Sirloin at Initial and Subsequent Contact Time Points. Reported in CFU/ml recovered, Percent and Log₁₀ Reduction

Assay	Units	Inoculum Count	Count/cm ²	15 seconds	30 seconds	1 minute	2 minutes	15 seconds	30 seconds	1 minute	2 minutes
CONTROL				BEEF SURFACE				FAT SURFACE			
Aerobic Plate Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	2.5 x 10 ⁶	7.6 x 10 ⁵	1.2 x 10 ⁶	7.1 x 10 ⁵	4.1 x 10 ⁶	4.6 x 10 ⁶	4.8 x 10 ⁶	2.4 x 10 ⁶
Coliform* Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	1.3 x 10 ⁶	4.1 x 10 ⁵	5.6 x 10 ⁵	5.0 x 10 ⁵	2.7 x 10 ⁶	2.9 x 10 ⁶	1.9 x 10 ⁶	1.1 x 10 ⁶
<i>Escherichia coli</i> Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	8.5 x 10 ⁵	3.0 x 10 ⁵	3.6 x 10 ⁵	3.8 x 10 ⁵	2.3 x 10 ⁶	1.7 x 10 ⁶	1.4 x 10 ⁶	8.2 x 10 ⁵
<i>Escherichia coli</i> O157:H7 (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	1.4 x 10 ⁶	6.0 x 10 ⁵	7.8 x 10 ⁵	4.4 x 10 ⁵	2.6 x 10 ⁶	3.5 x 10 ⁶	4.1 x 10 ⁶	1.2 x 10 ⁵

* Results read at 24 hours.

Table 1 cont'd: Results of Time-Kill for AquaFew Acidic Water ORP: 900+ on Top Sirloin at Initial and Subsequent Contact Time Points. Reported in CFU/ml recovered, Percent and Log₁₀ Reduction

Assay	Units	Inoculum Count	Count/cm ²	15 seconds	30 seconds	1 minute	2 minutes	15 seconds	30 seconds	1 minute	2 minutes
TAP WATER TREATMENT				BEEF SURFACE				FAT SURFACE			
Aerobic Plate Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	4.5 x 10 ⁵	5.1 x 10 ⁵	1.7 x 10 ⁵	1.9 x 10 ⁵	7.2 x 10 ⁴	1.9 x 10 ⁵	1.6 x 10 ⁵	1.6 x 10 ⁵
	% reduction	NA	NA	98.393	98.179	99.393	99.321	99.743	99.321	99.429	99.429
	Log ₁₀ reduction	NA	NA	1.797	1.742	2.220	2.171	2.593	2.171	2.246	2.246
Coliform* Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	3.1 x 10 ⁵	2.6 x 10 ⁵	1.4 x 10 ⁵	1.2 x 10 ⁵	3.2 x 10 ⁴	8.4 x 10 ⁴	6.8 x 10 ⁴	6.0 x 10 ⁴
	% reduction	NA	NA	98.893	99.071	99.500	99.571	99.886	99.700	99.757	99.786
	Log ₁₀ reduction	NA	NA	1.959	2.035	2.304	2.371	2.945	2.526	2.617	2.672
<i>Escherichia coli</i> Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	2.3 x 10 ⁵	1.9 x 10 ⁵	1.1 x 10 ⁵	9.0 x 10 ⁴	2.2 x 10 ⁴	6.5 x 10 ⁴	5.0 x 10 ⁴	3.8 x 10 ⁴
	% reduction	NA	NA	99.179	99.321	99.607	99.679	99.921	99.768	99.821	99.864
	Log ₁₀ reduction	NA	NA	2.088	2.171	2.409	2.496	3.108	2.637	2.751	2.870
<i>Escherichia coli</i> O157:H7 (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	3.9 x 10 ⁵	3.3 x 10 ⁵	1.3 x 10 ⁵	1.8 x 10 ⁵	5.4 x 10 ⁴	8.8 x 10 ⁴	8.6 x 10 ⁴	1.0 x 10 ⁵
	% reduction	NA	NA	98.607	98.821	99.536	99.357	99.807	99.686	99.693	99.643
	Log ₁₀ reduction	NA	NA	1.859	1.931	2.336	2.195	2.718	2.506	2.516	2.450

* Results read at 24 hours.

Table 1 cont'd : Results of Time-Kill for AquaFew Acidic Water ORP: 900+ on Top Sirloin at Initial and Subsequent Contact Time Points. Reported in CFU/ml recovered, Percent and Log₁₀ Reduction

Assay	Units	Inoculum Count	Count/cm ²	15 seconds	30 seconds	1 minute	2 minutes	15 seconds	30 seconds	1 minute	2 minutes
AQUAFEW SPRAY TREATMENT				BEEF SURFACE				FAT SURFACE			
Aerobic Plate Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	1.1 x 10 ⁵	6.5 x 10 ⁴	4.8 x 10 ⁴	4.6 x 10 ⁴	2.6 x 10 ⁴	2.8 x 10 ⁴	1.8 x 10 ⁴	2.2 x 10 ⁴
	% reduction	NA	NA	99.607	99.768	99.829	99.836	99.907	99.900	99.936	99.921
	Log ₁₀ reduction	NA	NA	2.409	2.637	2.769	2.787	3.035	3.003	3.195	3.108
Coliform* Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	5.0 x 10 ⁴	4.4 x 10 ⁴	2.2 x 10 ⁴	2.8 x 10 ⁴	1.4 x 10 ⁴	1.1 x 10 ⁴	7.2 x 10 ³	4.8 x 10 ³
	% reduction	NA	NA	99.821	99.843	99.921	99.900	99.950	99.961	99.974	99.983
	Log ₁₀ reduction	NA	NA	2.751	2.807	3.108	3.003	3.304	3.409	3.593	3.769
<i>Escherichia coli</i> Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	3.8 x 10 ⁴	3.3 x 10 ⁴	1.6 x 10 ⁴	2.1 x 10 ⁴	1.0 x 10 ⁴	8.5 x 10 ³	5.3 x 10 ³	3.5 x 10 ³
	% reduction	NA	NA	99.864	99.882	99.943	99.925	99.964	99.970	99.981	99.988
	Log ₁₀ reduction	NA	NA	2.870	2.931	3.246	3.128	3.450	3.521	3.726	3.906
<i>Escherichia coli</i> O157:H7 (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	4.8 x 10 ⁴	4.6 x 10 ⁴	3.8 x 10 ⁴	1.8 x 10 ⁴	1.6 x 10 ⁴	9.2 x 10 ³	8.2 x 10 ³	6.0 x 10 ³
	% reduction	NA	NA	99.829	99.836	99.864	99.936	99.943	99.967	99.971	99.979
	Log ₁₀ reduction	NA	NA	2.769	2.787	2.870	3.195	3.246	3.486	3.536	3.672

* Results read at 24 hours.

Table 2: Results of Time-Kill for AquaFew Acidic Water ORP: 900+ on Sirloin Butt at Initial and Subsequent Contact Time Points. Reported in CFU/ml recovered, Percent and Log₁₀ Reduction

Assay	Units	Inoculum Count	Count/cm ²	15 seconds	30 seconds	1 minute	2 minutes	15 seconds	30 seconds	1 minute	2 minutes
CONTROL				BEEF SURFACE				FAT SURFACE			
Aerobic Plate Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	1.0 x 10 ⁶	1.2 x 10 ⁶	5.9 x 10 ⁵	3.2 x 10 ⁵	2.4 x 10 ⁶	6.0 x 10 ⁶	2.8 x 10 ⁶	1.6 x 10 ⁶
Coliform* Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	6.4 x 10 ⁵	4.4 x 10 ⁵	3.3 x 10 ⁵	2.3 x 10 ⁵	1.6 x 10 ⁶	3.6 x 10 ⁶	1.4 x 10 ⁶	1.0 x 10 ⁶
<i>Escherichia coli</i> Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	4.2 x 10 ⁵	3.3 x 10 ⁵	2.4 x 10 ⁵	1.7 x 10 ⁵	1.2 x 10 ⁶	2.6 x 10 ⁶	1.0 x 10 ⁶	7.4 x 10 ⁵
<i>Escherichia coli</i> O157:H7 (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	8.0 x 10 ⁵	6.2 x 10 ⁵	4.5 x 10 ⁵	3.0 x 10 ⁵	2.8 x 10 ⁶	5.9 x 10 ⁶	1.7 x 10 ⁶	1.5 x 10 ⁶

* Results read at 24 hours.

Table 2 cont'd: Results of Time-Kill for AquaFew Acidic Water ORP: 900+ on Sirloin Butt at Initial and Subsequent Contact Time Points. Reported in CFU/ml recovered, Percent and Log₁₀ Reduction

Assay	Units	Inoculum Count	Count/cm ²	15 seconds	30 seconds	1 minute	2 minutes	15 seconds	30 seconds	1 minute	2 minutes
TAP WATER TREATMENT				BEEF SURFACE				FAT SURFACE			
Aerobic Plate Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	9.3 x 10 ⁵	5.4 x 10 ⁵	1.2 x 10 ⁶	9.2 x 10 ⁵	6.0 x 10 ⁵	2.8 x 10 ⁵	6.4 x 10 ⁵	4.1 x 10 ⁵
	% reduction	NA	NA	96.679	98.071	95.714	96.714	97.857	99.000	97.714	98.536
	Log ₁₀ reduction	NA	NA	1.482	1.718	1.371	1.486	1.672	2.003	1.644	1.837
Coliform* Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	5.4 x 10 ⁵	4.0 x 10 ⁵	7.2 x 10 ⁵	7.0 x 10 ⁵	3.4 x 10 ⁵	2.0 x 10 ⁵	3.1 x 10 ⁵	2.4 x 10 ⁵
	% reduction	NA	NA	98.071	98.571	97.429	97.500	98.786	99.286	98.893	99.143
	Log ₁₀ reduction	NA	NA	1.718	1.848	1.593	1.605	1.919	2.149	1.959	2.070
<i>Escherichia coli</i> Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	3.0 x 10 ⁵	3.0 x 10 ⁵	5.0 x 10 ⁵	4.3 x 10 ⁵	2.0 x 10 ⁵	1.3 x 10 ⁵	2.6 x 10 ⁵	1.8 x 10 ⁵
	% reduction	NA	NA	98.929	98.929	98.214	98.464	99.286	99.536	99.071	99.357
	Log ₁₀ reduction	NA	NA	1.973	1.973	1.751	1.817	2.149	2.336	2.035	2.195
<i>Escherichia coli</i> O157:H7 (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	8.0 x 10 ⁵	6.0 x 10 ⁵	9.4 x 10 ⁵	9.6 x 10 ⁵	4.4 x 10 ⁵	2.2 x 10 ⁵	5.0 x 10 ⁵	1.8 x 10 ⁵
	% reduction	NA	NA	97.143	97.857	96.643	96.571	98.429	99.214	98.214	99.357
	Log ₁₀ reduction	NA	NA	1.547	1.672	1.477	1.468	1.807	2.108	1.751	2.195

* Results read at 24 hours.

Table 2 cont'd: Results of Time-Kill for AquaFew Acidic Water ORP: 900+ on Sirloin Butt at Initial and Subsequent Contact Time Points. Reported in CFU/ml recovered, Percent and Log₁₀ Reduction

Assay	Units	Inoculum Count	Count/cm ²	15 seconds	30 seconds	1 minute	2 minutes	15 seconds	30 seconds	1 minute	2 minutes
AQUAFEW SPRAY TREATMENT				BEEF SURFACE				FAT SURFACE			
Aerobic Plate Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	4.9 x 10 ⁵	2.3 x 10 ⁵	1.8 x 10 ⁵	2.6 x 10 ⁵	3.0 x 10 ⁴	2.4 x 10 ⁴	2.0 x 10 ⁴	3.8 x 10 ⁴
	% reduction	NA	NA	98.250	99.179	99.357	99.071	99.893	99.914	99.929	99.864
	Log ₁₀ reduction	NA	NA	1.760	2.088	2.195	2.035	2.973	3.070	3.149	2.870
Coliform* Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	3.4 x 10 ⁵	1.6 x 10 ⁵	1.6 x 10 ⁵	2.1 x 10 ⁵	1.4 x 10 ⁴	1.7 x 10 ⁴	9.6 x 10 ³	1.7 x 10 ⁴
	% reduction	NA	NA	98.786	99.429	99.429	99.250	99.950	99.939	99.966	99.939
	Log ₁₀ reduction	NA	NA	1.919	2.246	2.246	2.128	3.304	3.220	3.468	3.220
<i>Escherichia coli</i> Count (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	2.8 x 10 ⁵	2.5 x 10 ⁵	1.5 x 10 ⁵	1.5 x 10 ⁵	1.0 x 10 ⁴	1.3 x 10 ⁴	7.1 x 10 ³	6.5 x 10 ³
	% reduction	NA	NA	99.000	99.107	99.464	99.464	99.964	99.954	99.975	99.977
	Log ₁₀ reduction	NA	NA	2.003	2.052	2.274	2.274	3.450	3.336	3.599	3.637
<i>Escherichia coli</i> O157:H7 (CFU/cm ²)	CFU/ml	2.8 x 10 ⁸	2.8 x 10 ⁷	4.8 x 10 ⁵	1.2 x 10 ⁵	1.4 x 10 ⁵	1.5 x 10 ⁵	1.3 x 10 ⁴	1.1 x 10 ⁴	1.3 x 10 ⁴	1.8 x 10 ⁴
	% reduction	NA	NA	98.286	99.571	99.500	99.464	99.954	99.961	99.954	99.936
	Log ₁₀ reduction	NA	NA	1.769	2.371	2.304	2.274	3.336	3.409	3.336	3.195

* Results read at 24 hours.

Table 2: Neutralizer Effectiveness Test Results (CFU/ml)

Gram Reaction	Challenge Organism	AquaFew Acidic Water		Control	
		0 minute contact time	30 minute contact time	0 minute contact time	30 minute contact time
Gram Negative	<i>Klebsiella pneumoniae</i> ATCC 10031	32	34	44	45

CONCLUSIONS

Based on the results presented in this study report, the challenge organisms evaluated in this Time Kill study demonstrated a minimum of 96.5% reduction in *E.coli* O157:H7 on the Top Sirloin Beef Surface and a minimum of 99.3% reduction in *E. coli* O157:H7 on the Top Sirloin fat surface and both surfaces of the Sirloin Butt within 2 minutes after exposure to AquaFew Acidic Water. All controls met the criteria established for a valid test.

APPENDIX 1

SIGNED PROTOCOL



An Evaluation of Antimicrobial Activity of One Test Product On Beef Primals

Using a Time Kill Procedure

Protocol # 09364-1B

Prepared for:

Cascade Ridge Industries, LLC (Sponsor)
PO Box 607
Quincy, Washington 98848

Ameristar Meats (Co-Sponsor)

Prepared by:

Q Laboratories, Inc. (Testing Facility)
1400 Harrison Avenue
Cincinnati, Ohio 45214
(513) 471-1300

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December 30, 2009

Protocol # 09364-1B

1.0 Title: An Evaluation of Antimicrobial Activity of One Test Product on Beef Primals Using a Time Kill Procedure

2.0 Sponsor: Cascade Ridge Industries, LLC
PO Box 607
Quincy, Washington 98848

3.0 Testing Facility: Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, OH 45214

4.0 Study Director: Erin S. Crowley

5.0 Purpose:

This study will evaluate the bactericidal properties of a product formulation by measuring the changes of a population of aerobic microorganisms on the surface of beef primal samples within a specified sampling time when tested against an antimicrobial test material provided by Cascade Ridge Industries, LLC. All testing will be performed in accordance with FDA Good Laboratory Practices, as specified in 21 CFR Part 58.

6.0 Scope:

A time kill evaluation of one (1) batch of electrolyzed water will be performed using a modification of the ASTM Committee E35.15 standard test method E2315-03 "The Assessment of Antimicrobial Activity Using a Time-Kill Procedure". The bactericidal properties of the test product versus the challenge microorganisms (listed in Section 8.0) inoculated on the surface of two types of beef primal will be tested at 15 seconds, 30 seconds, 1 minute and 2 minutes post inoculation.

7.0 Test Material:

One batch of the electrolyzed water to be evaluated will be provided to the Testing Facility by the Study Sponsor, complete with appropriate documentation. The Study Sponsor is responsible for the determination of the identity, strength, purity, composition, stability and solubility of the test product, as well as responsibility for the retention of the test product.

Test Product: AquaFew Acidic Water (pH 2.5 to 3.0)

Active Ingredient: Hypochlorous Acid

Test Matrices:

2 Beef primals both obtained from carcasses 24 hours post mortem, will be used in this evaluation and provided by Ameristar Meats.

8.0 Challenge Microorganisms:

- 8.1 *Escherichia coli* O157:H7 ATCC 43895
- 8.2 *Escherichia coli* O157:H7 ATCC 35150
- 8.3 *Escherichia coli* O157:H7 ATCC 51657
- 8.4 *Klebsiella pneumoniae* ATCC 10031

9.0 Equipment:

- 9.1 Incubators, Temperature Range $35 \pm 2^{\circ}\text{C}$ and $25 \pm 2^{\circ}\text{C}$
- 9.2 Incubator Thermometers- NIST Traceable
- 9.3 Biological Safety Cabinet
- 9.4 Steam Autoclaves
- 9.5 Vortex Mixers
- 9.6 Water Bath $47 \pm 2^{\circ}\text{C}$
- 9.7 Water Bath Thermometers, NIST Traceable
- 9.8 Refrigerator $2-8^{\circ}\text{C}$
- 9.9 Refrigerator Thermometers, NIST Traceable
- 9.10 Continuously adjustable pipettors, 20 μl – 200 μl capacity
- 9.11 Continuously adjustable pipettors, 100 μl – 1000 μl capacity
- 9.12 Calibrated, Traceable Minute/Second Timers
- 9.13 Quebec Colony Counter
- 9.14 Hand Tally
- 9.15 Electronic balance sensitive to 0.1 g.
- 9.16 Plate Turntable for spread plating
- 9.17 Hach Colorimeter
- 9.18 pH Meter

10.0 Supplies:

- 10.1 Sterile Serological Pipettes
- 10.2 Sterile 1.0 ml and 0.1 ml Micropipette tips
- 10.3 Test tubes, sterilized
- 10.4 Sterile Disposable Petri dishes, 100 x 15 mm
- 10.5 Disposable spreaders

11.0 Media/Reagents:

- 11.1 Lethen Blanks (LB)
- 11.2 Soybean-Casein Digest Media/Microbial Content Test Agar (MCT)
- 11.3 Tryptic Soy Agar (TSA)
- 11.4 Phosphate Buffer Solution (PBS)
- 11.5 Sorbitol Maconkey Agar (SMAC)
- 11.6 Tryptic Soy Agar with 5% Sheep Blood (SBA)
- 11.7 Neutralizer (Based on Sponsor's Recommendation)
- 11.8 0.85% Saline solution
- 11.9 Gram Stain Reagents
- 11.10 Petrifilm (Aerobic Plate Count and Coliform)
- 11.11 Chlorine Standards
- 11.12 Tap Water

12.0 Test Microorganism Preparation:

- 12.1 All isolates will be cultured on Tryptic Soy Agar (TSA) and incubated three (3) to five (5) days prior to testing at appropriate temperature and conditions. Daily transfers of each culture will be performed by aseptically transferring growth from each tube to a fresh agar slant. (Note: If only one daily transfer is missed, it will not be necessary to repeat the consecutive transfers).
- 12.2 A twenty-two to twenty-six hour culture of each challenge organism will be used for testing.
- 12.3 A standardized cocktail suspensions of the challenge organisms will be prepared by washing agar slants with sterile phosphate buffer solution (PBS). Organism concentration will be adjusted to a minimum target concentration of approximately 10^6 organisms per ml of test material. Inoculum suspensions may be centrifuged and reconstituted in PBS to achieve desired concentration.
- 12.4 Initial populations will be determined by preparing ten-fold dilutions of each challenge organism suspension in duplicate by standard microbiological procedures and incubated at 35°C for 48 ± 2 hrs.
- 12.5 Colonies will be counted and recorded as cfu/plate to determine organisms count. Duplicate plates will be averaged and multiplied by the dilution factor to calculate the microbial population (cfu/ml) of the control suspension.

13.0 Test Procedure:

- 13.1 The AquaFew acidic water containing Hypochlorous acid of 15-25 ppm at a pH of 2.5 to 3.0 (confirmed upon receipt) will be filled in a spray bottle and prepared in a sterile sampling dish prior to application using both the spray and dip processes.
- 13.2 A 0.1 ml aliquot of the cocktail suspension will be used to inoculate a 1" x 1" x 0.25" portion of each beef primal test portion in a sterile stomacher bag. The inoculum/beef sample will be mixed thoroughly by hand kneading to ensure and even distribution of organisms.
- 13.3 Following inoculation, two test portions (1 high fat portion and 1 lean portion) of each cut will be exposed to each of three treatments: Control (C), Tap Water (W) and Electrolyzed Water applied by spray (S).
- 13.4 The treated test portions will be samples using the swab method at 15 seconds, 30 seconds, 1 minute and 2 minute intervals and appropriate dilutions will be made in Lethen Blanks.

- 13.5 At each time point, Aerobic plate counts, coliform counts, *E. coli* counts and *E.coli* O157:H7 counts will be determined by plating appropriate dilutions will be plated onto APC and Coliform Petrifilm as well as spread-plated onto SMAC agar in duplicate and incubated at appropriate time and temperature.
- 13.5 Typical colonies will be counted and raw data recorded as cfu/plate. Duplicate plates will be averaged and multiplied by the dilution factor to arrive at cfu/ml of the test system.
- 13.6 A logarithmic transformation of the measured initial microbial population of the challenge organism and the measured surviving microbial populations will be performed. The log reduction will be calculated as follows:

\log_{10} reduction (LR) = \log_{10} (measured initial microbial population) – \log_{10} (surviving microbial population)

- 13.7 Percent reduction will be calculated as follows:

$$\text{Percent reduction (\%)} = 100 \times (1 - 10^{-LR})$$

- 13.8 A neutralization study will be conducted to demonstrate the adequacy of the neutralizer incorporated into the dilution and plating media.

14.0 Study Controls:

- 14.1 The MCT and SMAC plating media will be inoculated with an aliquot of each test organism suspension. These plates will be incubated at $35 \pm 2^{\circ}\text{C}$, 48 and 24 hours respectively and will serve as positive growth controls.
- 14.2 The acceptance criterion for these Study Controls is “growth”
- 14.3 For negative sterility controls, two tubes each of LB, PBS and TSA and three plates each of MCT and SMAC will be incubated at $35 \pm 2^{\circ}\text{C}$ for 48 hours or 24 hours respectively.
- 14.4 The acceptance criterion for these Study Controls is “negative for growth”.
- 14.5 Gram stains will be performed on representative colonies from each challenge organism for confirmation.
- 14.6 A pH and a % chlorine evaluation will be conducted on the AquaFew acidic water to verify protocol specifications.

15.0 Interpretation of Results/Acceptance Criteria:

15.1 The test will be acceptable for evaluation of the test results if a percent reduction from the initial microbial population present on the beef primal surfaces, calculated from the \log_{10} baseline counts of the surviving population is observed within the time intervals for each challenge organisms tested.

16.0 Reference:

16.1 ASTM Standard E2315-03, “Standard Guide for Assessment of Antimicrobial Activity Using a Time Kill Procedure”, ASTM International, 2003.

17.0 Final Report:

A Final Report will be prepared, including a tabularized summary of data and description of results of the study upon completion of testing.

18.0 Exceptional Conditions:

The Study Sponsor will be notified of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded.

19.0 Documentation and Record-Keeping:

All documentation and records will be compiled, analyzed, and retained by Q Laboratories, Inc. at its facility in Cincinnati, Ohio. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least seven (7) years.

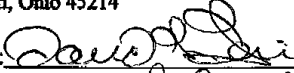
20.0 Product Disposition:

It is the responsibility of the Sponsor to retain a sample of the test substance(s) for future audit or evaluation. All unused test material will be disposed within 30 days following the study completion unless otherwise indicated by the Sponsor prior to initiation of the study.

22.0 **Acceptance:**

An Evaluation of Antimicrobial Activity of One Test Product On Beef Primals Using a Time Kill Procedure

Q Laboratories, Inc. (Testing Facility)
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Cincinnati, Ohio 45214

President: 
David G. Gains

1-7-10
Date


Study Director: 
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Date of Study Initiation

Director of Quality: 
James R. Agin

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1/6/2010
Date