Microorganism Recovery Equivalence from Cast Iron and Food Grade Stainless Steel

Final Report

December 18, 2019

Version 1

Project Identification Number

QL # 19269-2B

Test Articles

Cast Iron Cookware and Food Grade Stainless Steel Carriers

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Study Sponsor

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South Pittsburgh, TN 37380

Performing Laboratory

Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204

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Test Summary

Title: Microorganism Recovery Equivalence from Cast Iron and Food Grade Stainless Steel

Study Design: This study was designed to demonstrate that microorganisms can be removed from cast iron cookware with similar effectiveness as from stainless steel surfaces. The equivalence of recovery was demonstrated by inoculating both materials with equivalent numbers of each microorganism. For this study the following microorganisms were used: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* Enteritidis, *Listeria monocytogenes*, and *Clostridium perfringens*. Following inoculation, surfaces were sampled.

Test Articles:

The test articles evaluated were provided to the testing facility by the study sponsor, complete with appropriate documentation. Test articles were sterilized via autoclave upon receipt and stored at ambient temperature (20 - 25 °C) in autoclaved aluminum foil.

1. Cast Iron Cookware
   - 1.1 14 Ounce Round Cast Iron Mini Server (SKU: HMSRD)
   - 1.2 12 Ounce Cast Iron Mini Serving Bowl (SKU: HMSB)
   - 1.3 16 Ounce Oval Cast Iron Mini Server (SKU: HM16OS)
   - 1.4 9 Ounce Oval Cast Iron Mini Server (SKU: HMSOV)
   - 1.5 14 Ounce Rectangular Cast Iron Mini Server (SKU: HMS14RC)
   - 1.6 10 Ounce Square Cast Iron Mini Server (SKU: HMSS)
2. Food Grade Stainless Steel Carriers (18 GA 300 series, brush finish)

Sponsor: Lodge Manufacturing
204 East 5th Street
South Pittsburgh, TN 37380

Testing Conditions

Challenge Microorganisms:

1. *Staphylococcus aureus* American Type Culture Collection (ATCC) 6538
2. *Escherichia coli* ATCC 8739
3. *Salmonella* Enteritidis ATCC 13076
4. *Listeria monocytogenes* ATCC 7644
5. *Clostridium perfringens* ATCC 12915

Note: Appropriate laboratory safety conditions was employed while working with enriched culture suspensions. These conditions included, but were not limited to, the use of appropriate PPE (including disposable gloves, beard nets, hair nets, and lab coats), Biological Safety Cabinets, and protective eyewear.

Testing Conditions:

The evaluation was conducted at ambient temperature (20 - 25 °C).

Media/Reagents:

1. Tryptic Soy Agar with 5% Sheep Blood (SBA) (Fisher Scientific, PN 221261) or equivalent
2. Microbial Content Test (MCT) agar MP107
3. Tryptic Soy Broth (TSB) MP058
4. Phosphate Buffered Saline (PBS) MP416
5. Columbia Blood Agar (CBA) with 5% Sheep Blood MP086
6. Reinforced Clostridial Medium (RCM) MP158

Equipment/Supplies:

1. Incubator, temperature range 35 ± 1 °C
2. Incubator thermometer, NIST traceable
3. Sterile containers
4. Steam autoclave
5. Vortex mixer
6. Calibrated, traceable minute/second timer
7. Refrigerator, temperature range 2 - 8 °C
8. Refrigerator thermometer, NIST traceable
9. Traceable thermometer/clock/humidity monitor
10. Adjustable pipettor, 1 µL - 200 µL capacity
11. Adjustable pipettor, 100 µL - 1000 µL capacity
12. Sterile serological pipettes
13. Sterile 100 µL and 1000 µL micropipette tips
14. Reichert Quebec® Colony Counter, or equivalent
15. Hand tally

16. Test tubes, sterilized
17. Sterile disposable Petri dishes, 100 x 15 mm
18. Sterile polyurethane tip swabs
19. Sterile disposable loops
20. Rotator/shaker
21. Anaerobic Sachets, BBL GasPaks or equivalent

Study Dates and Facility

The analysis phase of this test was conducted at Q Laboratories in the Microbiology Research and Development Laboratory, 1930 Radcliff Drive, Cincinnati, Ohio 45204, from 10-28-19 to 11-11-19. The study sponsor and study director signed the protocol on 10-31-19. The final report was released 12-16-19.

Records to be Maintained

All testing data, 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, 1930 Radcliff Drive, Cincinnati, Ohio 45204 for a period of at least seven (7) years.

Test Procedure

Test Microorganism Preparation:

Staphylococcus aureus ATCC 6538, *Escherichia coli* ATCC 8739, *Salmonella* Enteritidis ATCC 13076, and *Listeria monocytogenes* ATCC 7644 were propagated on Tryptic Soy Agar with 5% Sheep Blood (SBA) from a Q Laboratories frozen stock culture stored at -70 °C. SBA plates were incubated aerobically at 35 ± 1 °C for 24 ± 2 hours. After incubation, an isolated colony was picked to Tryptic Soy Broth (TSB) and incubated at 35 ± 1 °C for 24 ± 2 hours. Test articles were inoculated with the 24 hour TSB culture.

Clostridium perfringens ATCC 12915 was propagated on SBA from a Q Laboratories frozen stock culture stored at -70 °C. The SBA plate was incubated anaerobically at 35 ± 1 °C for 24 ± 2 hours. After incubation, an isolated colony was transferred to pre-reduced Reinforced Clostridial Medium (RCM) and incubated anaerobically at 35 ± 1 °C for 24 ± 2 hours. Test articles were inoculated with the 24 hour RCM culture.

Pre-Inoculation Preparation:

The study sponsor reported that the test articles were pre-cleaned using one cycle in an industrial dishwasher prior to shipping.

Test articles and stainless-steel control carriers were placed in a sterile container and autoclaved after receipt by the testing facility. This step was done to ensure there is no residual bioburden prior to inoculation.

Using sterile gloves, the test article was placed on a disinfected flat surface. One (1) 1” x 1” location on each test article was marked for evaluation, depicted as red squares in Figures 1 - 4.

Inoculation of Test Articles:

A 100 µL aliquot of each test culture was applied to the 1” x 1” marked areas. The culture was uniformly spread over the sample area using 100 - 1000 µL micropipette tip to prevent areas of pooling.

After inoculation, the test articles were allowed to dry for 18 - 24 hours at ambient temperature (20 - 25 °C). After 18-24 hours, the test article was visually inspected to ensure the test culture suspension was uniformly dried and testing was initiated.

The inoculation steps above were repeated for the stainless-steel control carriers.

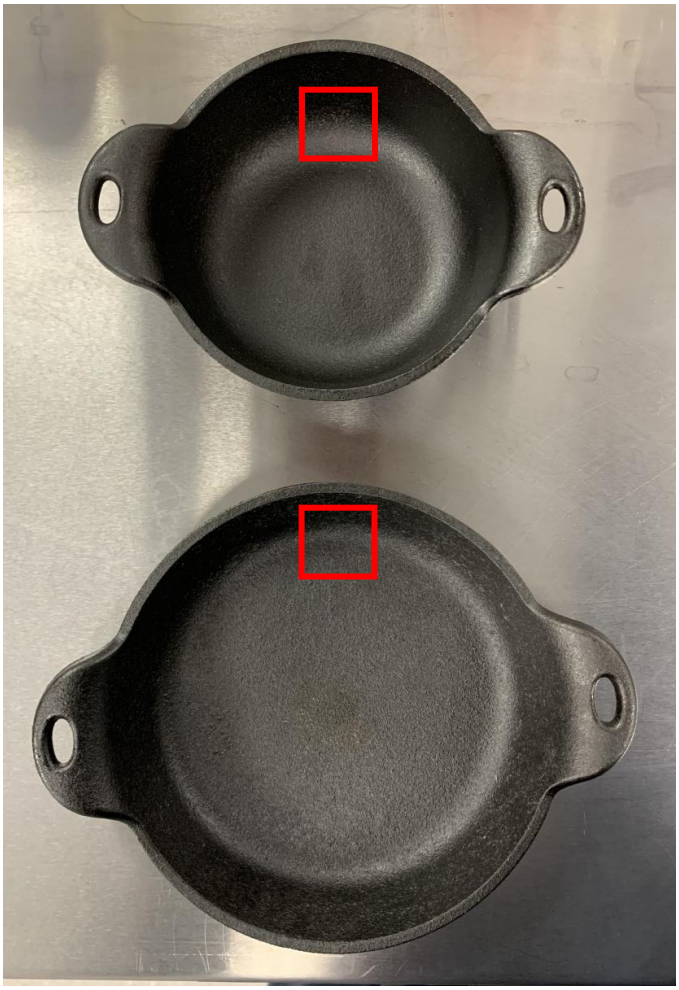

Figure 1. 12 Ounce Cast Iron Mini Serving Bowl and 14 Ounce Round Cast Iron Mini Server Sample Areas.

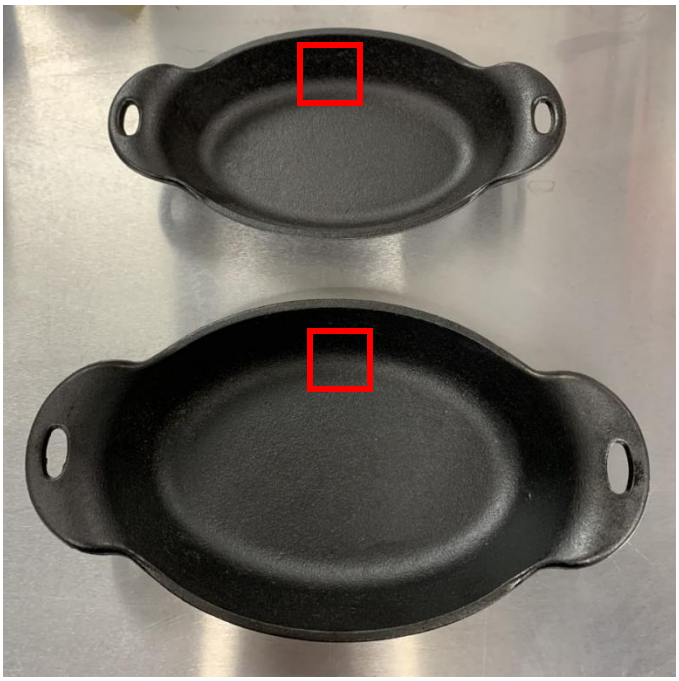

Figure 2. 9 Ounce Oval Cast Iron Mini Server and 16 Ounce Oval Cast Iron Mini Server Sample Areas.

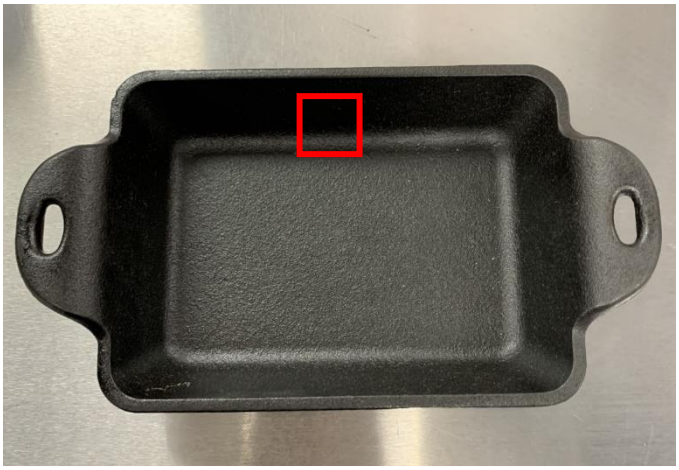

Figure 3. 14 Ounce Rectangular Cast Iron Mini Server Sample Area.

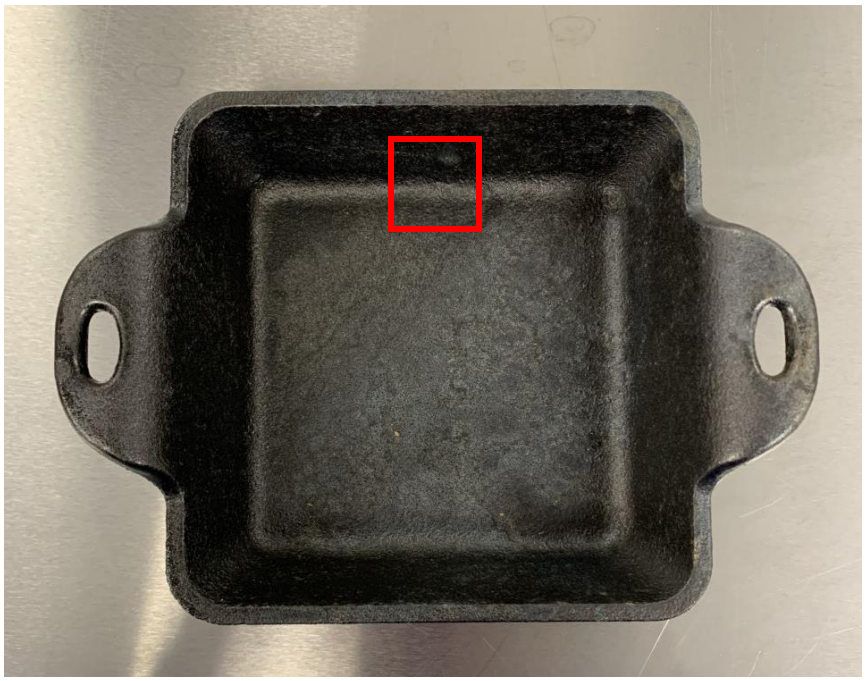

Figure 4. 10 Ounce Square Cast Iron Mini Server Sample Area.

Three (3) replicates of the test articles and three (3) replicates using food grade stainless steel carries were evaluated for each microorganism. A summary of the recovery study parameters is presented in Table 1.

Table 1. Summary of Recovery Study Parameters

Test Organisms	Test Article	No. of Test Replicates	No. of Stainless-Steel Control Replicates
S. aureus, *E. coli*, *S.* Enteritidis, *L. monocytogenes*, *C. perfringens*	14 Ounce Round Cast Iron Mini Server	3	3
	12 Ounce Cast Iron Mini Serving Bowl	3	3
	16 Ounce Oval Cast Iron Mini Server	3	3
	9 Ounce Oval Cast Iron Mini Server	3	3
	14 Ounce Rectangular Cast Iron Mini Server	3	3
	10 Ounce Square Cast Iron Mini Server	3	3

Recovery and Enumeration Procedure:

A 1.0 mL aliquot of PBS was added to a sterile swab. The marked 1" x 1" sample area was thoroughly swabbed in an up and down vertical motion and a left and right horizontal motion. This process was designed to remove viable microorganisms from the surface of the test article for enumeration.

The swab was placed in a test tube containing 9.0 mL of PBS. The swab was expressed into the test tube and thoroughly vortexed for 30 ± 5 seconds. Ten-fold serial dilutions of the sample were prepared by transferring 1.0 mL from the initial dilution into 9.0 mL of PBS.

For *S. aureus*, *E. coli*, *S.* Enteritidis and *L. monocytogenes*, each dilution was plated into duplicate sterile Petri dishes and 12 - 15 mL of tempered MCT was added. Plates were mixed thoroughly and allowed to solidify. Plates were inverted and incubated at 35 ± 1 °C for 48 ± 2 hours.

For *C. perfringens* each dilution was spread plated with sterile plating beads onto duplicate pre-poured plates of Columbia Blood Agar (CBA) with 5% Sheep Blood (CBA). Plates were inverted and incubated anaerobically at 35 ± 1 °C for 48 ± 2 hours.

After incubation, typical colonies were enumerated, and raw data was recorded as CFU/plate. Duplicate plates were averaged and multiplied by the dilution factor to arrive at CFU/test article. Raw values were recorded and used for the calculations in Tables 2-6.

Study Controls:

Food Grade Stainless Steel Controls – Three (3) 4" x 4" food grade stainless steel test articles were inoculated according to the test procedure. The recovered microorganisms were determined following the procedures found in Recovery and Enumeration. In order for the testing to be considered acceptable, the recovery data from the cast iron test articles had to be comparable to the food grade stainless steel.

Statistical Analysis

A logarithmic transformation measuring surviving microbial populations of the positive control article and test replicates for each microorganism were performed.

Equivalence of Recovery was calculated as follows:

ΔLog10 = Equivalence Recovery
TR1 = Test Article Replicate 1
TR2 = Test Article Replicate 2
TR3 = Test Article Replicate 3
SS1 = Stainless Steel 1
SS2 = Stainless Steel 2
SS3 = Stainless Steel 3

$$\left(\frac{TR1 + TR2 + TR3}{3}\right) - \left(\frac{SS1 + SS2 + SS3}{3}\right) = \Delta\text{Log10}$$

Media Quality Controls

The MCT plating media was inoculated with an aliquot of each *S. aureus, E. coli, S.* Enteritidis, and *L. monocytogenes* suspension and incubated at 35 ± 1 °C for 48 ± 2 hours. These plates served as positive growth controls for the media.

The CBA and RCM media were inoculated with an aliquot of the *C. perfringens* suspension and incubated anaerobically at 35 ± 1 °C for 48 ± 2 hours. These served as positive growth controls for the media.

The acceptance criterion for these bacterial media controls was "typical growth" of the organisms.

For negative sterility controls, two tubes each of TSB, PBS, and three plates of MCT were incubated at 35 ± 2 °C for 48 ± 2 hours.

The acceptance criterion for these uninoculated media controls was "negative for growth".

References

U. S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 3 *Aerobic Plate Count* (January 2001). (Accessed October 2019)
http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm

Summary of Results

The results of the initial microorganism recovery comparison are presented in Tables 2-6. The results of the retested test articles are presented in Tables 7-10. The mean Log values were obtained from duplicate plates. The Equivalence of Recovery was calculated as follows:

ΔLog10 = Equivalence Recovery
TR1 = Test Article Replicate 1
TR2 = Test Article Replicate 2
TR3 = Test Article Replicate 3
SS1 = Stainless Steel 1
SS2 = Stainless Steel 2
SS3 = Stainless Steel 3

$$\left(\frac{TR1 + TR2 + TR3}{3}\right) - \left(\frac{SS1 + SS2 + SS3}{3}\right) = \Delta\text{Log10}$$

Results

Table 2: *Staphylococcus aureus* ATCC 6538 Recovery Comparison
Reported in CFU/mL recovered.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
14 Ounce Round Cast Iron Mini Server	CFU/mL	2.6E+05	3.2E+05	3.8E+05	1.1E+05	2.6E+05	1.1E+05	0.3340
	Log CFU/mL	5.4150	5.5051	5.5798	5.0414	5.4150	5.0414	
12 Ounce Cast Iron Mini Serving Bowl	CFU/mL	3.3E+05	4.2E+05	2.8E+05	1.2E+05	1.2E+05	1.1E+05	0.4630
	Log CFU/mL	5.5185	5.6232	5.4472	5.0792	5.0792	5.0414	
16 Ounce Oval Cast Iron Mini Server	CFU/mL	3.0E+05	2.4E+05	2.1E+05	1.7E+05	1.5E+05	1.4E+05	0.2090
	Log CFU/mL	5.4771	5.3802	5.3222	5.2304	5.1761	5.1461	
9 Ounce Oval Cast Iron Mini Server	CFU/mL	4.2E+05	5.0E+05	1.2E+05	1.6E+05	1.1E+05	1.2E+05	0.3589
	Log CFU/mL	5.6232	5.6990	5.0792	5.2041	5.0414	5.0792	
14 Ounce Rectangular Cast Iron Mini Server	CFU/mL	4.6E+05	5.0E+05	4.9E+05	1.5E+05	8.4E+04	1.5E+05	0.5918
	Log CFU/mL	5.6628	5.6990	5.6902	5.1761	4.9243	5.1761	
10 Ounce Square Cast Iron Mini Server	CFU/mL	2.7E+05	3.0E+05	2.8E+05	7.4E+04	1.2E+05	1.3E+05	0.4311
	Log CFU/mL	5.4314	5.4771	5.4472	4.8692	5.0792	5.1139	

Table 3: *Escherichia coli* ATCC 8739 Recovery Comparison
Reported in CFU/mL recovered.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
14 Ounce Round Cast Iron Mini Server	CFU/mL	1.2E+04	1.7E+04	6.0E+03	5.0E+03	1.6E+04	5.6E+03	0.1455
	Log CFU/mL	4.0792	4.2304	3.7782	3.6990	4.2041	3.7482	
12 Ounce Cast Iron Mini Serving Bowl	CFU/mL	6.6E+03	3.0E+03	9.2E+03	4.4E+03	6.6E+03	7.0E+03	-0.0159
	Log CFU/mL	3.8195	3.4771	3.9638	3.6435	3.8195	3.8451	
16 Ounce Oval Cast Iron Mini Server	CFU/mL	5.4E+03	1.0E+04	5.8E+03	2.6E+04	2.6E+04	3.0E+04	-0.6038
	Log CFU/mL	3.7324	4.0000	3.7634	4.4150	4.4150	4.4771	
9 Ounce Oval Cast Iron Mini Server	CFU/mL	6.4E+03	8.0E+03	8.4E+03	1.7E+04	2.7E+04	3.6E+04	-0.5282
	Log CFU/mL	3.8062	3.9031	3.9243	4.2304	4.4314	4.5563	
14 Ounce Rectangular Cast Iron Mini Server	CFU/mL	4.7E+03	4.1E+03	4.2E+03	4.0E+03	5.6E+03	4.6E+03	-0.0350
	Log CFU/mL	3.6721	3.6128	3.6232	3.6021	3.7482	3.6628	
10 Ounce Square Cast Iron Mini Server	CFU/mL	5.4E+03	6.0E+03	1.0E+04	3.1E+03	9.2E+03	8.3E+03	0.0454
	Log CFU/mL	3.7324	3.7782	4.0000	3.4914	3.9638	3.9191	

Table 4: *Salmonella* Enteritidis ATCC 13076 Recovery Comparison
Reported in CFU/mL recovered.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
14 Ounce Round Cast Iron Mini Server	CFU/mL	7.0E+04	7.2E+04	3.9E+04	1.4E+04	3.6E+04	3.8E+04	0.3371
	Log CFU/mL	4.8451	4.8573	4.5911	4.1461	4.5563	4.5798	
12 Ounce Cast Iron Mini Serving Bowl	CFU/mL	2.6E+04	1.3E+04	1.4E+04	8.9E+03	5.2E+04	4.6E+04	-0.2177
	Log CFU/mL	4.4150	4.1139	4.1461	3.9494	4.7160	4.6628	
16 Ounce Oval Cast Iron Mini Server	CFU/mL	9.9E+03	8.7E+03	2.8E+04	4.6E+03	1.3E+04	8.8E+03	0.2204
	Log CFU/mL	3.9956	3.9395	4.4472	3.6628	4.1139	3.9445	
9 Ounce Oval Cast Iron Mini Server	CFU/mL	3.2E+04	4.2E+04	3.4E+04	2.8E+04	1.2E+04	1.4E+04	0.3291
	Log CFU/mL	4.5051	4.6232	4.5315	4.4472	4.0792	4.1461	
14 Ounce Rectangular Cast Iron Mini Server	CFU/mL	4.3E+04	3.4E+04	3.8E+04	1.2E+04	1.4E+04	2.7E+04	0.3627
	Log CFU/mL	4.6335	4.5315	4.5798	4.0792	4.1461	4.4314	
10 Ounce Square Cast Iron Mini Server	CFU/mL	6.3E+04	4.9E+04	5.8E+04	1.1E+04	1.7E+04	2.0E+04	0.5600
	Log CFU/mL	4.7993	4.6902	4.7634	4.0414	4.2304	4.3010	

Table 5: *Listeria monocytogenes* ATCC 7644 Recovery Comparison
Reported in CFU/mL recovered.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
14 Ounce Round Cast Iron Mini Server	CFU/mL	1.1E+04	5.6E+03	1.6E+04	4.6E+03	1.3E+04	6.8E+03	0.1282
	Log CFU/mL	4.0414	3.7482	4.2041	3.6628	4.1139	3.8325	
12 Ounce Cast Iron Mini Serving Bowl	CFU/mL	1.5E+04	5.8E+03	1.0E+03	6.3E+03	1.0E+03	6.4E+03	0.1133
	Log CFU/mL	4.1761	3.7634	3.0000	3.7993	3.0000	3.8062	
16 Ounce Oval Cast Iron Mini Server	CFU/mL	1.1E+04	8.2E+03	1.3E+04	7.0E+02	3.2E+03	2.6E+03	0.7680
	Log CFU/mL	4.0414	3.9138	4.1139	2.8451	3.5051	3.4150	
9 Ounce Oval Cast Iron Mini Server	CFU/mL	2.6E+04	3.0E+04	2.6E+04	1.2E+03	2.1E+03	3.4E+03	1.1247
	Log CFU/mL	4.4150	4.4771	4.4150	3.0792	3.3222	3.5315	
14 Ounce Rectangular Cast Iron Mini Server	CFU/mL	1.2E+04	5.4E+03	5.5E+03	3.8E+03	3.0E+03	2.8E+03	0.3493
	Log CFU/mL	4.0792	3.7324	3.7404	3.5798	3.4771	3.4472	
10 Ounce Square Cast Iron Mini Server	CFU/mL	2.4E+03	1.7E+03	4.3E+03	1.8E+03	9.6E+02	1.7E+03	0.2587
	Log CFU/mL	3.3802	3.2304	3.6335	3.2553	2.9823	3.2304	

Table 6: *Clostridium perfringens* ATCC 12915 Recovery Comparison
Reported in CFU/mL recovered.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
14 Ounce Round Cast Iron Mini Server	CFU/mL	2.3E+05	2.7E+05	3.9E+05	1.0E+05	1.3E+05	1.6E+05	0.3554
	Log CFU/mL	5.3617	5.4314	5.5911	5.000	5.1139	5.2041	
12 Ounce Cast Iron Mini Serving Bowl	CFU/mL	2.9E+05	4.5E+04	2.9E+05	9.0E+04	1.0E+05	1.2E+05	0.1815
	Log CFU/mL	5.4624	4.6532	5.4624	4.9542	5.0000	5.0792	
16 Ounce Oval Cast Iron Mini Server	CFU/mL	2.5E+05	2.7E+05	1.5E+05	1.6E+05	1.9E+05	1.6E+05	0.1061
	Log CFU/mL	5.3979	5.4314	5.1761	5.2041	5.2788	5.2041	
9 Ounce Oval Cast Iron Mini Server	CFU/mL	3.7E+05	4.7E+05	2.6E+05	1.1E+05	1.8E+05	1.7E+05	0.3761
	Log CFU/mL	5.5682	5.6721	5.4150	5.0414	5.2553	5.2304	
14 Ounce Rectangular Cast Iron Mini Server	CFU/mL	5.2E+05	3.8E+05	3.9E+05	1.7E+05	1.0E+05	2.6E+05	0.4138
	Log CFU/mL	5.7160	5.5798	5.5911	5.2304	5.0000	5.4150	
10 Ounce Square Cast Iron Mini Server	CFU/mL	1.9E+05	3.2E+05	2.6E+05	1.1E+05	8.0E+04	1.9E+05	0.3252
	Log CFU/mL	5.2788	5.5051	5.4150	5.0414	4.9031	5.2788	

Table 7: *Staphylococcus aureus* ATCC 6538 Recovery Comparison
Reported in CFU/mL recovered – Retested.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
14 Ounce Rectangular Cast Iron Mini Server	CFU/mL	2.1E+05	2.9E+05	4.5E+05	5.3E+05	4.1E+05	3.9E+05	-0.1635
	Log CFU/mL	5.3222	5.4624	5.6532	5.7243	5.6128	5.5911	

Table 8: *Escherichia coli* ATCC 8739 Recovery Comparison
Reported in CFU/mL recovered - Retested.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
16 Ounce Oval Cast Iron Mini Server	CFU/mL	1.3E+04	1.8E+04	2.4E+04	1.2E+04	3.4E+04	2.6E+04	-0.0921
	Log CFU/mL	4.1139	4.2553	4.3802	4.0792	4.5315	4.4150	
9 Ounce Oval Cast Iron Mini Server	CFU/mL	1.5E+04	2.3E+04	2.7E+04	3.3E+04	2.9E+04	2.4E+04	-0.1306
	Log CFU/mL	4.1761	4.3617	4.4314	4.5185	4.4624	4.3802	

Table 9: *Salmonella* Enteritidis ATCC 13076 Recovery Comparison
Reported in CFU/mL recovered - Retested.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
10 Ounce Square Cast Iron Mini Server	CFU/mL	5.5E+04	3.2E+04	6.2E+04	2.2E+04	2.5E+04	3.4E+04	0.2554
	Log CFU/mL	4.7404	4.5051	4.7924	4.3424	4.3979	4.5315	

Table 10: *Listeria monocytogenes* ATCC 7644 Recovery Comparison
Reported in CFU/mL recovered - Retested.

Test Article	Units	Cast Iron Replicate A	Cast Iron Replicate B	Cast Iron Replicate C	Stainless Steel Control A	Stainless Steel Control B	Stainless Steel Control C	Equivalence Recovery (ΔLog10)
16 Ounce Oval Cast Iron Mini Server	CFU/mL	1.8E+04	2.6E+04	1.1E+04	3.4E+04	2.3E+04	3.8E+04	-0.2538
	Log CFU/mL	4.2553	4.4150	4.0414	4.5315	4.3617	4.5798	
9 Ounce Oval Cast Iron Mini Server	CFU/mL	2.8E+04	3.9E+04	1.7E+04	2.0E+04	1.4E+04	4.5E+05	-0.2772
	Log CFU/mL	4.4472	4.5911	4.2304	4.3010	4.1461	5.6532	

Conclusion

Based on the results presented in this study report, the microorganism recovery equivalence from cast iron products and food grade stainless met the performance criteria for 2 of the 6 test articles. The performance criteria states that for equivalent recovery, the cast iron test articles must be within 0.5 Log of the stainless-steel carrier controls. Both 14 Ounce Round Cast Iron Mini Server and 12 Ounce Cast Iron Mini Serving Bowl met the performance criteria for each inoculum. The 9 Ounce Oval Cast Iron and 16 Ounce Oval Cast Iron did not meet the performance criteria for *Listeria monocytogenes,* and *Escherichia coli.* The 14 Ounce Rectangle Cast Iron Mini Server did not meet the performance criteria for *Staphylococcus aureus.* The 10 Ounce Square Cast Iron Mini server did not meet the performance criteria for *Salmonella* Enteritidis.

Since failure to meet the performance criteria could have been caused by variable inoculum levels due to homogenization of the test culture or by variable die off rate during the overnight drying, any test articles that did not meet the performance criteria were retested. Upon retesting all test articles met the performance criteria. The performance criteria states that for equivalent recovery, the cast iron test articles must be within 0.5 Log of the stainless-steel carrier controls.

Appendix 1

Signed Protocol

Microorganism Recovery Equivalence from Cast Iron and Food Grade Stainless Steel

Protocol # QL19269-2B

Version 2

Prepared for:

Lodge Manufacturing (Study Sponsor)
204 East 5th Street
South Pittsburgh, TN 37380

Prepared by:

Q Laboratories (Testing Facility)
1930 Radcliff Drive
Cincinnati, OH 45204
(513) 471-1300

Table of Contents

1.0 **Title:** Microorganism Recovery Equivalence from Cast Iron and Food Grade Stainless Steel

2.0 **Sponsor:** Lodge Manufacturing
204 East 5th Street
South Pittsburgh, TN 37380

3.0 **Testing Facility:** Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204

4.0 **Study Director:** Benjamin J. Bastin

5.0 **Purpose:**

This study is designed to demonstrate that microorganisms can be removed from cast iron cookware with similar effectiveness as from stainless steel surfaces.

6.0 **Scope:**

The equivalence of recovery will be demonstrated by inoculating both materials with equivalent numbers of each microorganism. For this study the following microorganisms will be used: *Staphylococcus aureus, Escherichia coli, Salmonella* Enteritidis, *Listeria monocytogenes,* and *Clostridium perfringens*. Following inoculation, surfaces will be sampled.

7.0 **Test Articles:**

The test articles to be evaluated will be provided to the testing facility by the study sponsor, complete with appropriate documentation. Test articles will be sterilized via autoclave upon receipt.

7.1 Cast Iron Cookware
- 7.1.1 14 Ounce Round Cast Iron Mini Server (SKU: HMSRD)
- 7.1.2 12 Ounce Cast Iron Mini Serving Bowl (SKU: HMSB)
- 7.1.3 16 Ounce Oval Cast Iron Mini Server (SKU: HM16OS)
- 7.1.4 9 Ounce Oval Cast Iron Mini Server (SKU: HMSOV)
- 7.1.5 14 Ounce Rectangular Cast Iron Mini Server (SKU: HMS14RC)
- 7.1.6 10 Ounce Square Cast Iron Mini Server (SKU: HMSS)

7.2 Food Grade Stainless Steel Carriers (18 GA 300 series, brush finish)

8.0 **Testing Conditions:**

8.1 The evaluation will be conducted at ambient temperature (20 - 25 °C).

9.0 Test Microorganisms:

9.1 *Staphylococcus aureus* American Type Culture Collection (ATCC) 6538
9.2 *Escherichia coli* ATCC 8739
9.3 *Salmonella* Enteritidis ATCC 13076
9.4 *Listeria monocytogenes* ATCC 7644
9.5 *Clostridium perfringens* ATCC 12915

Note: Appropriate laboratory safety conditions will be employed while working with enriched culture suspensions. These conditions will include, but are not limited to, the use of appropriate PPE (including disposable gloves, beard nets, hair nets, and lab coats), Biological Safety Cabinets, and protective eyewear.

10.0 Media/Reagents:

10.1 Tryptic Soy Agar with 5% Sheep Blood (SBA) Commercially available from BD 221261 or equivalent
10.2 Microbial Content Test (MCT) agar MP107
10.3 Tryptic Soy Broth (TSB) MP058
10.4 Phosphate Buffered Saline (PBS) MP416
10.5 Columbia Blood Agar (CBA) with 5% Sheep Blood MP086
10.6 Reinforced Clostridial Medium (RCM) MP158

11.0 Equipment/Supplies:

11.1 Incubator, temperature range 35 ± 1 °C
11.2 Incubator thermometers, NIST traceable
11.3 Sterile containers
11.4 Steam autoclave
11.5 Vortex mixer
11.6 Calibrated, traceable minute/second timer
11.7 Refrigerator, temperature range 2 - 8 °C
11.8 Refrigerator thermometer, NIST traceable
11.9 Traceable thermometer/clock/humidity monitor
11.10 Adjustable pipettor, 1 µL - 200 µL capacity
11.11 Adjustable pipettor, 100 µL - 1000 µL capacity
11.12 Sterile serological pipettes
11.13 Sterile 100 µL and 1000 µL micropipette tips
11.14 Reichert Quebec® Colony Counter, or equivalent
11.15 Hand tally
11.16 Test tubes, sterilized
11.17 Sterile disposable Petri dishes, 100 x 15 mm
11.18 Sterile polyurethane tip swabs
11.19 Sterile disposable loops
11.20 Rotator/shaker
11.21 Anaerobic Sachets, BBL GasPaks or equivalent

12.0 Test Microorganism Preparation:

12.1 *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Salmonella* Enteritidis ATCC 13076, and *Listeria monocytogenes* ATCC 7644 will be propagated on Tryptic Soy Agar with 5% Sheep Blood (SBA) from a Q Laboratories frozen stock culture stored at -70 °C. SBA plates will be incubated aerobically at 35 ± 1 °C for 24 ± 2 hours. After incubation, an isolated colony will be picked to Tryptic Soy Broth (TSB) and incubated at 35 ± 1 °C for 24 ± 2 hours.

12.2 *Clostridium perfringens* ATCC 12915 will be propagated on SBA from a Q Laboratories frozen stock culture stored at -70 °C. The SBA plate will be incubated anaerobically at 35 ± 1 °C for 24 ± 2 hours. After incubation, an isolated colony will be transferred to pre-reduced Reinforced Clostridial Medium (RCM) and incubated anaerobically at 35 ± 1 °C for 24 ± 2 hours.

13.0 Microorganism Recovery Study Parameters:

13.1 Three (3) total replicates of the test articles will be evaluated for each microorganism. A summary of the recovery study parameters is presented in Table 1.

13.2 Three (3) total replicates using food grade stainless steel carries will be evaluated for each microorganism as controls. A summary of the antimicrobial activity study parameters is presented in Table 1.

Table 1. Summary of Recovery Study Parameters.

Test Organisms	Test Article	No. of Test Replicates	No. of Stainless-Steel Control Replicates
S. aureus, *E. coli*, *S.* Enteritidis, *L. monocytogenes*, *C. perfringens*	14 Ounce Round Cast Iron Mini Server	3	3
	12 Ounce Cast Iron Mini Serving Bowl	3	3
	16 Ounce Oval Cast Iron Mini Server	3	3
	9 Ounce Oval Cast Iron Mini Server	3	3
	14 Ounce Rectangular Cast Iron Mini Server	3	3
	10 Ounce Square Cast Iron Mini Server	3	3

14.0 Test Procedure:

Preconditioning:

14.1 The study sponsor reported that the test articles were pre-cleaned using one cycle in an industrial dishwasher prior to shipping.

14.2 Test articles and stainless-steel control carriers will be placed in a sterile container and autoclaved after receipt by the testing facility. This step will be done to ensure there is no residual bioburden prior to inoculation.

Inoculation:

14.3 Using sterile gloves, place the test article on a disinfected flat surface. One (1) 1" x 1" location on the test article will be marked for evaluation as depicted in Figures 1 - 4.

14.4 Apply 100 µL of each test culture to the 1" x 1" marked areas. The culture will be uniformly spread over the sample area using 100 - 1000 µL micropipette tip to prevent areas of pooling.

14.5 After inoculation, the test articles will be allowed to dry for 18 - 24 hours at ambient temperature (20 - 25 °C). After 18-24 hours, the test article will be visually inspected to ensure the test culture suspension is uniformly dried and testing will be initiated.

14.6 Repeat inoculation steps 14.2 to 14.4 for the stainless-steel control carriers.

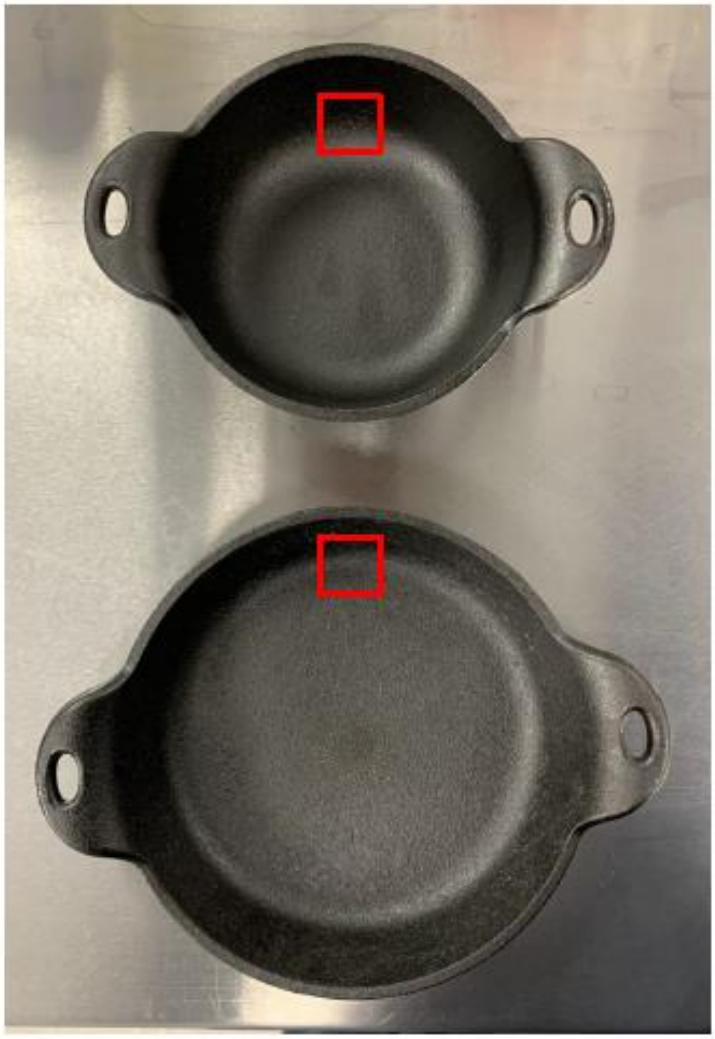

Figure 1. 12 Ounce Cast Iron Mini Serving Bowl and 14 Ounce Round Cast Iron Mini Server Sample Areas.

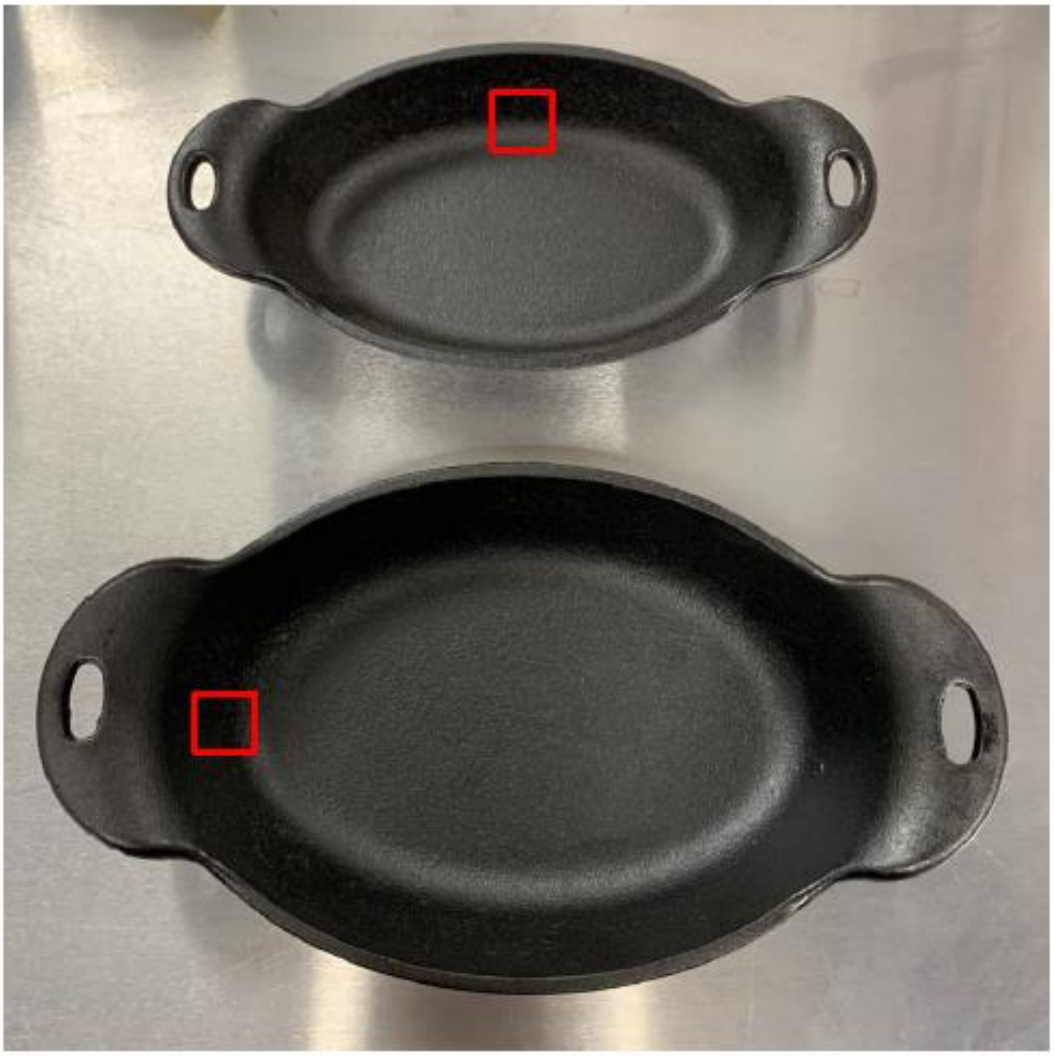

Figure 2. 9 Ounce Oval Cast Iron Mini Server and 16 Ounce Oval Cast Iron Mini Server Sample Areas.

Figure 3. 14 Ounce Rectangular Cast Iron Mini Server Sample Area.

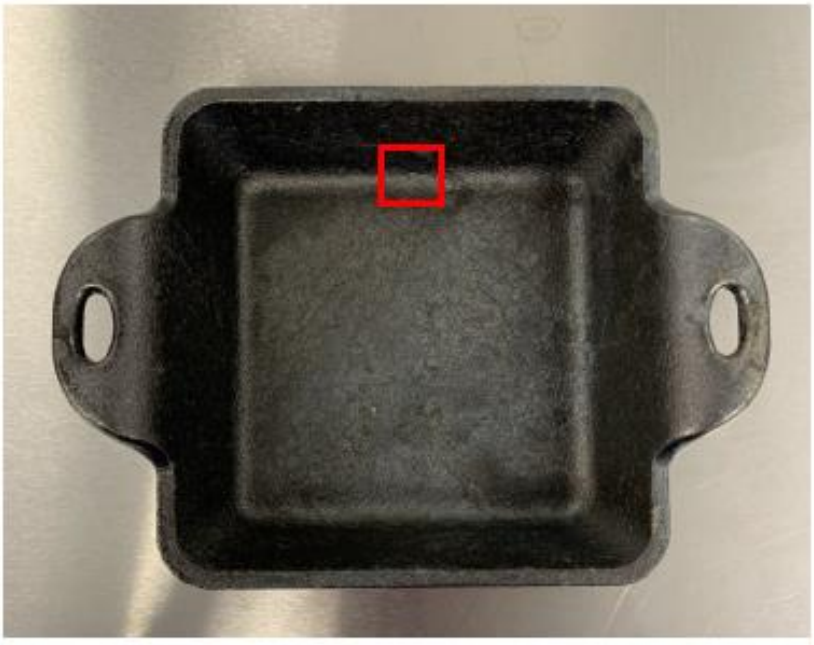

Figure 4. 10 Ounce Square Cast Iron Mini Server Sample Area.

15.0 Recovery and Enumeration Procedure:

15.1 Add 1.0 mL of PBS to a sterile swab. Thoroughly swab the 1" x 1" sample area in a both an up and down vertical motion and in a left and right horizontal motion. This process is designed to remove viable microorganisms from the surface of the test article for enumeration.

15.2 Place the swab in a test tube containing 9.0 mL of PBS. Express the swab into the test tube and thoroughly vortex. Prepare ten-fold serial dilutions of the sample by transferring 1.0 mL from the initial dilution into 9.0 mL of PBS.

15.3 For *S. aureus, E. coli, S.* Enteritidis and *L. monocytogenes*, plate each dilution into duplicate sterile Petri dishes and add 12 - 15 mL of tempered MCT to the Petri dishes. Mix thoroughly and allow the plates to solidify. Invert plates and incubate at 35 ± 1 °C for 48 ± 2 hours.

15.4 For *C. perfringens* spread plate each dilution on duplicate pre-poured plates of CBA. Spread inoculum with a sterile L-shaped spreader or sterile plating beads. Invert plates and incubate anaerobically at 35 ± 1 °C for 48 ± 2 hours.

15.5 After incubation, typical colonies will be enumerated and raw data recorded as CFU/plate. Duplicate plates will be averaged and multiplied by the dilution factor to arrive at CFU/test article. Raw values will be recorded and used for the calculations in section 18.0.

16.0 Study Controls:

16.1 Food Grade Stainless Steel Controls – Three (3) 4" x 4" food grade stainless steel test articles will be inoculated according to the procedures outlined in Section 14.0. The recovered microorganisms will be determined following the procedures in Section 15.0. In order for the testing to be considered acceptable, recovery data comparable to the cast iron test articles much be achieved.

17.0 Statistical Analysis:

17.1 A logarithmic transformation measuring surviving microbial populations of the positive control article and test replicates for each microorganism will be performed.

17.2 Equivalence of Recovery will be calculated as follows:
ΔLog10 = Equivalence Recovery
TR1 = Test Article Replicate 1
TR2 = Test Article Replicate 2
TR3 = Test Article Replicate 3
SS1 = Stainless Steel 1
SS2 = Stainless Steel 2
SS3 = Stainless Steel 3

$$\left(\frac{TR1 + TR2 + TR3}{3}\right) - \left(\frac{SS1 + SS2 + SS3}{3}\right) = \Delta\text{Log10}$$

18.0 Media Quality Controls:

18.1 The MCT plating media will be inoculated with an aliquot of each *S. aureus, E. coli, S.* Enteritidis, and *L. monocytogenes* suspension. The MCT plates will be incubated at 35 ± 1 °C for 48 ± 2 hours. These plates will serve as positive growth controls for the media.

18.2 The CBA and RCM media will be inoculated with an aliquot of the *C. perfringens* suspension. The CBA and RCM will be incubated anaerobically at 35 ± 1 °C for 48 ± 2 hours. These will serve as positive growth controls for the media.

18.3 The acceptance criterion for these bacterial media controls is "typical growth" of the organisms.

18.4 For negative sterility controls, two tubes each of TSB, PBS, and three plates of MCT will be incubated at 35 ± 2 °C for 48 ± 2 hours.

The acceptance criterion for these uninoculated media controls is "negative for growth".

19.0 Performance Criteria:

19.1 In order to demonstrate equivalent recovery the cast iron test articles must be within 0.5 Log of the stainless-steel carrier controls.

20.0 Acceptance Criteria:

20.1 The study controls below must perform according to the criteria detailed for the data to be considered acceptable.

20.1.1 Comparable growth acceptance will be within 50 - 200 % between the media. Sterility acceptance is no growth.

20.1.2 The acceptance criteria are growth from inoculated streaks and no growth from the sterility controls.

21.0 References:

21.1 U. S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 3 *Aerobic Plate Count (*January 2001). (Accessed October 2019) http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm

22.0 Final Report:

A final validation report will be prepared upon completion of the study, including a tabularized summary of data and a description of results of the study.

23.0 Documentation and Record-Keeping:

All documentation and records will be compiled, analyzed, and retained by Q Laboratories at its facility in Cincinnati, Ohio. All raw data for this study, as well as the final report, will be sent to the study sponsor and retained in safe storage by the testing facility for a period of at least seven (7) years (20 –ADMN-ISO-008D, Control of Records).

24.0 Quality Compliance:

Q Laboratories has developed and implemented a quality management system that enhances our ability to provide testing services that consistently meet client expectations and regulatory requirements. Q Laboratories quality documentation requirements are defined by ISO 17025, FDA Quality System Regulations (QSR), FDA Current Good Manufacturing Practices (cGMPs), FDA Good Laboratory Practices (GLP), and EPA Good Laboratory Practices standards (GLPs).
Q Laboratories applies the following standards as applicable:

- ISO 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories
- FDA 21 CFR Part 820 Quality System Regulation
- FDA 21 CFR Part 58 Good Laboratory Practice for Non Clinical Laboratory Studies
- FDA 21 CFR Part 211 Current Good Manufacturing Practice for Finished Pharmaceuticals
- FDA 21 CFR Part 210 Current Good Manufacturing Practice in Manufacturing Processing, Packing or Holding of Drugs; General
- EPA 40 CFR Part 160 FIFRA Good Laboratory Practice Standards

25.0 Protocol Modifications:

During the testing phase, changes to the protocol may be required. The study sponsor will be notified immediately of any modifications to the protocol. Approval of the modifications is required before any additional analysis is conducted. The modifications will be added to the protocol as an amendment and approved by both the study director and study sponsor.

26.0 Test Article Disposition:

All unused test material will be offered for return to the Study Sponsor at expense of Study Sponsor. If not desired by Study Sponsor, all unused test material to be disposed of within 90 days following the study completion.

27.0 Acceptance of Study Protocol:

Microorganism Recovery Equivalence from Cast Iron and Food Grade Stainless Steel

Q Laboratories (Testing Facility)
1930 Radcliff Drive
Cincinnati, OH 45204

Laboratory Supervisor: _____ 10/31/19
Benjamin J. Bastin — Date
Microbiology R&D Laboratory Supervisor

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204 East 5th Street
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David Fletcher — 10/31/19
Representative — Date

QA Supervisor
Title