



Haley & Aldrich, Inc.
70 Blanchard Road
Suite 204
Burlington, MA 01803
617.886.7400

19 November 2015
File No. 41741

Corrugated Packaging Alliance
500 Park Blvd., Suite 985
Itasca, IL 60143

Attention: Mr. Dennis Colley
Executive Director

Subject: Effectiveness of the Time and Temperature Profile of Corrugation to Eliminate Microbial Loads

Dear Mr. Colley:

As corrugated containers are commonly used to store and transport fresh produce from farm to table, the Corrugated Packaging Association's (CPA) member companies have historically monitored the microbial cleanliness of corrugated containers for many years. However, due to recent information citing the potential of reusable plastic containers (RPCs) to harbor excessive microbial loads, the CPA sponsored the current study by NSF International (NSF) to verify the microbial cleanliness of corrugated containers. The results of the CPA sponsored study are summarized in this report, which details the effects of the time and temperature profile of corrugation and the corresponding 5-log reduction of thermotolerant microorganisms on corrugated coupons exposed to the profile, effectively sanitizing the material.¹

Background Information

CORRUGATED DATA

The corrugated industry has historically evaluated the microbial cleanliness of corrugated containers via multiple pathways. Each of these efforts detailed below has provided information that supports the industries' position on the microbial cleanliness of corrugated containers.

- The High temperature short time (HTST), and Higher heat short time (HHST) curves, commonly used by the dairy industry to assess temperatures that would result in the destruction of pathogens were reviewed against the time/temperature profile of a typical corrugation process.

In a typical corrugation process, the containerboard attains a temperature of 190°F +/- 10 °F for approximately 8-9 seconds; HHST and HTST curves indicate that a temperature of 191°F for 1.0 second will result in a 99.999% (5-log) reduction (IDFA, 2014). Taking into consideration

¹ Sanitization as defined by the U.S. Environmental Protection Agency (USEPA) requires a 5-log reduction of organisms after the application of a sanitizer under standardized laboratory conditions.

differences between the dairy matrix and the corrugated material, the time/temperature profile of the corrugation process was assessed to be sufficient to effectively eliminate microbial contamination (Sanders, 2011).²

- The establishment of routine microbial testing of finished products to confirm microbial cleanliness. Routine testing of the finished product for overall aerobic organisms as well as pathogenic microbes verified that the microbial loads present on the corrugated containers were below those considered by industry experts to be acceptable, even after storage at the production facility for up to two months (Sanders, 2014a).

As no specific regulatory limits are available for containers used for food transport, the number of microorganisms detected was evaluated against those quoted by Dr. Keith Warriner of the University of Guelph to evaluate the cleanliness of containers used in the transport of fresh produce. Per Dr. Warriner, acceptable levels of organisms include up to 10,000 total organisms/container and no more than 1,000 pathogenic indicator organisms/container (Warriner, 2013). These levels are consistent with European regulatory guidelines (New South Wales Food Authority, 2013; European Commission, 2011).

- The performance of an industry wide field test of corrugated containers to assess the microbial cleanliness of the containers at the distribution facilities.

The field testing, following a protocol established by Dr. Trevor Suslow of the University of California - Davis, included sampling of over 360 different containers from 12 unique shipments and multiple corrugated manufacturers at five customer locations in three states, showed that all containers evaluated in the field study met the sanitation standards defined by Dr. Keith Warriner. Specifically, all containers sampled had microbial loads of less than 10 microorganisms/container (Sanders, 2015a).

FOOD-BORNE ILLNESS AND PACKAGING

The Centers for Disease Control and Prevention (CDC) has indicated that fresh produce is a potential source of contamination leading to food-borne illness (CDC, 2015). In fact, produce has been estimated to have contributed 46% of domestically-acquired illnesses and 23% of the deaths between 1998 and 2008 (Painter et al, 2013). Despite the fact that there is no documented evidence for transport containers to be the source of food-borne illness, there is evidence that microorganism loads can reach over 10,000,000 organisms per container and that the transfer of organisms from containers to fresh produce can occur (Sanders, 2014b; Danyluk, 2012). Based on these findings, as well as observations showing dirty, wet RPCs arriving for use at the field, confidence that shipping and transport containers will not serve to contribute to potential microbial loads has been somewhat eroded. The potential for RPCs to harbor significant levels of microorganisms has recently been detailed in the press (Zuraw, 2015; Williams, 2015; Andrews, 2014). Multiple field studies conducted to determine the potential microbial loads on RPCs showed that up to 49% of those containers failed to meet the identified sanitation standards (Warriner, 2013; Warriner, 2014; Sanders, 2015a). Further, bench scale testing conducted to evaluate the ability of organisms to establish biofilms, which resist sanitization by common antimicrobial substances used by the industry, showed that organisms can readily adhere to the RPC surfaces and that

² The HHST/HTST curves were evaluated taking into consideration not only the published HHST/HTST values, but also the difference between the corrugation medium and dairy products where it is commonly used.

those organisms are not readily removed (Clayborn, 2015, Sanders, 2015b). Understanding the potential for produce shipping containers to harbor microorganisms is critical for growers, distributors retailers and food service companies as they try to meet the intent of the U.S. Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA), which focuses on preventing potential food safety risks rather than reacting to issues after they occur (FDA, undated).

Specifically, the microbial hazards and the potential for cross-contamination associated with inanimate objects, including totes and bins have been recognized by the U.S. Food and Drug Administration (FDA, 1998). Internationally, a United Nations Food and Agriculture Organization technical document, *Management of reusable plastic crates in fresh produce supply chains* (Rapusas and Rolle, 2009) highlights the need for special attention on transport containers to contribute to product decay or spoilage, and/or human foodborne illness. These recent regulatory actions serve to elevate grower, shipper, and affiliated industries awareness of the need for science-based programs to manage these risks.

Study Goal/Purpose

The study as summarized in this report was conducted to confirm that the time/temperature profile of a typical corrugation process is sufficient to mitigate microbial contamination and effectively sanitize the coupons. To test the hypothesis, an organism inoculum was applied to corrugated container board and heated; the level of organisms before and after heating was assessed and the log reduction was calculated. If the testing resulted in a 5-log reduction in organisms, the test result was deemed acceptable.

A time/temperature profile consistent with that found in a typical corrugating manufacturing process, where container board top liners reach temperatures of 190°F +/- 10 °F for 8-9 seconds, was employed in the study (O'Banion, 2015). This top liner typically represents the food contact surface of the container.

Testing was established to replicate the corrugation process from the single-facer through the hot plates, excluding the bridge. In the manufacturing process, the top liner is joined with the medium at the single-facer at a temperature of approximately 200°F though a web distance of 22 feet. The single-face board accumulates on the bridge before being combined with the bottom liner at the double-backer at a top liner temperature between 190 – 207°F. The web length through the double-backer is 24 feet. The combined board then travels 66 feet through the hot plate section where the top liner temperature reaches between 190-200°F. The exposure time of 8.4 seconds is calculated using an average run speed of 800 fpm over a distance of 112 feet (22+24+66). To mimic the corrugation profile, the laboratory sandwiched the corrugated coupons between two, 1" thick aluminum plates pre-heated to 215°C for 22 seconds. Under these conditions, the liner board reached the desired temperatures (180 - 200°F) for 9 seconds (See Figure 1).

To evaluate the effects of the corrugation process on the viability of microorganisms, organisms were selected that may be found on the fresh produce, are recognized human pathogens that have resulted in illness attributed to fresh produce, or have been assessed to be equally or more thermotolerant than such organisms. Based on these criteria, a cocktail of *Escherichia coli* (ATCC 25922), two strains of *Escherichia coli* (O157:H7) (ATCC 51657 & ATCC 43890), and *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* (ATCC 13076) was used to inoculate the corrugated material in the study. The organisms were

chosen based on either their status as a human pathogen or due to the fact they have been identified as surrogates for certain thermotolerant pathogens.³

The microbial reductions attained following exposure of the inoculated corrugated material to the time/temperature requirements of the corrugation process were then compared to EPA requirements for chemical sanitizers (5-log reduction) to confirm that the process would be sufficient to mitigate the presence of pathogenic organisms on corrugated containers.

Test Protocol

A brief synopsis of the procedure used by NSF to evaluate the temperature of the corrugation process for its ability to mitigate the presence of microorganisms is included herein. For more details on the protocol, please see Appendix 1 (Corrugator Effect on Microbial Contamination, 2015).

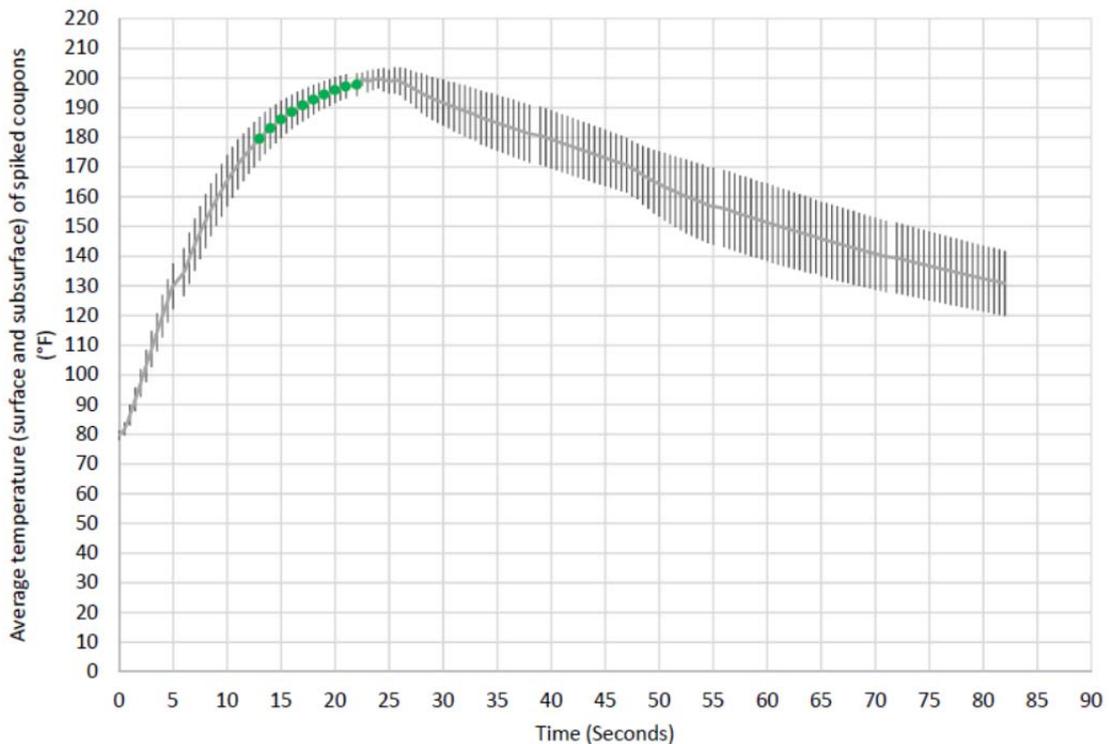
1. NSF International received two lots of corrugated material from two different corrugated manufacturers for testing. The corrugated material was, upon receipt, cut into 4" square sections (coupons) for the testing. 22 coupons/lot were assigned to 1 of 3 groups as follows:
 - 2 coupons/lot (Blanks);
 - 10 unheated coupons/lot; and
 - 10 heated coupons/lot
2. Coupon blanks were evaluated to confirm that the pretest sanitization protocol (UV radiation) was sufficient to generate a consistent baseline showing the absence of organisms on the coupons.
3. 0.5 mL of a cocktail containing four different organisms was spread across the surface of both the heated and unheated coupon subsets. The organisms included in the cocktail include *Escherichia coli* (ATCC 25922), two strains of *Escherichia coli* (O157:H7) (ATCC 51657 & ATCC 43890), and *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* (ATCC 13076). The organisms were chosen based on either their status as a human pathogen or due to the fact they have been identified as surrogates for certain thermotolerant pathogens.⁴
4. The number of organisms present in the cocktail was established so that the final level of recoverable organisms from unheated coupons would meet or exceed a 5-log/milliliter (mL). After inoculation, each coupon was allowed to air dry for approximately 10 minutes before processing.
 - a. Coupons designated for "heating" were placed between two, 1" thick aluminum plates for 22 seconds allowing the top liner of the board to reach temperatures between 180° and 200 °F for 9 seconds. To confirm the temperature profile of the coupons, thermocouples were

³ The two *E. coli* (O157:H7) strains and the *Salmonella* strain used are known human pathogens, the other *E. coli* strain used was used as a surrogate to model the heat resistance of the *Salmonella* Montevideo and Poona (Eblen, 2005).

⁴ The two *E. coli* (O157:H7) strains and the *Salmonella* strain used are known human pathogens, the other *E. coli* strain used was used as a surrogate to model the heat resistance of the *Salmonella* Montevideo and Poona (Eblen, 2005).

placed in contact with the surface and subsurface of the inoculated liner. Figure 1 represents the time/temperature curve employed in the study.

Figure 1: Corrugated Coupon Time/Temperature Curve



- b. After heating, each coupon was placed into 100 mL of Letheen broth within 1 minute for processing.
- c. Concurrently, a paired unheated coupon was processed alongside a corresponding heated coupon; each unheated coupon was also placed into a separate 100 mL of Letheen broth for processing.
- d. Viable organisms were then eluted from the coupons via stomaching.
- e. Dilutions from each coupon eluent were plated on selective media (Petrifilm® and XLD agar) to determine the residual level of *E. coli* and *Salmonella* spp., respectively.
- f. Microbial levels before and after heating were assessed to determine the log reductions for each matched pair of coupons from each lot.

Results

The study performed by NSF showed that the time/temperature profile of a typical corrugator resulted in a 5-log reduction of a cocktail of two *E. coli* O-157 strains, *Salmonella enteridis* and an *E. coli* strain with

similar thermotolerance to heat-labile *Salmonella* Montevideo and *Salmonella* Poona, when heating to 180 – 200°F for 9 seconds.

- The log reductions for both *E.coli* and *Salmonella* attained using a time/temperature profile consistent with the time/temperature of the corrugation process met or exceeded the EPA's requirement for chemical sanitizers (5-log reduction).
- None of the heated samples exhibited any microbial growth.
- The blanks (pretest coupons) indicate that the process employed prior to inoculation and heat treatment was sufficient to sanitize the coupons, eliminating confounding organism contamination.
- Results between the two different lots of corrugated material did not show any significant difference in values.

Tables 1 and 2 provide a summary of the study data. Table 1 is provided as Logarithmic values while Table 2 provides information in Arithmetic terms.

Table 1: Study Results (Log basis) as compared to EPA Requirements

| Sample | Organism | Blank Coupon Avg. (Log CFU/ml) | Unheated Coupon Avg. (Log CFU/ml) | Heated Coupon Avg. (Log CFU/ml) | Avg. Log Reduction | Meets EPA Sanitizer Log Reduction Requirement (5-Log) |
|--------|-------------------|--------------------------------|-----------------------------------|---------------------------------|-----------------------|---|
| Lot 1 | <i>E. coli</i> | <0.1 Log | 6.33 Log | <0.1 Log | 6.03 Log | Yes |
| | <i>Salmonella</i> | <0.1 Log | 5.59 Log ⁵ | <0.1 Log | 5.49 Log ⁵ | Yes |
| Lot 2 | <i>E. coli</i> | <0.1 Log | 6.42 Log | <0.1 Log | 6.12 Log | Yes |
| | <i>Salmonella</i> | <0.1 Log | 5.31 Log ⁵ | <0.1 Log | 5.21 Log ⁵ | Yes |

Table 2: Study Results (Arithmetic basis)

| Sample | Organism | Blank Coupon Avg. (CFU/ml) | Unheated Coupon Avg. (CFU/ml) | Heated Coupon Avg. (CFU/ml) |
|--------|-------------------|----------------------------|-------------------------------|-----------------------------|
| Lot 1 | <i>E. coli</i> | <1 | 2,140,000 | <1 |
| | <i>Salmonella</i> | <1 | 390,000 ⁵ | <1 |
| Lot 2 | <i>E. coli</i> | <1 | 1,260,000 | <1 |
| | <i>Salmonella</i> | <1 | 204,000 ⁵ | <1 |

⁵ The data shows that the two *Salmonella* spp. used in the study were likely more susceptible to desiccation which occurred during the 10 minutes between organism inoculation and organism elution.

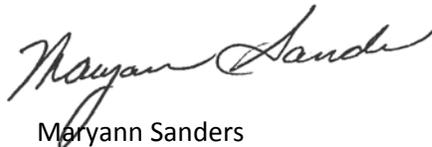
Conclusions

This bench scale evaluation of the time/temperature profile of a typical corrugator confirms that the profile is sufficient to result in a 5-Log reduction in organisms, effectively sanitizing the combined board. The results support the prior work of the corrugated industry which shows that single-use corrugated containers would not introduce microorganisms into the food chain and lead to food-borne illness.

Sincerely yours,
HALEY & ALDRICH, INC.



Mark Jackson
Senior Toxicologist
Regulatory Compliance Specialist



Maryann Sanders
Senior Regulatory Compliance Specialist
Microbiologist

Attachment 1:

Corrugator Effect on Microbial Contamination, NSF International

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Send to: Fibre Box Association
25 Northwest Point Boulevard, Suite 510
Elk Grove Village, Illinois 60007

Result: COMPLETE

Report Date: 04-November-2015

Customer Name: Fibre Box Association
Location of Testing: NSF Ann Arbor
Description: Corrugator Effect on Microbial Contamination
Test Type: Test Only
Job Number: J-00184630
Project Number: 10014843
NSF Corporate: C0262787
Project Manager: J. Vantine

Executive Summary:

Fibre Box Association contracted the Applied Research Center at NSF International to determine if the corrugation process is sufficient to mitigate microbial contamination on the container board that occurs prior to corrugation.

The surface of 2 sample lots of containerboard material were inoculated with a microbial challenge population of thermotolerant bacteria. This inoculated containerboard was then exposed to heat at a timed interval to simulate the corrugation process. The exposure of 185 ± 5 °F for 8-9 seconds was sufficient to eliminate microbial contamination.

Thank you for working with the Applied Research Center! We hope to collaborate again with you soon!

Please contact your Project Manager if you have any questions or concerns pertaining to this report.

Report Authorization:



Digitally signed by Dr. Robert Donofrio /jv
DN: cn=Dr. Robert Donofrio /jv, o=NSF
International, ou=Director - Applied Research
Center, email=Donofrio@NSF.ORG, c=US
Date: 2015.11.04 09:06:52 -05'00'

Robert Donofrio – Director, Applied Research Center

Scope of Test Report

The objective of the study was to understand if the process of corrugation eliminates microbial contamination on the containerboard material. A selection of thermotolerant organisms representative of foodborne pathogens that are of concern in the produce industry were selected.

Organism cocktail:

Escherichia coli ATCC 25922*

Escherichia coli ATCC 43890 (O157:H7)

Escherichia coli ATCC 51657 (O157:H7)

Salmonella enterica subsp. *enterica* serovar Enteritidis ATCC 13076

* According to Eblen et al. (J. Food Prot., 2005). *E. coli* ATCC 25922 is, relative to tested pathogenic *E. coli* strains, heat-labile and may be used as a surrogate to model the heat resistance of the heat-labile *Salmonella* strains like *Salmonella* Montevideo G4639 and *Salmonella* Poona RM 2350

The corrugation process itself was reviewed and the exposure of 185 ± 5 °F for 8-9 seconds was selected to replicate the hot plate processing segment of the corrugation process. In the manufacturing process the component materials are pre-heated and brought together for a final pressing. In representative facilities this pressing has been measured to be between 193 °F (Top Liner) and 240 °F (Bottom Liner) for an average of 66 feet at a rate of 700 fpm. In our simulation the containerboard material was not be pre-heated.

In order to replicate the desired temperature and time exposure, NSF conducted method verification (See Figure 1) to determine the correct placement and removal times to achieve the 185 ± 5 °F for 8-9 seconds.

One inch thick aluminum plaques were heated in an oven at various temperatures, ranging from 180F to 220F until the desired effect on the containerboard was achieved.

The organism cocktail was applied to the 4" × 4" area of containerboard material and allowed to dry for 10 minutes. The coupons were then carefully wrapped in foil and exposed to the simulated corrugation process. At the completion of the exposure the coupons were allowed to cool for 1 minute to replicate the material moving along the production line and then placed into a stomacher bag containing buffer. After simulated processing the containerboard plaques and foil press material were stomached to determine the remaining population of challenge organisms.

Proposed Sampling

- 2 lots of container board were evaluated; each tested in 22 locations (4 × 4 inch cut-outs; “coupons”).
 - 20 spiked (10 sampled without heating, 10 sampled after heating)
 - 2 unspiked (1 sampled without heating, 1 sampled after heating)

The level of the inoculated surrogate organisms on the container board before and after the simulated corrugation process was used to demonstrate the efficacy of the corrugation process to eliminate organisms.

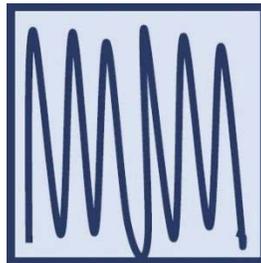
Methodology

Methods:

1. Thermocouple Temperature Profile Study
 - a. 4” × 4”. cardboard coupons were spiked on the outer liner surface (rough material side) with 500 µL of sterile BNaCIPT (per liter: FLUKA Peptone Hy-Soy® T, 1 g; Tween 80, 1 mL; KH₂PO₄, 3.6 g; Na₂HPO₄, 7.2 g; NaCl, 4.3 g; pH 7.0 +/- 0.2) containing 50 mM Trehalose. 50 mM Trehalose was included to protect the inoculum against desiccation (die-off) during the drying process (S.B. Leslie et al. 1995 Appl. Environ. Microbiol.). The solution was immediately spread across the liner using a T-spreader and then allowed to dry for 10 minutes.
 - b. A thermocouple was affixed to the coupons in order to monitor and document the temperature profile of the cardboard coupons during the heating process and one minute of cooling.
 - c. Note: After affixing the thermocouple, the coupon surface was topped with a 4” x 4” segment of heavy duty aluminum foil and then wrapped in heavy duty aluminum foil.
 - d. Phase 1: the thermocouple was affixed to the outer portion of the upper cardboard liner. This phase was deemed complete when an oven temperature was identified which quickly heated three consecutive replicate coupons to 185 ± 5 °F and maintained that temperature for 8-9 seconds. The selected oven temperature was used in Phase 2.
 - e. Phase 2: the thermocouple was affixed to the inner portion of the upper cardboard liner and the heating process repeated on new spiked coupons.
 - f. The aim of these two phases was to capture the temperature profile of the outer liner from both of its sides.
 - g. As long as the temperature profile observed in phase 2 was within 10°F of that observed within phase 1, the oven temperature observed in phase 1 was used in the full study plan. If the observed phase 2 temperature profiles were more than 10°F different than that of phase 1, NSF and the client discussed the results and decided on how to proceed.

2. Full Study

1. Each of the following samples was UV-sterilized for ten minutes:
 - a. One side of a 10" X 6" piece of heavy duty aluminum foil (enough for all coupons)
 - b. Both sides of a 4" x 4" piece of Heavy Duty Aluminum foil (enough for all coupons)
 - c. Both sides of a 4" x 4" cardboard coupon from each lot (11 of each type for unheated sampling and heated sampling)
 - i. This totaled 44 coupons (22 of each type)
2. A master spike suspension mixture of the following organisms in BNaCIPT + 50 mM Trehalose was created:
 - a. *Escherichia coli* ATCC 25922
 - b. *Escherichia coli* ATCC 43890 (O157:H7)
 - c. *Escherichia coli* ATCC 51657 (O157:H7)
 - d. *Salmonella enterica* ATCC 13076
3. All organisms from step 2 were made to target densities of 1×10^9 CFU/mL in BNaCIPT + 50 mM Trehalose. Equal parts of the cell suspensions were mixed together. This master suspension was used to inoculate all 44 coupons.
4. Using a calibrated pipette, the inoculum was added onto the surface of the cardboard coupon following the pattern shown below and immediately spread using a T-spreader to inoculate the coupon.



5. Only two coupons of each type and treatment were inoculated at a time to minimize variability in drying and processing time (i.e., only 2 coupons of each lot for each treatment type; 4 total per round of testing).
6. Coupons were allowed to dry for ten minutes and were then covered with the 4" X 4" piece of corresponding aluminum foil.

7. The large piece of aluminum foil was folded to cover the samples that were to be heated:
 - a. Covered coupons were placed between two pre-heated aluminum blocks held at 215 °F
 - b. After 22 seconds the coupons were removed, allowed to rest for 1 minute and then processed using the same procedure in step 8 (below)
8. The unheated coupons along with the 4” × 4” piece of corresponding aluminum foil was placed in a pre-labeled stomacher bag that contained 100 mL of Lethen broth and stomached for 30 seconds.
9. Unheated samples were diluted to 10⁻³, 10⁻⁴, and 10⁻⁵ and plated on 3M™ Petrifilm *E.coli*/Coliform Count Plates for detection and quantification of *E. coli* and to XLD agar for detection and quantification of *Salmonella*.
10. Heated samples were diluted to “neat (zero)”, 10⁻¹, and 10⁻² and plated on 3M™ Petrifilm *E.coli*/Coliform Count Plates for detection and quantification of *E. coli* and to XLD agar for detection and quantification of *Salmonella*.
11. 3M™ Petrifilm *E.coli*/Coliform Count Plates and XLD agar plates were incubated for 48 ± 4 hours at 35 ± 1 °C.

Results and Discussion

Thermocouple Temperature Profile Study:

With an aluminum block temperature of 215 °F, the average temperature of the upper cardboard liner (measured from above and below the liner surface) reached 180 – 200 °F in 13 seconds and was maintained in that temperature range for 8-9 seconds (Figure 1). These results were communicated to the client and this exposure protocol (22 second heated-plaque exposure) was deemed appropriate for the full study.

Full Study:

As can be seen from the data shown in Table 1 in Appendix A, the reduction of total cells from the cardboard coupons under the conditions tested is between 6.41 and 6.46 Log₁₀, which equates to a ~99.9999% reduction (6 Log₁₀) in viable cells on the substrate. In fact, each sample tested no challenge organism could be detected as can be seen in Table 3 in Appendix A.

Additional raw data are provided in Appendix A, including the temperature profile for the test as well as raw data for each sample tested.

Scope of Work Revisions

- Scope of work authorized: September 9th, 2015
- Version: 10014843 1 10012015 authorized 10/01/2015 contained the following revisions:
 - Amended from Proposal Letter format to Attachment/Annex format to track changes.
 - Updated **Methodology** (2) to include details clarifying the use of ‘T’ spreader.
 - Updated **Methodology** (3) to decrease the time span from inoculation to sampling to under 10minutes.
 - Updated **Methodology** to clarify that plating will be completed in duplicate.
 - Updated **Methodology** (6) to remove reference to swabbing and update with stomaching.
 - Deleted **Figure 2** – which detailed swabbing approach.
 - Updated **Sample Processing** section to accurately detail methods. (Remove references to swabbing.)
 - Updated footer to include page numbering new (project) version number 10014843 1
- Version: 10014843 2 10192015 authorized 10/19/2015 contained the following revisions:
 - Changes Made: Addition of new header and details to Method Development and Verification section
Revision of costs to accommodate additional method verification and laboratory services.
 - Updated footer to include new (project) version number 10014843 2

Appendix A

Result Tables and Figures

Table 1: Reduction in viable organisms from the heating process.

The following table displays the number of organisms present after heat treatment and for coupons that received no heat treatment. Reduction percentages are shown as a calculation of heated organisms remaining to unheated.

| Lot | Received Date | Description | Total cells (E. coli and Salmonella) (Log CFU/mL in eluent) (Avg. ± SD) | | | E. coli (Log CFU/mL in eluent) (Avg. ± SD) | | | Salmonella (Log CFU/mL in eluent) (Avg. ± SD) | | |
|-----|---------------|------------------------------------|--|--------------------|----------------|--|--------------------|----------------|---|--------------------|----------------|
| | | | Unheated (n = 10) | Heated (n = 10) | Reduction % | Unheated (n = 10) | Heated (n = 10) | Reduction % | Unheated (n = 10) | Heated (n = 10) | Reduction % |
| 1 | 9/9/2015 | 23 PC'S OF 12"X12" CARDBOARD | 6.41 ± 0.23 | <1 ^{a,b} | 99.9999 | 6.33 ± 0.21 | <1 ^a | 99.9999 | 5.59 ± 0.34 | <1 ^b | 99.999 |
| 2 | 9/15/2015 | 15 PC'S OF 24"X24" CARDBOARD | 6.46 ± 0.25 | <1 ^{a,b} | 99.9999 | 6.42 ± 0.25 | <1 ^a | 99.9999 | 5.31 ± 0.39 | <1 ^b | 99.999 |

^aThe limit of detection for *E. coli* was 50 CFU (i.e., 50 CFU of viable challenge organism could have survived the simulated corrugation procedure and the enumeration protocol was not sensitive enough to detected them).

^bThe limit of detection for *Salmonella* was 100 CFU (i.e., 100 CFU of viable challenge organism could have survived the simulated corrugation procedure and the enumeration protocol was not sensitive enough to detected them).

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Table 2: Organism numbers on unheated lots (Raw Data).

The following table displays the number of organisms present for coupons that received no heat treatment.

| Total Log CFU/mL (all organisms) | | | | | Total Log CFU/mL (all organisms) | | | | |
|----------------------------------|-----------|----------------|-------------------|------|----------------------------------|-----------|----------------|-------------------|------|
| Sample | Replicate | <i>E. coli</i> | <i>Salmonella</i> | Sum | Sample | Replicate | <i>E. coli</i> | <i>Salmonella</i> | Sum |
| Lot 1 | 1 | 6.60 | 5.68 | 6.65 | Lot 2 | 1 | 6.39 | 5.17 | 6.42 |
| | 2 | 6.37 | 5.69 | 6.45 | | 2 | 6.49 | 5.10 | 6.51 |
| | 3 | 6.22 | 5.65 | 6.32 | | 3 | 6.41 | 5.60 | 6.48 |
| | 4 | 6.63 | 6.12 | 6.75 | | 4 | 6.28 | 5.33 | 6.32 |
| | 5 | 6.08 | 5.13 | 6.13 | | 5 | 6.66 | 5.14 | 6.67 |
| | 6 | 6.26 | 5.46 | 6.32 | | 6 | 6.28 | 5.30 | 6.32 |
| | 7 | 6.57 | 5.94 | 6.66 | | 7 | 6.58 | 5.60 | 6.63 |
| | 8 | 6.21 | 5.58 | 6.30 | | 8 | 5.85 | 4.41 | 5.87 |
| | 9 | 6.32 | 5.65 | 6.40 | | 9 | 6.56 | 5.62 | 6.60 |
| | 10 | 6.05 | 4.97 | 6.08 | | 10 | 6.72 | 5.79 | 6.76 |
| | | | Avg. | 6.41 | | | | Avg. | 6.46 |
| | | | St.Dev. | 0.23 | | | | St.Dev. | 0.25 |

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Table 3: Organism numbers on heated lots (Raw Data).

The following table displays the number of organisms present for coupons that received heat treatment.

| Total Log CFU/mL (all organisms) | | | | | Total Log CFU/mL (all organisms) | | | | |
|----------------------------------|-----------|----------------|-------------------|-----|----------------------------------|-----------|----------------|-------------------|-----|
| Sample | Replicate | <i>E. coli</i> | <i>Salmonella</i> | Sum | Sample | Replicate | <i>E. coli</i> | <i>Salmonella</i> | Sum |
| Lot 1 | 1 | <1 | <1 | <1 | Lot 2 | 1 | <1 | <1 | <1 |
| | 2 | <1 | <1 | <1 | | 2 | <1 | <1 | <1 |
| | 3 | <1 | <1 | <1 | | 3 | <1 | <1 | <1 |
| | 4 | <1 | <1 | <1 | | 4 | <1 | <1 | <1 |
| | 5 | <1 | <1 | <1 | | 5 | <1 | <1 | <1 |
| | 6 | <1 | <1 | <1 | | 6 | <1 | <1 | <1 |
| | 7 | <1 | <1 | <1 | | 7 | <1 | <1 | <1 |
| | 8 | <1 | <1 | <1 | | 8 | <1 | <1 | <1 |
| | 9 | <1 | <1 | <1 | | 9 | <1 | <1 | <1 |
| | 10 | <1 | <1 | <1 | | 10 | <1 | <1 | <1 |
| | | | Avg. <1 | | | | Avg. <1 | | |
| | | | St.Dev. 0.00 | | | | St.Dev. 0.00 | | |

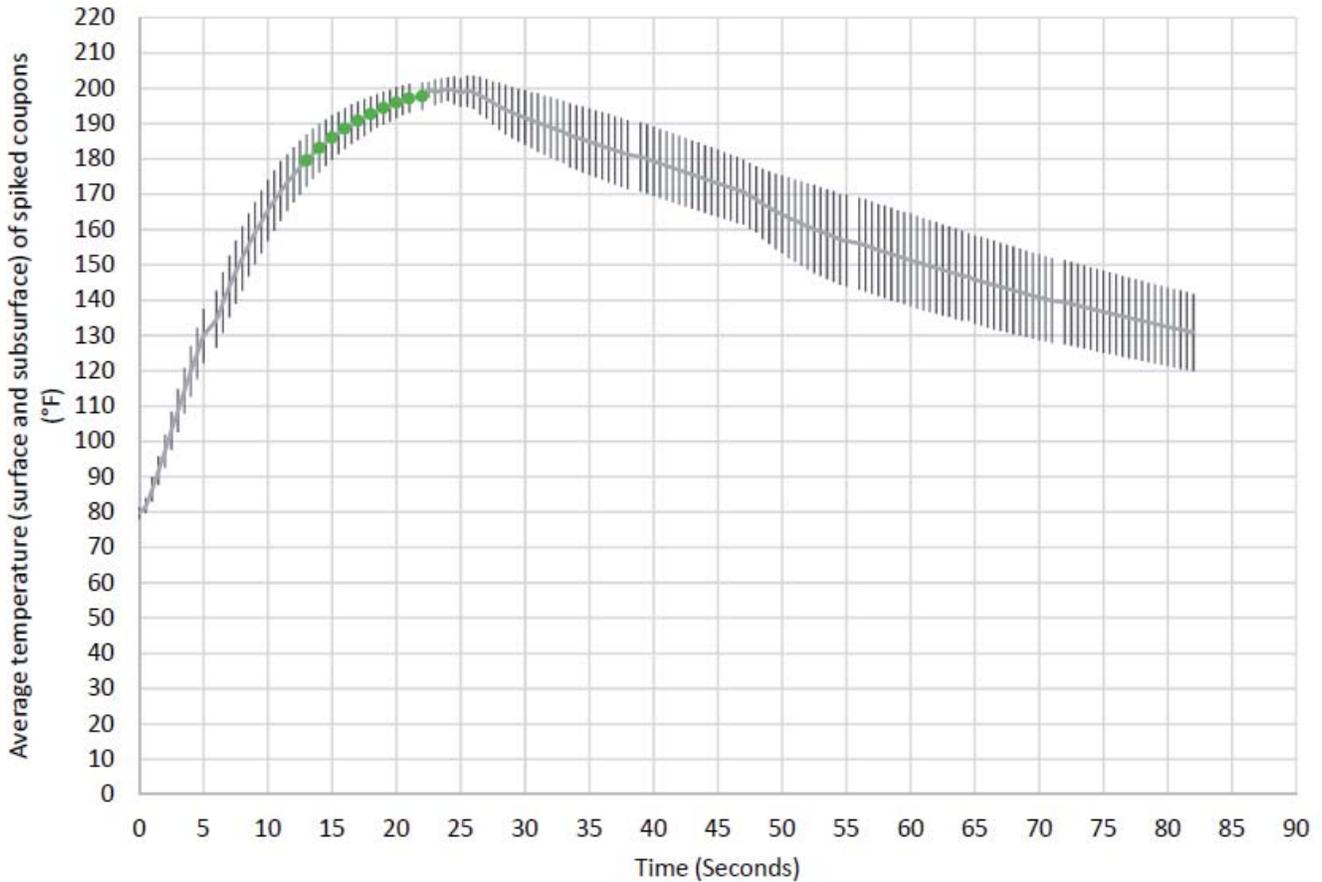


Figure 1: Temperature profile of ideal simulated corrugation.

The following chart shows the average \pm standard deviation of 6 representative sample coupons, 3 monitored from the outer portion of the upper cardboard liner and 3 monitored from the inner portion of the upper cardboard liner). The coupons were wetted with 0.5mL of sterile BNaCIPT with 50 mM Trehalose and dried for 10 minutes prior to testing. Green dots indicate the time points during the 22 second heated-plaque exposure, from 13 to 22 seconds, during which the upper cardboard liner reached the desired temperature required to simulate the corrugation process (180 – 200 °F).