A Simulation Study to Evaluate the Impact of Temperature on Microbiological Quality of Food Served During Field Trips

The Center of Excellence for FOOD SAFETY RESEARCH IN CHILD NUTRITION PROGRAMS

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Summary Report

The Center of Excellence for Food Safety Research in Child Nutrition Programs

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Executive Summary

Field trips to off-site locations present a challenge to school nutrition programs to provide a nutritious meal that is stored and handled properly to ensure food safety. These meals are often prepared as sack lunches, stored in coolers, and taken with teachers and students to the off-site location. Coolers of sack lunches might remain on school buses without temperature regulation. On days with elevated outdoor temperatures (e.g. above 80^{o}F [26.7^{o}C]), school buses can reach extreme temperatures, subjecting coolers and sack lunches to extreme storage conditions. Bacteria grow rapidly when exposed to temperatures between 41^{o}F and 135^{o}F (5^{o}C to 57.2^{o}C); subsequently, the risk that foodborne pathogens will grow in poorly-stored sack lunches is concerning. The research described herein investigates this concern.

Temperature data within school buses during the spring and summer months were collected and used to develop a simulated school field trip when coolers filled with sack lunches are stored on a bus under extreme temperatures. Preliminary research identified the two riskiest cooler-packing scenarios with regard to product temperature within each cooler. Inoculation studies were then performed using the school bus temperature profiles and the two riskiest cooler-packing scenarios to investigate this risk. The inoculation study evaluated *Salmonella* and *Listeria monocytogenes* growth on turkey sandwiches, sliced apples, and baby carrots. Inoculated products were packaged into individual sack lunches and placed into two coolers: a cooler with no ice and a cooler with one layer of ice (approximately 2 inches thick) on the bottom. Both coolers were placed in a commercial electronically controlled thermal processing unit (ECTPU), and subjected to a 5-hour cycle that simulated the natural increase in temperature that would occur in a school bus on a day with elevated temperatures. The highest temperature reached during the 5-hour cycle was specifically chosen to simulate high-risk case storage conditions. Populations of *Listeria monocytogenes* and *Salmonella* were enumerated from inoculated sack lunches stored in the bottom, middle, and top of each cooler.

With the exception of *Salmonella* on carrots, *Listeria monocytogenes* and *Salmonella* populations did not vary ($p>0.05$) based on the cooler type the lunches were stored in, their location within the cooler, or the interaction of cooler type x location within the cooler. *Salmonella* populations on carrots varied ($p<0.05$) by sack lunch location within the cooler, with the largest *Salmonella* population recovered from the untreated control sample, which was sampled immediately upon removal from cold storage at 39.2°F (4^{o}C).

Thermocouple data collected from both coolers indicate that sack lunches were held at temperatures conducive for foodborne pathogen growth. However, *Listeria monocytogenes* and *Salmonella* populations did not significantly increase during the 5-hour simulation. These data suggest that, in most cases, relying on time (<5 hours) as a public health control may reduce risk of *Salmonella* and *Listeria monocytogenes* growth in deli sandwiches, apple slices, and baby carrots stored in coolers during field trips.

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Background

School nutrition programs are required to have a food safety program based on Hazard Analysis and Critical Control Point (HACCP) principles wherever food is prepared, stored, or served. Meals that are provided by the school nutrition program and served on field trips are included in this requirement ("Healthy, Hunger Free Kids Act of 2010" [HHFKA], 2010).

Serving meals outside the cafeteria poses specific temperature-control challenges. Once meals leave the school nutrition operation, equipment may not be available to maintain the temperatures that ensure the safety of time/temperature control for safety (TCS) foods, and high ambient air temperatures might pose a risk to food safety. TCS foods are defined as a food that requires time /temperature control for safety to limit pathogenic microorganism growth or toxin formation (United States Food and Drug Administration (FDA), 2013). Accordingly, the United States Department of Agriculture (USDA) Food and Nutrition Service (FNS) research question asks if using time itself—up to a maximum of four hours—as a public health control for food with an initial temperature of ≤ 41°F (5°C), allows only minimal growth of pathogens of concern, especially when using current practices for serving meals outside the school.

The Center of Excellence for Food Safety Research in Child Nutrition Programs conducted two studies to determine current practices for field trip and Summer Food Service Programs (SFSP) meals. The first study was conducted to determine what foods are served for field trip meals, how those foods are transported and stored, and what standard operating procedures are in place to guide school nutrition personnel and teachers in providing safe meals (Sneed & Patten, 2015). Two written electronic questionnaires were developed and delivered via email to obtain information from school nutrition managers and teachers. A total of 192 school nutrition managers and 80 teachers responded.

Sixty-seven percent of school nutrition managers indicated they frequently or occasionally provided deli sandwiches as part of field trip meals. Twenty-one percent reported serving lettuce-based salads with toppings such as deli meat, cheese, or cut tomatoes. Eighty-six percent of school nutrition managers reported serving individual, prepackaged fruits and vegetables, and 15% reported using fruit prepared in the school kitchen. Fifty percent of the teachers reported frequently receiving sandwiches made with turkey, ham, or other deli meat; 46% reported these foods as the item brought second-most frequently from home. As reported by school nutrition managers and teachers, TCS foods are frequently served for field trip meals, so using time and temperature control is important in keeping these foods safe (Sneed & Patten, 2015).

Most managers (71%) reported using insulated containers, typically made of plastic, to transport cold foods, and 69% of school nutrition managers also reported sending meals in individual sacks or boxes. Seventy-seven percent of managers reported using ice or ice packs. More teachers (63%) reported that meals were placed in a cardboard box, bag, or plastic tub than in coolers (33%). School buses were almost always used for field trip transportation, and 50% of teachers stated the school buses served as the storage site for meals (Sneed & Patten, 2015).

The second study was conducted to identify current food safety practices implemented at the SFSP serving sites, specifically the types of food offered, time and temperature control, and where and how food was served (Patten, Alcorn, Watkins, Cole, & Paez, 2017). Observations were conducted at 28 SFSP sites across the United States. A convenience sample of four sites from one state in each of the seven USDA FNS regions was selected based on program service dates. The average number of meals prepared at each production center during the observations ranged from 20 to 5,000 meals per day. Meals were served at community centers, parks, housing complexes, churches, schools, camps, libraries, recreation centers, and town halls.

Meals served during observations included TCS foods such as cantaloupe, deli sandwiches, and chicken, potato, and tuna salads. Data loggers were used to record the temperature of hot and cold food items. Overall, fewer hot food items were held in the temperature danger zone (TDZ), between 41 °F (5°C) – 135 °F.(57°C), and spent less cumulative time in the TDZ than cold food items. Two hot food items and eight cold food items were held in the TDZ for more than two hours, with one of the cold food items (potato salad) remaining in the TDZ for more than four hours (Paez, Cole, Alcorn, Patten, & Watkins, 2016).

The 2013 Food Code (FDA, 2013) states that time can be used as a public health control for a maximum of four hours when foods have an internal temperature of 41°F (5°C) or less when removed from cold storage. Bacteria grow readily in foods held at ambient temperatures, and can reproduce in as little as 20 minutes when held within the TDZ (United States Department of Agriculture Food Safety and Inspection Service (FSIS), 2013). Foodborne pathogens have varied infectious doses, which makes it difficult to establish a universal threshold of concern regarding growth within a food (Montville, Matthews, & Kniel, 2012). While temperature dramatically impacts microbial growth and lag periods, the interaction of time, intrinsic factors (i.e. pH), and extrinsic factors (i.e. humidity) must also be considered with regard to microbial generation times and lag periods (FDA, 2013). Therefore, it is important to evaluate the behavior of each pathogen of concern within a food matrix when subjected to specific extrinsic factors in order to evaluate risk.

Listeria outbreaks are traditionally associated with ready to eat meats, a food product commonly used to prepare lunches served on field trips (Sneed & Patten, 2015), however increasingly *Listeria* outbreaks are associated with fresh produce (Garner & Kathariou, 2016). Since the implementation of HHFKA, US children are consuming 23% more fruit and 16% more vegetables at lunch (FNS, 2017). The success of HHFKA has brought more fresh fruits and vegetables into the school lunch environment and along with it increased concern for food safety in fresh produce. *Listeria* contamination has occurred in many vegetables, including celery, tomatoes, sprouts, cabbage, corn, lettuce, bagged salad, parsley, cucumber, and carrots (Garner & Kathariou, 2016). *Listeria* outbreaks have occurred less commonly in fresh fruits; but cantaloupe, stone fruits, and apple outbreaks have occurred (Garner & Kathariou, 2016).

Though *Listeria* outbreaks in US schools are uncommon (Centers for Disease Control and Prevention [CDC], 2017) foods served in schools have been involved in recent recalls. In 2015, apple slices recalled for possible *Listeria* contamination were served in the Palm Beach County School District (FDA, 2015a). In 2015, 3-oz single serving Blue Bell ice creams distributed to hospitals, nursing homes, and schools in 23 states were recalled (FDA, 2015b). In 2016, pre-prepared sandwiches sold to school foodservice distributors in 29 states were recalled due to possible *Listeria* contamination (FDA, 2016).

Salmonella is a major foodborne illness causing organism in the US, and since 1998, there have been 42 *Salmonella* outbreaks in schools, colleges, and universities; resulting in over 2400 illnesses and 180 hospitalizations (CDC, 2017). Though traditionally associated with eggs, many other foods have been implicated in outbreaks. According to the CDC's 2015 Annual Summaries of Foodborne Outbreaks (CDC, 2017), *Salmonella* associated with seeded vegetables, pork, and vegetable row crops were responsible for three of the top five food-germ pairs causing outbreak-associated illness. School *Salmonella* outbreaks have been associated with many different kinds of foods as well, with 11 of the 42 reported outbreaks associated with fresh produce (CDC, 2016). *Listeria* and *Salmonella* are two pathogens that could represent a potential risk for schools.

Temperature control of food served away from a food preparation site is often challenging, especially during the summer season when many field trip and summer food service program meals are provided to children. The USDA FNS Office of Food Safety is concerned about the safety of school meals served outside the school cafeteria where equipment may not be available to maintain adequate temperatures for TCS foods. This may be a particular concern for meals served on school field trips through the National School Lunch Program or in the Summer Food Service Program. Thus, there is a need to determine if the use of time as a public health control allows for minimal growth of pathogens of concern when using current practices in the service of National School Lunch Program meals outside of the school. Some programs may use time as a public health control for TCS foods, but little research has examined the microbiological growth that may occur under these types of temperature abuse situations. Thus, the purpose of this study is to determine the growth of foodborne pathogens in school lunch meals served off-site, packaged in insulated coolers, and exposed to extreme environmental temperatures.

Objectives

This study was designed to specifically address the research question posed by the USDA FNS Office of Food Safety regarding the use of time as a public health control to restrain foodborne pathogen growth in meals served outside of the school. The experimental procedures are intended to determine the growth of foodborne pathogens in school lunch meals served off-site, packaged in insulated coolers, and exposed to extreme environmental temperatures. More specifically, the objective was to determine population changes among *Listeria monocytogenes* and *Salmonella* on carrots, turkey sandwiches, and apple slices placed in coolers and held under conditions that simulate storage on a school bus.

Methods

Food Preparation

Products frequently consumed on school field trips were specifically chosen for this study. Individual sack lunches were prepared to meet the requirements for National School Lunch Program meals. Supplies to prepare turkey sandwiches, sliced apples, and baby carrots were purchased at a Manhattan, KS, grocery store and used to make individual sack lunches. Sandwiches were prepared with two slices of oven roasted white turkey (approximately 45 g; Oscar Mayer, Madison, WI) and two slices of whole grain white bread (Sara Lee, Downers Grove, IL) and then stored in a re-sealable plastic sandwich bag. Riveridge Red Delicious apples were cored and sliced by hand. Approximately 50 g of sliced apples were packed in plastic, non-filtered stomacher bags and then sealed. Approximately 50 g of baby carrots (Grimmway Farms, Bakersfield, CA) were packed in plastic, non-filtered stomacher bags and then sealed. Each sack lunch consisted of one deli turkey sandwich, one stomacher bag of sliced apples, and one stomacher bag of baby carrots; products were packed in a brown paper bag.

Preliminary Data Collection

This simulation study intended to represent a high-risk scenario with regards to temperatures on school buses that sack lunches may be exposed to during a field trip. Preliminary data was collected to understand the temperature profiles on school buses and within sack lunches stored in coolers on school buses. These preliminary data were used to inform the design and execution of the subsequent inoculation study.

School Bus Temperature Profiling

In order to effectively simulate cooler storage on a bus during a field trip, it was necessary to first determine the temperature profiles on a school bus from morning into early afternoon, as this is commonly when school lunch coolers are stored during a field trip. To obtain this data, multiple schools across the US were invited to participate. Three school districts agreed to collect data; temperature data loggers were distributed to school personnel in two districts in North Carolina and one district in Arkansas during May and June of 2015. It was anticipated that these locations would represent a warmer climate and, therefore, a high-risk scenario. Four data loggers (LogTag TRIX-8 Temperature Data Recorder) recorded internal and external bus temperatures at two-minute intervals for a minimum of two days at each location. Interior data loggers were placed on the two interior sides of the bus. Exterior data loggers were placed in sheltered locations near the bus. Magnetic clips and zip ties were provided to ensure data loggers were positioned with the temperature sensors facing away from walls to accurately record air temperatures. Average, minimum, and maximum interior and exterior air temperatures were determined for times most likely to correspond with packing, transporting, and serving sack lunches on field trips. These data were then used to create a commercial electronically controlled thermal processing unit (ECTPU)$_{cycle}$ to mimic high-risk scenario temperature changes on a school bus (Appendix A). For this reason, ECTPUtemperatures exceeded the temperatures recorded by the school bus temperature data loggers. The relative humidity was maintained at 80% throughout the ECTPUcycle, and the combination of relative humidity and temperature was intended to simulate conditions experienced during a hot, humid day.

Cooler Temperature Profiling

Simulating a high-risk scenario of temperature and cooler-packing methods required a preliminary evaluation of five packing/storage methods to determine the scenarios most likely to favor pathogen growth. Temperature profiles were collected on non-inoculated turkey sandwiches, carrots, and apple slices packed in coolers with ice. Ice was layered in the following ways:

1. Ice on bottom, middle, and top of cooler
2. Ice on top of cooler
3. Ice on bottom of cooler
4. Ice on top and bottom of cooler
5. No ice in cooler

Thermocouples (Measurement Computing USB-TC with MCC DAQ Software, Norton, MA; MultiPaq21 Data logger with Food Tracker® Software, Datapaq, Inc., Derry, NH) were placed in each product packed in the bottom, middle, and top of the coolers for the preliminary study. The coolers were then placed in a commercial ECTPUat the Biosecurity Research Institute on the Kansas State University campus in Manhattan, KS. Once in the ECTPU, coolers were subjected to an ECTPU cycle (Appendix A) specifically designed to simulate extreme, high-risk temperature increases. The ultimate goal was to simulate a high risk case scenario on a school bus during a field trip on a day with temperatures above 80^{o}F (26.7^{o}C). A 5-hour ECTPU cycle was chosen to: a) simulate the length of time that coolers would be stored on a bus before food was consumed (i.e., 8:00 am departure with a 1:00 pm lunch); and b) evaluate a period of time in excess of the maximum (4 hours) allowed to use time as a public health control by the 2013 Food Code (FDA, 2013). All thermocouples recorded temperatures every 10 minutes throughout the 5-hour cycle. Resulting data were saved and analyzed using data logger software and Microsoft Excel. Graphs illustrating the temperature profiles were generated and used to identify the two packing methods that pose the greatest risk for pathogen growth (those with the highest product temperatures) for use in inoculation studies, which represent the next stage of this simulation study.

Inoculation Study

Understanding how *Salmonella* and *Listeria monocytogenes* behave on baby carrots, turkey sandwiches, and sliced apples under simulated field trip storage conditions was the overall objective of this inoculation study. Temperature and cooler packing data obtained in the preliminary study were used to simulate high-risk sack lunch storage scenarios.

Bacterial Cultures

Five-strain cocktails of *Listeria monocytogenes* [SLR 2249 (Cornell University laboratory developed strain), B-33043 (USDA-ARS isolate from turkey/ham luncheon meats), B-33260 (USDA-ARS isolate from beef sausage), B-33054 (USDA-ARS isolate from cucumbers), B-33245 (USDA-ARS environmental isolate)] and *Salmonella enterica,* subspecies *enterica* [serotypes Heidelberg (F5038BG1; CDC isolate from stuffed ham/chad slicer), Tennessee (ATCC 10722), Typhimurium (ATCC 14028), Newport (ATCC 6962), Senftenberg (ATCC 43845)] were prepared. Briefly, frozen stocks of the individual strains were streaked onto Tryptic Soy Agar (TSA; Difco™, Becton, Dickinson, and Company, Sparks, MD) and allowed to incubate at 98.6°F (37°C) for 18-24 hours. One isolated colony was transferred to 45 mL of Tryptic Soy Broth (TSB; Difco™, Becton, Dickinson, and Company, Sparks, MD) and incubated at 98.6˚F (37˚C) for 18-24 hours. A separate cocktail was prepared for both pathogens by combining all five strains (total of 225 mL) and then centrifuging for 15 minutes at 5,520 x g at 39.2˚F (4°C). The supernatant was decanted, and the pellet re-suspended in 225 mL of 0.1% peptone water (Bacto™, Becton, Dickinson and Company, Sparks, MD). Following rehydration, 1 mL of each five-strain cocktail was combined with 0.1% peptone water for a total volume of 1 L at a target concentration of 1.0 x 10^6 CFU/mL.

Inoculation and Preparation of Food Products

Food products were inoculated, prepared, and stored at the Food Safety and Defense Laboratory on the Kansas State University campus in Manhattan, KS. Turkey lunch meat, sliced apples, and baby carrots were spray inoculated with the prepared *Listeria monocytogenes* or *Salmonella* cocktail to achieve a target concentration of approximately 1.0 x 10^4 CFU/g on the product. According to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (National Advisory Committee on Microbiological Criteria for Foods, 2009), aerosolizing/spraying inoculum is a suitable means for inoculating foods products in order to apply inoculum that is no more than 1% of the total volume of the food. The NACMCF also states that an inoculum concentration between 2 and 3 $\log_{10}$ CFU/g is commonly used in growth challenge studies (National Advisory Committee on Microbiological Criteria for Foods, 2009). A higher target inoculation concentration (approximately 4 $\log_{10}$ CFU/g) was chosen for the present study due to concerns that antimicrobial interventions previously applied to the commercially purchased food products may result in population declines below the limit of detection. Following inoculation, all products were allowed to rest at ambient temperature for 30 minutes to facilitate pathogen attachment. All inoculated food products were prepared and packaged as previously described and then stored for 12-15 hours at 39.2 °F (4°C). In the study conducted by

Sneed and Patten, (2015), 32% of respondents reported frequently preparing the field trip meals the day before. Non-inoculated sack lunches were also prepared to fill a single cooler with 30 sack lunches (i.e., approximately the average number of meals required for field trips (Sneed & Patten, 2015). On the day of the study, the sack lunches were removed from storage at 39.2 °F (4°C) and packed into coolers. Two Coleman brand coolers (approximately 62.2 cm x 31.8 cm x 35.6 cm) were required for each replication of this study. One cooler was packed with a single layer of ice on the bottom (approximately 2 inches thick), while the second cooler was packed without ice. Each cooler contained 3 inoculated lunches and 27 non-inoculated lunches. Seven inoculated lunches were prepared for each pathogen. One of these lunches served as the inoculated control and was sampled immediately following the 15 hours of storage at 39.2^{o}C. The remaining six lunches were stored in coolers in the following ways (Figure 1):

1. Top layer, cooler without ice
2. Middle layer, cooler without ice
3. Bottom layer, cooler without ice
4. Top layer, cooler with a layer of ice on the bottom
5. Middle layer, cooler with a layer of ice on the bottom
6. Bottom layer, cooler with a layer of ice on the bottom

Two thermocouples were placed in each cooler: in one lunch on the bottom layer and in one lunch on the top layer. Following packing and thermocouple placement, the coolers were transported from the Food Safety and Defense Laboratory to the Biosecurity Research Institute.

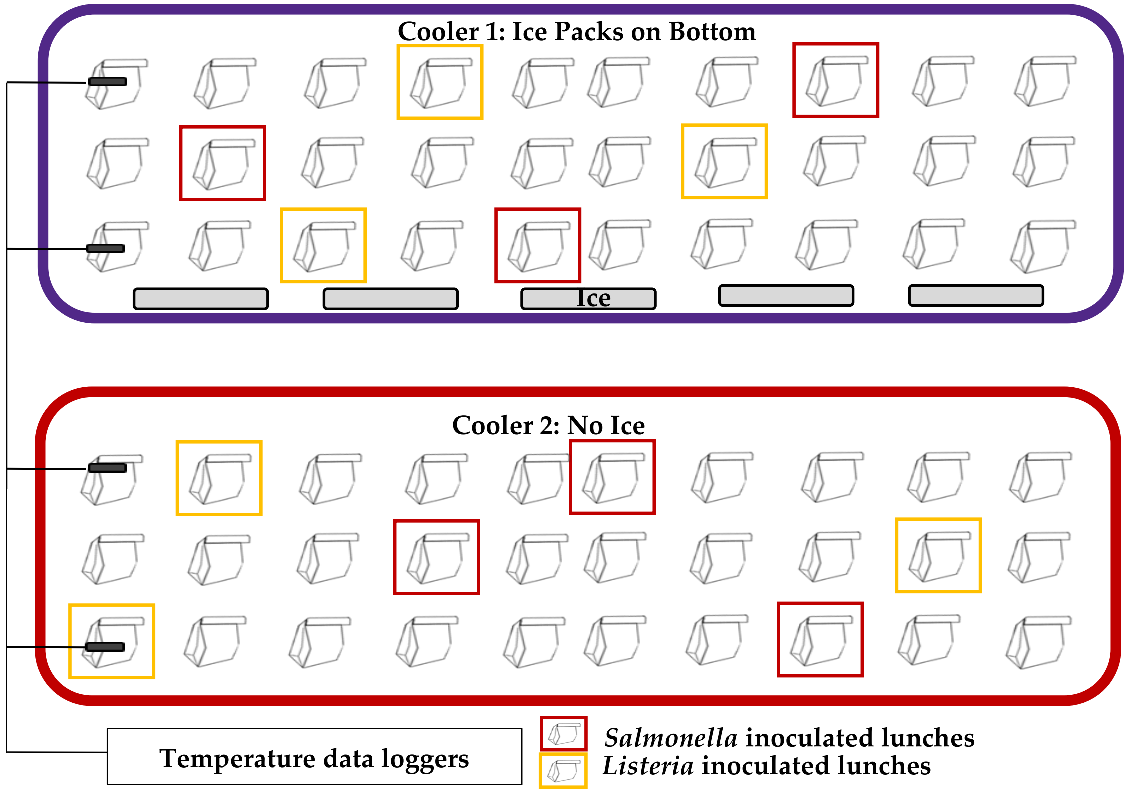


Figure 1. Cooler packing scenarios. Inoculated lunches were randomly assigned to a position within each layer of the cooler.

ECTPU Simulation

The packed coolers were placed in a commercial ECTPU at the Biosecurity Research Institute and subjected to a 5-hour ECTPU cycle (Appendix A). Thermocouples recorded temperatures of the inoculated lunches every 10 minutes throughout the 5-hour cycle, and these data were downloaded at the conclusion of data collection. Coolers were immediately removed from the ECTPU at the end of the 5-hour cycle. They were transported to the Food Safety and Defense Laboratory, for sampling and microbiological analyses.

Microbiological Analyses

The inoculated lunches were removed from the coolers and 75 mL of 0.1% peptone water was added to the apple and carrot stomacher bags, which were then pulsated (Pulsifier Model

PUL100; Microbiology International, Frederick, MD 21704) for 1 minute. For each inoculated sandwich, a coring device was used to remove approximately 50 g from the middle, which was combined with 75 mL of 0.1% peptone water in a stomacher bag and homogenized (Smasher™; AES CHEMUNEX, bioMérieux Inc., Hazelwood, MO) for 1 minute. Pulsated and homogenized serial dilutions in 0.1% peptone water were prepared for all samples. The *Listeria monocytogenes* dilutions were plated on MOX agar (Modified Listeria Selective Enrichment Supplement [Oxoid Ltd, Basingstoke, Hampshire, England]); Oxford (Difco™, Becton, Dickinson, and Company, Sparks, MD). *Salmonella* dilutions were plated on XLD agar (Xylose Lysine Deoxycholate; Difco™, Becton, Dickinson, and Company, Sparks, MD) and then enumerated. MOX plates were incubated at 86°F (30°C) for 24-48 hours; XLD plates were incubated at 95°F (35°C) for 18-24 hours. Following incubation, *Listeria monocytogenes* and *Salmonella* populations were recorded.

Statistical Analyses

All experimental procedures were replicated three times. Data collected from all three replications were analyzed using the MIXED procedure with Satterthwaite approximation of Statistical Analysis Software (SAS 9.4; Cary, NC). For each pathogen, the main factors (product, use of ice, and sack lunch location within the cooler) and interactions were evaluated for statistical significance at the $p \leq 0.05$ threshold. Because of differences in pathogen attachment to each product, data were also analyzed for each individual product (use of ice, sack lunch location, and the use of ice x sack lunch location interaction). The statistical model was designed by assigning the untreated control (inoculation concentration) for each product to a location. As previously mentioned, this sample was not packed in either cooler, and instead was sampled immediately upon removal from storage at 39.2°F (4°C), which allowed for effective comparison with the experimental samples to determine if significant changes in pathogen populations occurred as a result of the experimental variables.

Results and Discussion

Preliminary Data Collection

The highest temperature recorded in the cooler packed with no ice (Appendix B, Figure B1) was approximately 113°F (45°C), and was recorded in a bag of carrots at the top outside portion of the cooler. The highest temperature in the cooler packed with a layer of ice on the bottom (Appendix B, Figure B2) was approximately 100.4°F (38°C), which was recorded in a sandwich located in the top outside of the cooler. All products in the other cooler-packing scenarios remained below 68°F (20°C; Appendix B, Figures B3-B5). These data indicate that packing a cooler with no ice, or with a single layer of ice on the bottom, presents the greatest risk for temperature abuse and foodborne illness. Therefore, these two packing methods were chosen for use in the inoculation study.

Temperature data presented in Appendices C and D informed the creation of a commercial ECTPU program designed to increase in temperature in a manner similar to what was demonstrated by the school bus data loggers. More specifically, Appendix C lists the range of average interior and exterior bus temperatures recorded between 7:00 AM and 2:00 PM. The average interior temperatures during this period ranged from 72.8°F to 97.9°F (22.7°C to 36.6°C). The maximum recorded average interior temperature was 104.2°F (40.1°C), and the minimum recorded average interior temperature was 64.4°F (18°C).

The average exterior temperatures during the same period ranged from 68.8°F to 95.3°F (20.4°C to 35.2°C). The maximum recorded average exterior temperature was 106.8°F (41.6°C), and the minimum recorded average exterior temperature was 62.8°F (17.1°C). Appendix D lists the range of temperatures recorded from 7:00 AM to 2:00 PM by all interior and exterior data loggers. The highest interior temperature recorded was 108.9°F (42.7°C), and the highest exterior temperature recorded was 107.6°F (42°C). It should be noted that the majority of data points were collected from North Carolina, as data was collected on only one day in Arkansas.

Inoculation Study

Statistical analyses indicate significant (p<0.0001) variation in both *Salmonella* and *Listeria monocytogenes* populations on each product. This is not unexpected, as the intrinsic properties of foods (for instance, pH) greatly affect attachment, survival, and growth of microorganisms (Montville et al., 2012). Uniform attachment was not observed in this study, Appendix E figures illustrate inoculation levels variation across the products. In general, *Salmonella* and *Listeria monocytogenes* populations were highest on turkey, followed by apples and then carrots. Naturally occurring compounds in food products may also affect foodborne pathogens, which likely explains why *Listeria monocytogenes* was not readily recovered from carrots in this study. Previous studies have noted that carrots inhibit attachment and growth of *Listeria monocytogenes* (Babic, Nguyen-the, Amiot, & Aubert, 1994; L. R. Beuchat & Brackett, 1990; Larry R. Beuchat, Brackett, & Doyle, 1994). For example, Babic et al. (Babic et al., 1994) report that purified ethanolic extracts obtained from shredded and peeled carrots demonstrate antimicrobial activity against *Listeria monocytogenes*. Because of variation in pathogen attachment, survivability, and growth among foods, products were not statistically compared against one another and data were analyzed at the product level.

Temperature Profiles

Figures 2 and 3 illustrate that coolers packed with no ice, or with a layer of ice on the bottom, result in temperature abuse when exposed to the simulated school bus conditions. All products packed in a cooler without ice (Figure 2) reached temperatures above 68°F (20°C) at the end of five hours, with the highest temperature recorded approximately 95°F (35°C). According to thermocouple data collected from products stored at the top and bottom of this cooler, all products were within the danger zone throughout the entire 5-hour period.

In the cooler packed with a layer of ice on the bottom, a maximum temperature of 86°F (30°C) was observed for carrots (Figure 3). Those stored on the bottom of the cooler, in close proximity to the ice, maintained a temperature below 50°F (10°C) throughout the 5-hour simulation period. According to thermocouple data, only apples and sandwiches packed on the bottom of the cooler stayed out of the temperature danger zone for the majority (approximately 4.5 hours) of the 5-hour test period.

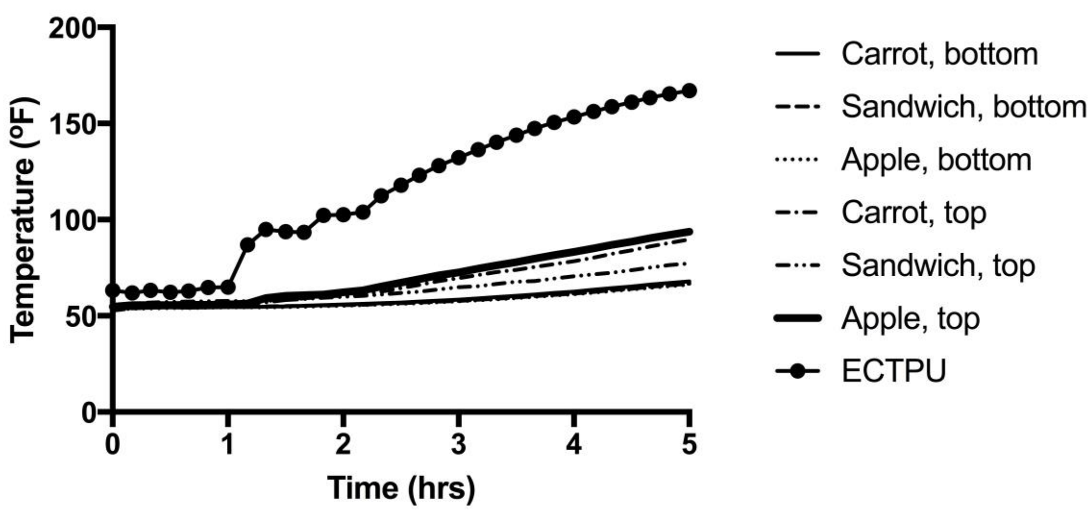


Figure 2. Exposure temperature for turkey sandwiches, sliced apples, and baby carrots packed in a cooler with no ice. Values represent the average temperature of three replications.

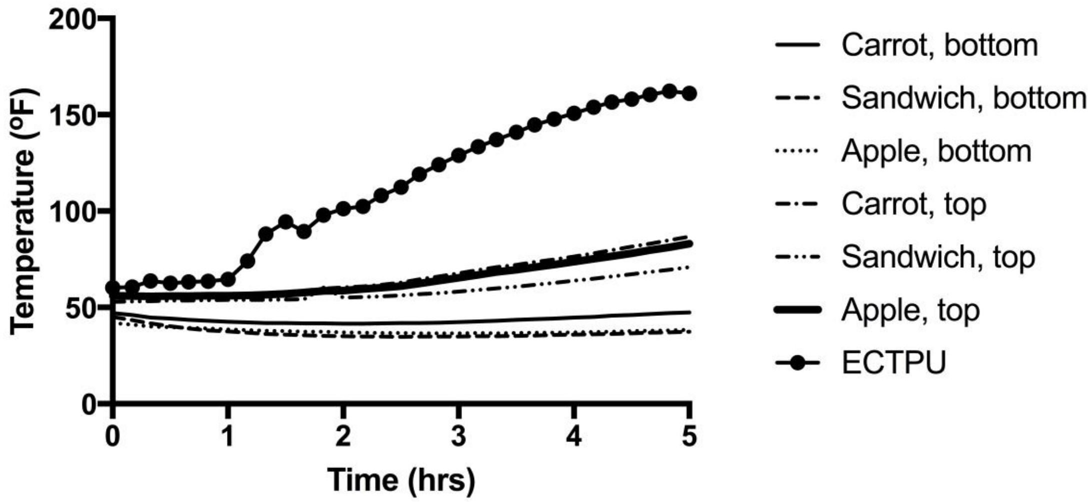
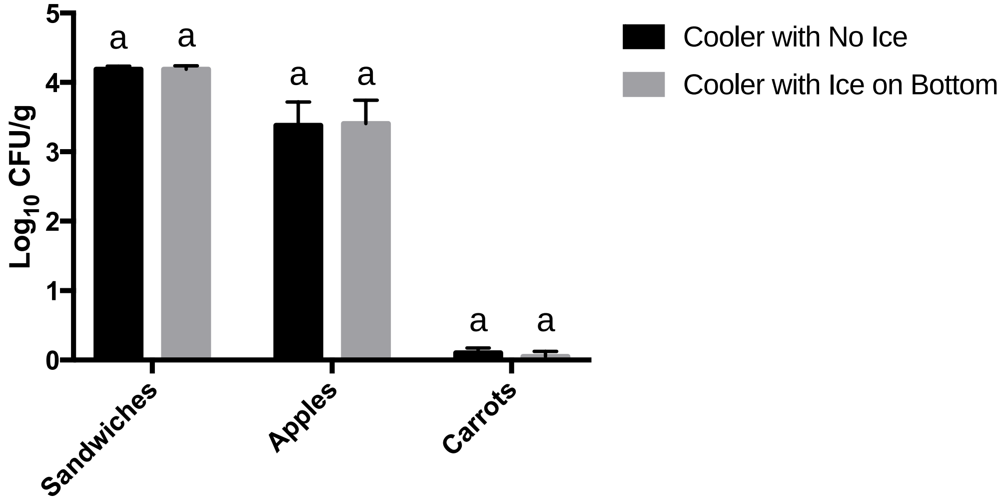


Figure 3. Exposure temperature for turkey sandwiches, sliced apples, and baby carrots packed in a cooler with a layer of ice on the bottom. Values represent the average temperature of three replications.

Foodborne pathogens require a variety of conditions for growth, and temperature is a very important factor that must be considered when assessing risk for foodborne illness *Listeria monocytogenes,* a psychrotroph, has optimal growth between 86-98.6°F (30-37°C) (Lado & Yousef, 2007). *Salmonella*, a mesophile, exhibits optimum growth at approximately 98.6°F (37°C) (Montville et al., 2012). The temperature profiles collected demonstrate a substantial risk for pathogenic growth. Based on temperature data alone, the risk for *Listeria monocytogenes* growth is particularly notable. During most of the 5-hour simulation period, product temperatures were within the optimum growth range for psychrotrophs.

Listeria on Sandwiches, Apples, and Carrots

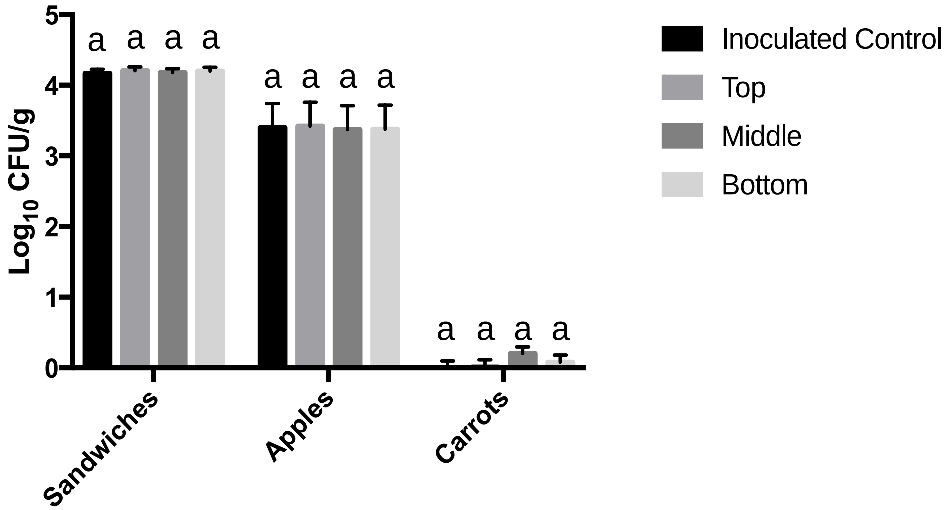
Use of ice (no ice versus one layer of ice on the bottom) (Figure 4), sack lunch location outside of the cooler (refrigerated control) or within the cooler (top, middle, bottom; Figure 5), and the use of ice x sack lunch location interaction (Appendix E) were not statistically significant (*p*>0.05; Appendix F) for sandwiches, apples, or carrots inoculated with *Listeria monocytogenes*. These data suggest that *Listeria monocytogenes* populations pose no difference in risk if school lunches are packed in coolers with no ice or with one layer of ice on the bottom regardless of location within the cooler.



* Error bars represent the standard error of the mean.
[a,b] Populations with different superscripts ($p \leq 0.05$) within each product are statistically different. Statistical significance is not compared between products.

Figure 4. *Listeria monocytogenes* populations on turkey sandwiches, apples, and baby carrots in a cooler packed with no ice and a cooler packed with one layer of ice on the bottom.

Although accompanying temperature data suggest the risk for growth, statistical analysis of the microbiological data indicate that five hours is not sufficient time for Listeria monocytogenes to grow under these simulated conditions. Perhaps most notable is the lack of any significant difference (p>0.05; Appendix F) in sack lunch location within coolers, which indicates that Listeria monocytogenes had the same population as the control products that were sampled immediately upon removal from cold storage at 39.2° F (4°C). Similarly, the lack of use of ice x sack lunch location interaction (Appendix E) indicates that populations did not vary depending on where a sack lunch was packed within a specific cooler, whether the cooler contained ice or not. For example, Listeria monocytogenes populations recovered from apples packed at the top of the cooler with no ice were statistically the same as populations on apples at the bottom of the cooler packed with a layer of ice.

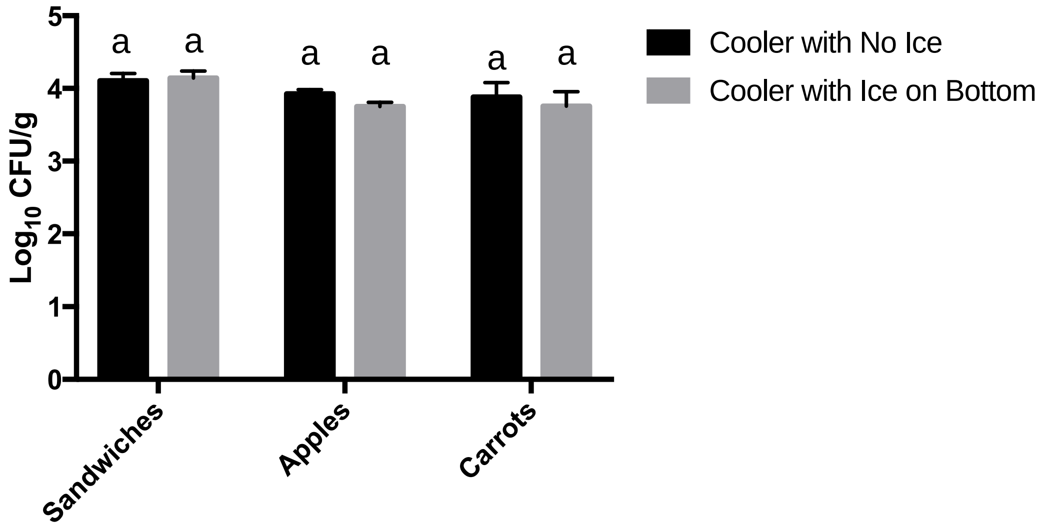


* Error bars represent the standard error of the mean.
a,b Populations with different superscripts (p<0.05) within each product are statistically different. Statistical significance is not compared between products.

Figure 5. *Listeria monocytogenes* populations on turkey sandwiches, apples, and baby carrots according to their location within the cooler.

Salmonella on Sandwiches, Apples, and Carrots

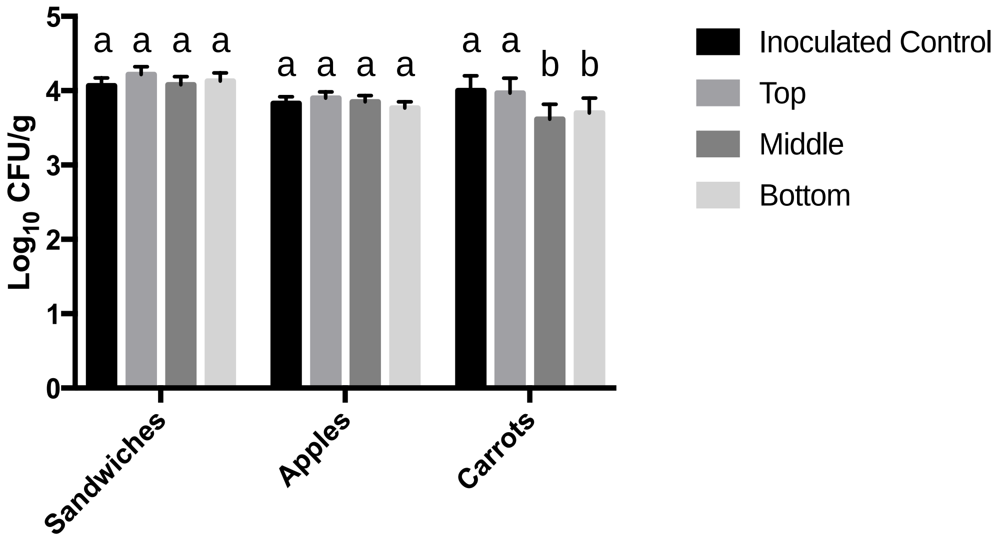
Use of ice, sack lunch location within the cooler, and use of ice x sack lunch location interaction were not statistically significant (p>0.05; Appendix F) for sandwiches and apples inoculated with *Salmonella* (Figures 6 and 7). Although the population difference between the two cooler types was marginal at 0.18 Log_{10} CFU/g, a use of ice effect was approaching statistical significance (p=0.0625) for *Salmonella* on apples (Figure 6). With a p value of 0.0833, a use of ice effect was not observed for *Salmonella* on carrots, with a difference in populations between the cooler with no ice and the cooler with a layer of ice of only 0.13 Log_{10} CFU/g. Not only are these p values not statistically significant, this small degree of variation could be due to natural variation in inoculation and attachment.



* Error bars represent the standard error of the mean.
[a,b] Populations with different superscripts ($p \leq 0.05$) within each product are statistically different. Statistical significance is not compared between products.

Figure 6. *Salmonella* populations on turkey sandwiches, apples, and baby carrots in a cooler packed with no ice and a cooler packed with one layer of ice on the bottom.

Statistical analyses indicate that *Salmonella* populations on carrots did vary statistically (p=0.0022) according to sack lunch location in the cooler (Figure 7). Indeed, the untreated control sample showed the largest *Salmonella* population at 4.00 Log_{10} CFU/g. Therefore, the significant differences observed were due to variation introduced by the smaller *Salmonella* populations on the experimental samples in the cooler, not *Salmonella* growth at various locations throughout the cooler. The use of ice x sack lunch location interaction was not significant (p=0.5817) for *Salmonella* on carrots. The lack of a use of ice x sack lunch location interaction for all products inoculated with *Salmonella* indicates that *Salmonella* populations did not vary by their location within a cooler, regardless of which use if ice method they were exposed to. Thus, the risk for *Salmonella* growth is the same throughout the cooler, whether it is packed with no ice or packed with one layer of ice on the bottom.



* Error bars represent the standard error of the mean.
[a,b] Populations with different superscripts ($p \leq 0.05$) within each product are statistically different. Statistical significance is not compared between products.

Figure 7. *Salmonella* populations on turkey sandwiches, apples, and baby carrots according to their location within the cooler.

As previously discussed, the temperature data in this study suggest that the simulated conditions present a risk for *Salmonella* growth on turkey sandwiches, sliced apples, and carrots. However, this anticipated risk is not sustained by the microbiological data. The lack of *Salmonella* growth on these products throughout the simulation period suggests that five hours is not ample time for the pathogen to grow on these food products under the simulated conditions.

Summary

Controlling time and temperature is critical in protecting food against foodborne pathogen growth during storage (United States Food and Drug Administration (FDA), 2013). School nutrition programs have the challenge of preparing and packing lunches for field trips, maintaining the cold chain, and avoiding the danger zone, particularly on days with outdoor temperatures above 80°F (26.7°C). Controlling the amount of time that school lunches are held at elevated outdoor temperatures (above 80°F [26.7°C]) during a field trip is possible, but the ambient temperature at which the coolers are stored cannot be controlled. The situation is worsened when coolers are packed with little or no ice and are stored on a school bus with temperatures far exceeding already elevated outdoor temperatures. According to the 2013 Food Code (United States Food and Drug Administration (FDA), 2013), time can be used effectively for a maximum of four hours as a public health control when foods have an internal temperature of 41°F (5°C) or less when removed from cold storage. This study was designed to simulate a high-risk scenario, and the recorded data generally suggest that *Salmonella* and *Listeria monocytogenes* populations do not significantly increase after five hours of exposure to extreme conditions. The initial USDA FNS research question was intended to probe the use of time (alone) as a public health control—up to a maximum of four hours—for foods with an initial temperature of ≤ 41°F (5°C) as a means to prevent growth of two pathogens of concern in meals served outside of school. This study evaluated microbial populations in foods held without temperature control in excess of four hours (5 hours) and pathogenic growth was not observed. Thus, the data presented herein provides evidence supporting the use of time as a public health control for storing sack lunches in portable coolers on field trips for a maximum of four hours.

Conclusions and Recommendations

Conclusions

This study was designed to investigate pathogen growth in food products served in school nutrition programs during a simulated high-risk scenario for storing sack lunches in coolers on a hot school bus during field trips. In general, data suggest that as long as the lunches are eaten within the 5-hour simulation period, the risk of foodborne illness as a result of microbial growth from temperature abuse is minimal, even when coolers are packed with little or no ice and subjected to elevated temperatures on a school bus. Thus, these data support the 2013 Food Code (United States Food and Drug Administration (FDA), 2013), which states that relying on time as a public health control is effective for a maximum of four hours when foods have an internal temperature of 41°F (5°C) or less when removed from cold storage. However, it is critical to note that the present study only evaluated populations of *Listeria monocytogenes* and *Salmonella* on turkey sandwiches, apple slices, and baby carrots. Thus, these data are somewhat limited in scope and can only function as an indication of how other pathogens (i.e. *Staphylococcus aureus* and *Escherichia coli*) might behave on sandwiches, sliced apples, carrots, and other food products under similar conditions. Additional studies are necessary to validate the behavior of other pathogens exposed to these products and conditions. The USDA FNS Office of Food Safety is also interested in whether pathogen modeling may be effectively used to address these research needs.

Although the risk for foodborne illness appears to be low under these simulated conditions, it is recommended that sack lunches be packed within insulated coolers. Three layers of ice or ice packs (at the bottom, middle, and top of the cooler) should be used to maintain an appropriate temperature and reduce the amount of time the food products are exposed to the TDZ before the food is consumed. Avoiding cooler storage on school buses with elevated internal temperatures whenever possible is also recommended.

School nutrition program personnel should be educated on the importance of maintaining the cold chain when packing sack lunches for field trips. Education should emphasize packing a cooler with three layers of ice or cold packs (at the bottom, middle, and top of the cooler) as the most effective method for avoiding the temperature danger zone. School bus drivers and educators should also be trained to be mindful of internal bus temperatures so that cooler storage on a bus with elevated temperatures can be avoided whenever possible. While the data suggest little risk if these best practices are not followed, it should be emphasized that this study did not exhaustively investigate all potential foodborne pathogen and food combinations.

Recommendations

The Center proposes the following categorical recommendations:

Research Opportunities

1. Continue investigating the research question originally posed by USDA FNS Office of Food Safety by evaluating additional pathogens using this experimental design to determine if the low risk for pathogenic growth associated with the simulated conditions applies to other foodborne pathogens.
2. Investigate other food products using this experimental design to determine if the low risk associated with the simulated conditions applies to other foods commonly used by school nutrition programs.
    a. Explore the use of pathogen modeling in order to address this research opportunity.
3. Using this experimental design, investigate additional abuse profiles to determine the time and temperature parameters necessary for significant pathogen growth.

Education/Application Opportunities

1. Develop educational materials emphasizing the importance of proper cooler packing and storage for field trips in order to prevent microbial growth.

2. Incorporate study findings and recommendations into Serving Up Science courses and other outreach efforts to disseminate the message further.

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Appendices

Appendix A:

ECTPU Simulation Program

ECTPU Program

Step	**Program**	House[a] Temperature (°F)	Time (minutes)	Relative Humidity (%)
1	Start		1	
2	Heat	75	33	80
3	Heat	85	33	80
4	Heat	95	33	80
5	Heat	105	33	80
6	Heat	115	33	80
7	Heat	125	33	80
8	Heat	135	33	80
9	Heat	145	33	80
10	Heat	150	33	80
11	Stop		1	

[a]The ECTPU program increases temperatures automatically for designated lengths of time. Every 33 minutes the temperature increases 10 degrees until it reaches the target temperature of 150

Appendix B:

Preliminary Data Collected from Various Cooler Packing Scenarios

Preliminary Data Collected from Various Cooler Packing Scenarios

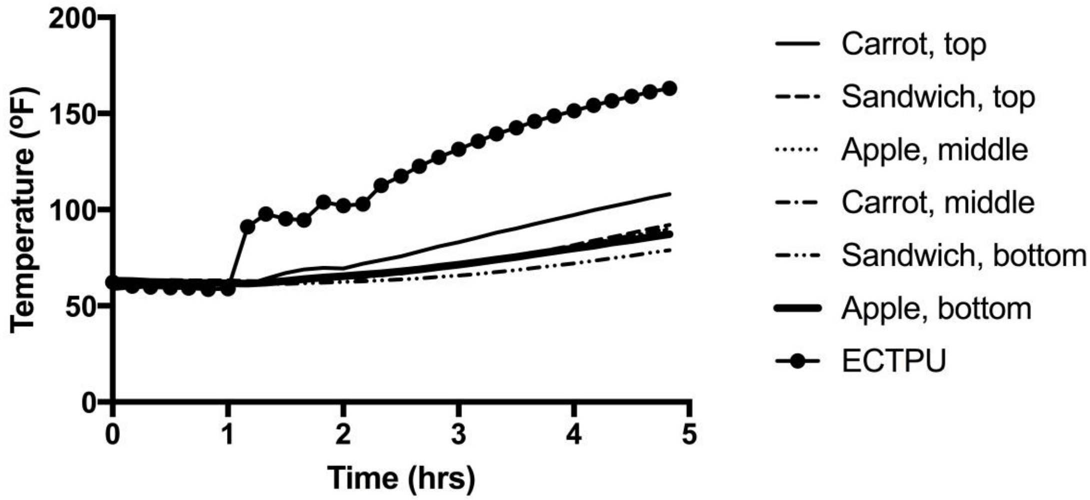


Figure B 1. Temperature profile of turkey sandwiches, baby carrots, and sliced apples in a cooler packed with no ice.

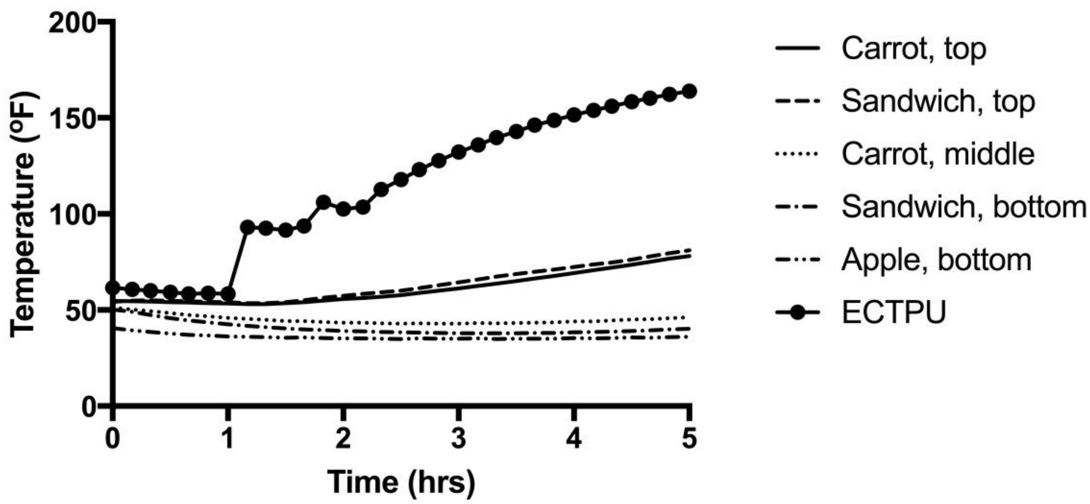
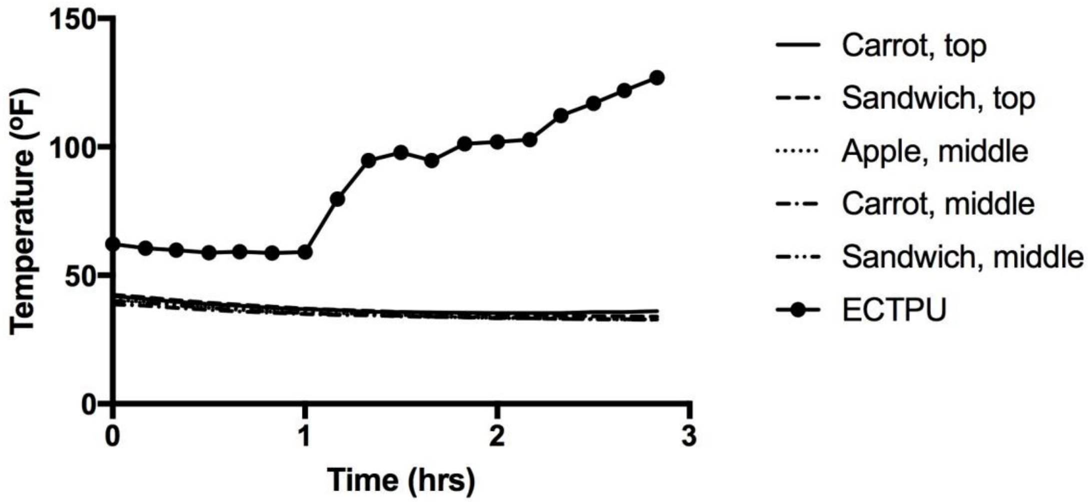


Figure B 2. Temperature profile of turkey sandwiches, baby carrots, and sliced apples in a cooler packed with a layer of ice on the bottom.



*Scenario used a 3 hours cycle to determine the temperature at 3 and 5 hours.

Figure B 3. Temperature profile of turkey sandwiches, baby carrots, and sliced apples in a cooler packed with three layers of ice (bottom, middle, and top)

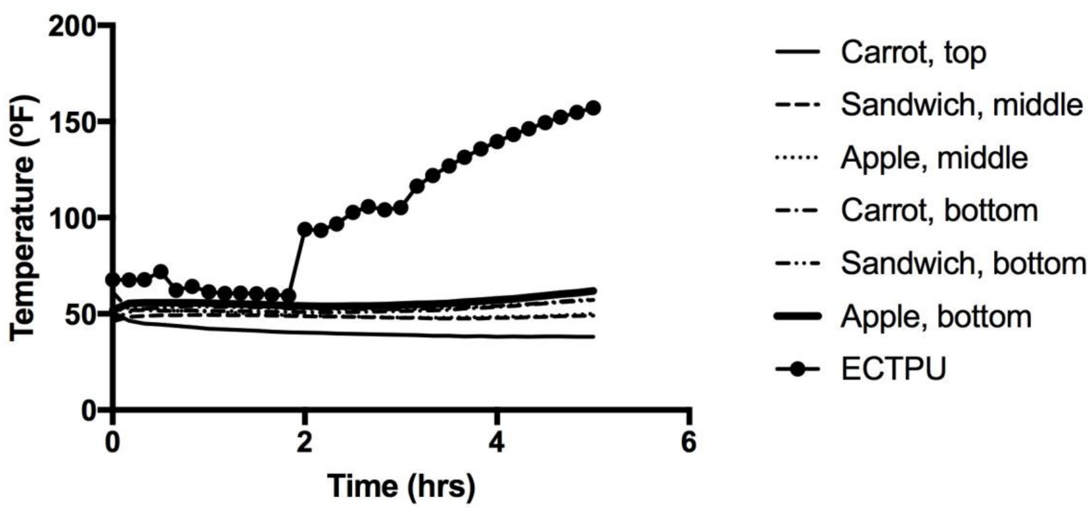


Figure B 4. Temperature profile of turkey sandwiches, baby carrots, and sliced apples in a cooler packed with a layer of ice on the top.

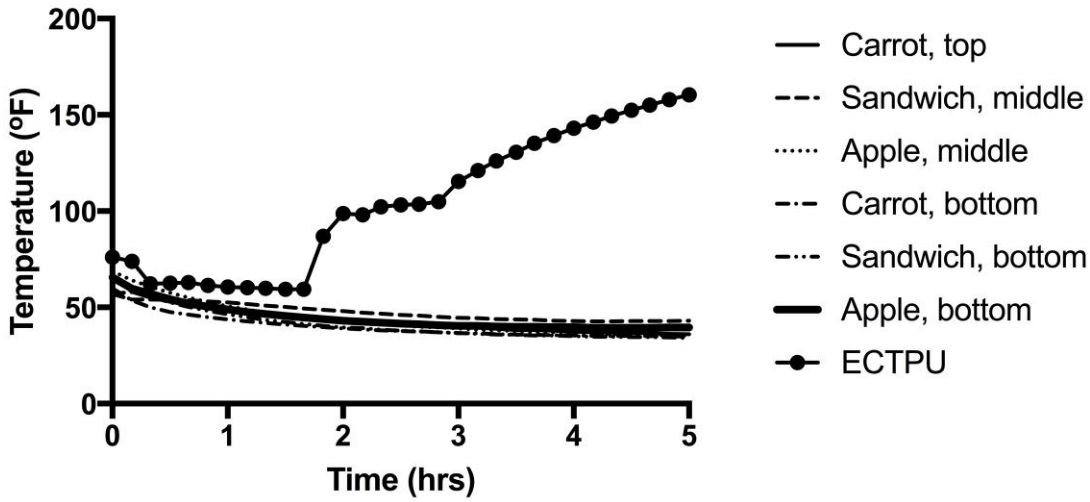


Figure B 5. Temperature profile of turkey sandwiches, baby carrots, and sliced apples in a cooler packed with a layer of ice on the top and on the bottom.

Appendix C:

Maximum, Minimum, and Average Internal and External

Bus Temperatures (°F)

Maximum, Minimum, and Average Internal and External Bus Temperatures (°F)

	North Carolina Interior Average 5/12/15	North Carolina Exterior Average 5/12/15	North Carolina Interior Average 5/13/15	North Carolina Exterior Average 5/13/15	North Carolina Interior Average 5/14/15	North Carolina Exterior Average 5/14/15	North Carolina Interior Average 5/15/15	North Carolina Exterior Average 5/15/15
Avg	93.9	91.3	91.6	86.8	81.7	78.8	82.5	79.0
Max	98.9	100.4	96.4	98.7	85.6	91.2	85.1	88.4
Min	90.9	76.8	85.9	72.5	78.5	62.8	77.6	65.4

	North Carolina Interior Average 5/16/15	North Carolina Exterior Average 5/16/15	North Carolina Interior Average 5/17/15	North Carolina Exterior Average 5/17/15	North Carolina Interior Average 5/18/15	North Carolina Exterior Average 5/18/15	North Carolina Interior Average 5/19/15	North Carolina Exterior Average 5/19/15
Avg	85.4	88.9	86.2	90.9	95.1	93.3	97.9	93.4
Max	92.7	100.3	94.7	101.4	100.1	106.5	101.5	103.1
Min	76.9	70	75.4	70.6	91.6	77.1	91.6	79.4

	North Carolina Interior Average 5/20/15	North Carolina Exterior Average 5/20/15	North Carolina Interior Average 5/21/15	North Carolina Exterior Average 5/21/15	North Carolina Interior Average 5/21/15	North Carolina Exterior Average 5/21/15	Arkansas Interior Average 5/27/15	Arkansas Exterior Average 5/27/15
Avg	96.4	95.3	92.9	90.6	82.0	79.3	79.0	82.4
Max	100.3	106.8	96.9	103.1	86.4	92	91.1	97.6
Min	89.9	78.6	88.1	76.4	78.2	64.5	64.4	71.5

	North Carolina Interior Average 6/2/15	North Carolina Exterior Average 6/2/15	North Carolina Interior Average 6/3/15	North Carolina Exterior Average 6/3/15	North Carolina Interior Average 6/4/15	North Carolina Exterior Average 6/4/15
Avg	97.1	86.4	72.8	68.8	82.7	76.5
Max	104.2	93.4	77.4	72.95	91.25	82
Min	77.3	69.2	69.3	65.7	73.1	70.4

Appendix D:

Internal and External Bus Temperatures:

7:00 AM – 2:00 PM (ºF)

Internal and External Bus Temperatures: 7:00 AM – 2:00 PM (ºF)

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2
	5/12/15	5/12/15	5/12/15	5/12/15	5/13/15	5/13/15	5/13/15	5/13/15
7:00 AM	90.8	77.7	93.8	75.9	89.5	72.5	82.3	72.4
7:10 AM	90.2	77.2	93.1	76.5	89.5	72.7	85.1	72.2
7:20 AM	89.3	78.3	94.4	77.6	89.2	72.7	86.9	72.4
7:30 AM	88.5	79.7	94.5	79.2	88.7	73.1	87.4	72.5
7:40 AM	88.1	80.4	93.9	79.6	88.1	74.4	88	74.2
7:50 AM	87.8	80.2	94	80.3	87.6	75.2	88.3	75.3
8:00 AM	87.7	80.8	94.8	81.2	87	76.1	88.9	76.2
8:10 AM	87.7	81.4	95.5	81.9	86.8	77.7	89.6	77.1
8:20 AM	87.7	82.8	96.4	82.9	86.7	76.9	89.9	76.5
8:30 AM	87.9	84.2	99	84.4	86.6	79	91.2	77.7
8:40 AM	88	85	101.2	84.8	86.7	79.7	90.9	78.7
8:50 AM	87.7	84.6	95.4	84.9	86.7	79.6	91	78.9
9:00 AM	88	86.2	94.1	86.3	86.7	81.1	91.4	80.5
9:10 AM	88.1	87.8	94.5	88.4	87	81.8	91.9	82
9:20 AM	88.2	87.2	94.4	88.5	87.2	85.6	92.6	84.7
9:30 AM	88.3	89.5	95.2	90.2	87.6	85.6	93.3	83.6
9:40 AM	88.6	88.9	94.8	90.2	87.7	85.8	94	84.8
9:50 AM	88.9	89.7	94.5	90.5	88.1	88.2	94.7	86.9
10:00 AM	89.3	90.3	94.9	91.5	88.3	88.1	95	86.8
10:10 AM	89.7	90.5	94.7	91.5	88.6	89.5	95.4	88.9
10:20 AM	90.2	91.1	94.7	92.2	89	88.7	95.6	89.2
10:30 AM	90.6	92.8	95.4	93.4	89.3	87.2	95.6	86.9
10:40 AM	90.8	95.2	95.4	94.9	89.6	88.1	95.4	86.9
10:50 AM	91.1	94.9	95.4	95.1	89.9	89.4	95.5	90.1
11:00 AM	91.8	94.4	94.9	95.4	90.3	93.6	95.5	92.5
11:10 AM	92.4	95.1	95.4	96.5	90.9	91.6	95.6	90.9
11:20 AM	93	98.7	96.2	98.8	91.4	92	95.8	91
11:30 AM	93.5	97.2	96.1	96.6	91.8	89.2	95.6	86.9
11:40 AM	93.8	96.2	96.5	97.1	92	90.4	95.5	91
11:50 AM	94.4	97.8	96.2	98.2	92.4	91.9	95.3	91.9
12:00 PM	94.6	97.7	96.5	98.1	92.6	93.9	95.1	91.6
12:10 PM	94.8	97.3	96.6	98.9	93	94.9	94.9	93.8
12:20 PM	95.5	98.8	96.5	99.1	93.6	94.3	94.9	93.4
12:30 PM	96.2	97.8	97	98.2	93.8	95.2	95.2	93.4
12:40 PM	96.9	98.3	97.1	100.5	94.4	96.8	95.4	95.7
12:50 PM	97.4	99.5	97.4	99.6	94.7	96.4	95.7	94.7
1:00 PM	97.9	97.9	97.7	99.1	95.1	93.4	95.9	93.5

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2
	5/12/15	**5/12/15**	**5/12/15**	**5/12/15**	**5/13/15**	**5/13/15**	**5/13/15**	**5/13/15**
1:10 PM	98.3	98.3	98.5	98.8	95.4	97.1	96.2	96.1
1:20 PM	98.6	99	98.5	100.3	95.5	96.2	96.5	96.1
1:30 PM	99.2	99	98.5	100.3	95.8	96.3	96.9	95.3
1:40 PM	98.5	98.6	94.9	99.4	93.7	98.5	96.3	95.1
1:50 PM	98.8	99.6	94.6	101.2	90.1	99.3	91.1	98
2:00 PM	100.4	98.2	94.8	99.6	93.2	99.1	88.8	98.2
Avg	92.1	91.1	95.8	91.6	90.3	87.2	92.9	86.4
Max	100.4	99.6	101.2	101.2	95.8	99.3	96.9	98.2
Min	87.7	77.2	93.1	75.9	86.6	72.5	82.3	72.2

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2
	5/14/15	**5/14/15**	**5/14/15**	**5/14/15**	**5/15/15**	**5/15/15**	**5/15/15**	**5/15/15**
7:00 AM	83.4	64.6	77.6	63.7	79	66.1	76.2	64.6
7:10 AM	82.7	63.6	80.2	62.7	79.5	66.8	80.5	64.9
7:20 AM	81.9	63.1	81.1	62.4	79.9	66.7	80.9	65.1
7:30 AM	81	63.6	81.3	63	79.8	67.3	80.8	66.3
7:40 AM	79.9	64.1	81.2	63.7	79.5	68.2	80.8	67
7:50 AM	79	64.8	81.2	64.5	79.3	68.1	81	67.3
8:00 AM	78.5	67.7	81.6	66.6	79.3	70	81.5	68.9
8:10 AM	78.3	68.1	80.3	67.7	79.3	71.4	81.7	69.8
8:20 AM	78.2	68	78.3	67.4	79.2	71.1	81.9	70.3
8:30 AM	78.2	70	79.8	69.7	79	73.1	82.2	71.8
8:40 AM	78.3	69.1	79.5	68.6	78.9	73.4	82.3	71.5
8:50 AM	78.1	75	80.1	73.7	78.7	75.7	82.8	73.8
9:00 AM	78.5	75	81	74.1	79	78.9	83.4	76.8
9:10 AM	78.7	76.1	81.2	74.3	79.4	79.1	84.1	76.6
9:20 AM	78.9	78.5	81.3	76.6	79.7	80.6	84.5	77.5
9:30 AM	79.2	76.1	81.4	75.5	80.1	80	84.8	78.8
9:40 AM	79.5	78.2	81.8	78	80.3	81.5	85.4	78.7
9:50 AM	80	78.3	82.9	76.9	80.6	84.3	86.1	82.4
10:00 AM	80.1	77.4	82.4	75.6	81	84.9	86.8	83.9
10:10 AM	80.2	78.6	81.7	78.6	81.5	86.2	87.6	84.4
10:20 AM	80.4	82	81.2	79.8	81.9	86.3	88.1	84.2
10:30 AM	80.8	81.8	80.9	79.2	82.6	88.1	85.1	88.1
10:40 AM	81	82.5	81.4	81.3	83.3	87.6	83.9	89.1
10:50 AM	81.5	82.5	80.3	80.8	84.2	85.1	84.2	86.3
11:00 AM	81.8	83.3	80.3	82	85	84.3	84.4	83.8

	North Carolina Interior 1 5/14/15	North Carolina Exterior 1 5/14/15	North Carolina Interior 2 5/14/15	North Carolina Exterior 2 5/14/15	North Carolina Interior 1 5/15/15	North Carolina Exterior 1 5/15/15	North Carolina Interior 2 5/15/15	North Carolina Exterior 2 5/15/15
11:10 AM	82.2	84.6	80.1	83.2	85.2	83.1	83.8	82.6
11:20 AM	82.8	85.6	80.3	83.2	85.1	82.1	82.6	80.9
11:30 AM	83.3	86.2	81.2	85.1	85	80.9	82.1	79.9
11:40 AM	83.6	86.8	80.8	84.5	85	83.9	83.1	83.5
11:50 AM	84.2	83.5	80.8	82.2	85.2	86.1	83.6	84.6
12:00 PM	84.8	85.9	81	84.7	85.4	85.9	82.9	84
12:10 PM	85.1	88.6	80.7	85.9	85.5	84.7	83.6	84.3
12:20 PM	85.6	88.8	81	85.6	85.6	84.4	82.5	82.2
12:30 PM	86.1	88.8	80.5	86.1	85.2	79.9	81.2	79.2
12:40 PM	86.7	87.6	81	85.1	84.7	77.8	80.3	76.3
12:50 PM	87	87.8	81	86.7	84	76.8	79.3	75.4
1:00 PM	87.7	89.7	81.7	87.8	83.5	77.7	79.6	77.5
1:10 PM	87.9	89.2	82	87.3	83.2	79.8	80.7	79.1
1:20 PM	88.3	88.4	82.8	86.9	83.2	83.9	81.3	83.7
1:30 PM	88.2	91	82.2	89.2	83.7	86.2	81.9	86.1
1:40 PM	86.3	90.9	84.1	88.1	83.9	87.6	83.1	85.6
1:50 PM	85.8	92.3	82.6	90	84.3	89.2	83.3	87.4
2:00 PM	86.6	91.4	81.7	88.9	85.5	88.6	84.6	87.9
Avg	82.3	79.5	81.1	78.1	82.2	79.6	82.8	78.4
Max	88.3	92.3	84.1	90	85.6	89.2	88.1	89.1
Min	78.1	63.1	77.6	62.4	78.7	66.1	76.2	64.6

	North Carolina Interior 1 5/16/15	North Carolina Exterior 1 5/16/15	North Carolina Interior 2 5/16/15	North Carolina Exterior 2 5/16/15	North Carolina Interior 1 5/17/15	North Carolina Exterior 1 5/17/15	North Carolina Interior 2 5/17/15	North Carolina Exterior 2 5/17/15
7:00 AM	75.5	72.4	79	67.6	73.7	72	77	69.2
7:10 AM	79.9	74.7	83.9	70.4	74.5	73.2	76.7	70.6
7:20 AM	81.4	77.1	86.6	73.2	78.9	75.7	84.7	73.5
7:30 AM	75.5	77.2	84.9	76.4	74.7	77.8	84.8	76.2
7:40 AM	74	78	81.7	77	73.7	77.8	81.5	76.5
7:50 AM	74.6	76.8	79.2	77.3	73.7	76.2	78.4	75.2
8:00 AM	75.7	77.9	78.3	78.4	74.9	81.3	79.5	79.9
8:10 AM	76.8	78.7	79.6	79.2	76.1	80.7	79.9	81.1
8:20 AM	77.8	81.8	79.9	81.6	77.4	81.5	81.5	81
8:30 AM	78.9	81.5	80.5	82.3	78.1	80.4	81.1	80.5
8:40 AM	80.1	83.3	84.4	82.7	79.3	85	86.5	85.4
8:50 AM	81.2	86.2	82.2	84.7	80.6	86.2	83.9	86.6
9:00 AM	82	85	81.6	85.3	81.9	86.1	83.6	87.5
9:10 AM	82.9	86.6	82.3	87.2	83.1	88.1	83.8	87.6

	North Carolina Interior 1 5/16/15	North Carolina Exterior 1 5/16/15	North Carolina Interior 2 5/16/15	North Carolina Exterior 2 5/16/15	North Carolina Interior 1 5/17/15	North Carolina Exterior 1 5/17/15	North Carolina Interior 2 5/17/15	North Carolina Exterior 2 5/17/15
9:20 AM	83.8	88.3	82.1	87.8	84.1	86.2	83.9	85.6
9:30 AM	85.1	90.1	83.5	87.4	84.9	87.8	84.7	88.9
9:40 AM	85.8	87.9	83.2	87	85.7	88.8	85.3	89.2
9:50 AM	86	87.7	83.1	88.5	86.3	90.5	85.6	90.2
10:00 AM	86.1	88.7	83.3	87.9	87	90.2	86.2	90.9
10:10 AM	86.3	88.8	83.1	89.5	87.6	89.7	86.7	91.4
10:20 AM	87	93.1	83.6	91.4	88.1	91.8	86.5	92.4
10:30 AM	87.7	93.8	84.5	94	88.4	93.2	87.2	94.9
10:40 AM	88.1	92	84.7	92.2	88.9	93.7	86.9	94.9
10:50 AM	88.2	94	85	94	89	93.8	87.3	94.5
11:00 AM	88.4	90.3	85.4	91.9	89.4	94.9	87.3	95.6
11:10 AM	88.9	94.9	85.2	93.3	89.8	98.7	87.6	96.6
11:20 AM	89.3	97.2	85.4	94.9	90.1	99	87.1	99.1
11:30 AM	90.2	97.1	85.5	95	90.4	99.8	87.7	98.6
11:40 AM	90.2	91.1	85.5	91.6	91.1	96.6	87.6	96.6
11:50 AM	90.2	96.2	85.2	96.6	91.4	96.8	88.1	97.7
12:00 PM	91.2	98.9	86.2	97.7	91.4	99.4	87.6	99.9
12:10 PM	91.9	101.5	87.2	99	92.1	100.1	88	99
12:20 PM	92.4	92.9	87.3	95.2	92.2	98.1	88.5	98.6
12:30 PM	92.8	92.8	87.7	93.3	92.8	100.9	87.9	98.7
12:40 PM	93.3	92.8	87.3	91.2	93	98.8	89.2	99.8
12:50 PM	93.5	97.3	88.1	97.6	93.9	100.9	88.9	98.3
1:00 PM	94	89.4	87.2	89	94.5	101.4	89	99.4
1:10 PM	94.1	91.2	86.7	90	94.9	98.9	89.7	99.7
1:20 PM	94.2	96.1	87.2	94.9	95.4	99.9	90.5	100.2
1:30 PM	94.5	99.8	88.3	98.7	95.9	100.8	91	100.5
1:40 PM	95.1	98.8	88.5	97.8	96.4	98.3	91.6	98.8
1:50 PM	95.8	98.2	88.3	95.1	97	100	91.7	99.6
2:00 PM	96.2	99.4	89.1	98.4	97.9	102.6	91.5	100.2
Avg	86.4	89.2	84.5	88.5	86.5	91.0	85.9	90.7
Max	96.2	101.5	89.1	99	97.9	102.6	91.7	100.5
Min	74	72.4	78.3	67.6	73.7	72	76.7	69.2

	North Carolina Interior 1 5/18/15	North Carolina Exterior 1 5/18/15	North Carolina Interior 2 5/18/15	North Carolina Exterior 2 5/18/15	North Carolina Interior 1 5/19/15	North Carolina Exterior 1 5/19/15	North Carolina Interior 2 5/19/15	North Carolina Exterior 2 5/19/15
7:00 AM	90	78.3	93.2	75.9	89.3	80.8	93.8	78
7:10 AM	89.4	78.4	96.9	76.8	89.7	81.1	98.4	79.1
7:20 AM	88.8	79.1	99	77.7	89.9	82	101	80.1

	North Carolina Interior 1 5/18/15	North Carolina Exterior 1 5/18/15	North Carolina Interior 2 5/18/15	North Carolina Exterior 2 5/18/15	North Carolina Interior 1 5/19/15	North Carolina Exterior 1 5/19/15	North Carolina Interior 2 5/19/15	North Carolina Exterior 2 5/19/15
7:30 AM	88.3	79.9	100.8	79.2	89.8	82.1	102.7	81
7:40 AM	87.9	81	98.2	80.5	89.1	83.9	102.3	82.7
7:50 AM	87.9	82	97	81.1	89.3	86.4	100	85.2
8:00 AM	88	81.8	97.2	81.5	89.3	87.5	100	87.3
8:10 AM	87.9	84.7	97.8	84.6	89.6	86.8	100.5	87.2
8:20 AM	88.1	88	96.3	86.9	89.3	85.6	100.6	86
8:30 AM	88.3	87.3	103.3	87.2	89.3	86.2	100.6	86.3
8:40 AM	88.5	86.9	102.3	87.7	89.3	88.2	101.1	88.3
8:50 AM	88.9	85.8	95.9	87.1	89.6	93.7	102.2	94.3
9:00 AM	88.9	88.8	94.5	88.4	90.2	91	103	93.4
9:10 AM	89.2	88.9	95.1	89.3	90.2	87.2	103	88.4
9:20 AM	89.3	90	94.4	90.2	90.2	88.5	103.3	89.2
9:30 AM	89.7	91.3	95.2	92.5	90.3	91.3	103.7	91.8
9:40 AM	90	92.4	95.1	93.1	90.6	91.6	103.9	93.2
9:50 AM	90.4	92	95.6	92.6	90.8	95	104.3	95.2
10:00 AM	90.8	94.8	96	94.5	91.4	96.3	104.5	96.6
10:10 AM	91.2	95.7	95.8	96.7	91.4	98.1	104.4	96.8
10:20 AM	91.8	93.7	95.8	94.8	91.8	97.9	104.3	98.2
10:30 AM	92.1	94.6	96.6	94.9	92.2	94.5	104.1	96.1
10:40 AM	92.6	94.8	96.3	95.2	92.6	97.2	104.1	97.5
10:50 AM	93	94.9	96.6	96.1	93	98.3	104	99.1
11:00 AM	93.4	97.4	96.2	98.1	93.6	97.2	103.9	99
11:10 AM	94	97	96.2	96.1	94.4	96.1	103.9	96.7
11:20 AM	94.5	99	96.3	99	94.6	96.8	103.8	96.5
11:30 AM	95	98.9	96.4	100	94.6	97.5	103.3	97.5
11:40 AM	95.8	99.8	96.9	100.7	94.9	101	102.9	99.3
11:50 AM	96.4	99	96.8	100.8	95.6	99.4	102.9	100.7
12:00 PM	97.1	100.1	97.1	101.1	96	99.4	103.1	101
12:10 PM	97.4	98.2	96.4	98.7	97.1	99.4	103.6	99.6
12:20 PM	98.1	96.6	95.8	95.8	97.4	93.2	103.9	93.4
12:30 PM	97.9	91.7	95.9	91.3	97.4	94	104.1	94.3
12:40 PM	97.9	100.8	96.2	100.3	97.7	95.5	104.4	95.5
12:50 PM	98.3	104.3	96.5	104	97.5	96.5	104.5	96.1
1:00 PM	98.9	101.6	97.2	103.4	97.5	98	104.6	97
1:10 PM	99.5	102.5	97.6	102.1	97.3	96.1	104.6	95.6
1:20 PM	100.2	106.3	97.6	106.6	97.3	95.6	104.7	96.1
1:30 PM	100.8	105.5	97.8	104.4	97.8	100.2	105.2	100.5
1:40 PM	101.5	101.9	97.6	101.9	97.8	103.3	105.1	101.9
1:50 PM	101.1	101.6	98.9	102.5	98.5	102.8	100.6	102.1
2:00 PM	101.6	101.9	98.5	101.7	99.6	103.5	96.7	102.6

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2
	5/18/15	**5/18/15**	**5/18/15**	**5/18/15**	**5/19/15**	**5/19/15**	**5/19/15**	**5/19/15**
Avg	93.3	93.2	96.9	93.3	93.1	93.4	102.7	93.4
Max	101.6	106.3	103.3	106.6	99.6	103.5	105.2	102.6
Min	87.9	78.3	93.2	75.9	89.1	80.8	93.8	78

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2
	5/20/15	**5/20/15**	**5/20/15**	**5/20/15**	**5/21/15**	**5/21/15**	**5/21/15**	**5/21/15**
7:00 AM	86.8	80.7	93.1	76.5	90.5	77	85.6	75.8
7:10 AM	90.9	81.7	98.8	78.6	90.7	77.3	88.2	75.9
7:20 AM	92.4	83.8	100.8	80.2	90.1	77.2	90.1	75.5
7:30 AM	92.9	84.2	102.5	81.7	89.9	79.8	91.3	77.6
7:40 AM	93	84.9	100.2	83.2	89.7	81.1	91.2	78.7
7:50 AM	93.1	86.3	99.4	84.4	89.7	80.4	91.1	78.8
8:00 AM	93.2	86	99.9	84.9	89.5	80.6	90.9	79.1
8:10 AM	92	87.4	100.7	86.1	89.1	80.4	91	79.4
8:20 AM	91.8	88.1	101.8	86.9	88.5	83.3	91.6	81.8
8:30 AM	91.7	89.7	108.9	88.3	88.5	84.5	92.2	82.8
8:40 AM	91.3	90.4	102.7	88.9	88.2	86.1	92.7	84
8:50 AM	91.8	91.9	98.4	90.4	88.3	86.9	93.3	85.2
9:00 AM	92	91.9	98.1	91.3	88.5	88.4	94.1	86.6
9:10 AM	92.1	94.5	97.7	93.6	88.8	91.7	94.7	89.5
9:20 AM	92.4	96.1	98.1	93.8	88.9	88	94.7	86.9
9:30 AM	92.5	93.6	97.5	92.1	88.9	88	94.5	86.6
9:40 AM	92.5	94.3	96.9	94	89.2	92.5	94.8	90.3
9:50 AM	92.6	97.3	97	94.5	89.6	92.5	93.2	91.4
10:00 AM	92.8	96.1	96.4	93.9	90.1	91.7	93.2	89.8
10:10 AM	93.1	96.5	97.1	96.4	90.2	91	92.6	90.5
10:20 AM	93.4	98.7	97.3	97.2	90.3	94.6	92.7	94.4
10:30 AM	93.8	98	96.9	98.1	90.7	97.2	93.7	94.4
10:40 AM	93.7	98.1	96.3	95.8	91.2	96.1	94.1	93.5
10:50 AM	93.9	102.1	96	101.4	91.8	95.7	93.6	93.5
11:00 AM	94.5	102.2	96.5	100.7	92.3	95.6	93.4	96.4
11:10 AM	94.6	102.5	96.2	100.1	92.6	96.2	94	97.1
11:20 AM	94.8	103.9	95.7	102.1	93	95.7	94.1	96.6
11:30 AM	95.5	104.4	96.1	101	93.7	97.1	94.6	98.7
11:40 AM	96	107.6	95.6	105.9	94.2	99.8	95.4	99.3
11:50 AM	96.6	106.6	96.7	103.7	95	104.3	95.7	101.9
12:00 PM	97.3	105.1	96.9	102	95.8	96.4	95.3	98.3
12:10 PM	98.2	104.2	97.5	103.2	96.5	97.5	95.6	98.7

	North Carolina Interior 1 5/20/15	North Carolina Exterior 1 5/20/15	North Carolina Interior 2 5/20/15	North Carolina Exterior 2 5/20/15	North Carolina Interior 1 5/21/15	North Carolina Exterior 1 5/21/15	North Carolina Interior 2 5/21/15	North Carolina Exterior 2 5/21/15
12:20 PM	98.7	101.6	97	102	96.9	98.2	96.1	97.4
12:30 PM	99	102.8	97.7	103.2	97.1	98.6	95.6	96.2
12:40 PM	99.2	100.1	98.2	99.6	97.2	100.3	96.5	99
12:50 PM	99.7	100.2	98.6	100	97.5	98	96	97.2
1:00 PM	99.6	98.9	98.2	99	97.1	96.2	95.9	96.2
1:10 PM	99.3	96.8	97.8	94.1	97.3	98.5	96.3	97.7
1:20 PM	99.1	98.2	97.6	98.8	97.3	96.7	96.2	96.6
1:30 PM	99.3	98.8	98	99.9	97.4	94.8	96.2	94.1
1:40 PM	100.6	98.8	96.6	98.7	97.1	96.2	96.4	96.9
1:50 PM	99.6	100	93.3	99.4	95.7	89.8	93.9	89.5
2:00 PM	100.6	101.3	91.7	102.5	95.7	86.2	88.4	85.9
Avg	94.8	96.0	97.9	94.6	92.3	91.1	93.5	90.1
Max	100.6	107.6	108.9	105.9	97.5	104.3	96.5	101.9
Min	86.8	80.7	91.7	76.5	88.2	77	85.6	75.5

	North Carolina Interior 1 5/22/15	North Carolina Exterior 1 5/22/15	North Carolina Interior 2 5/22/15	North Carolina Exterior 2 5/22/15	Arkansas Interior 1 5/27/15	Arkansas Exterior 1 5/27/15	Arkansas Interior 2 5/27/15	Arkansas Exterior 2 5/27/15
7:00 AM	82	64.5	74.3	64.4	78.2	71.5	77	71.5
7:10 AM	81.9	67.6	83.3	65.8	79.7	72.4	78.8	72.3
7:20 AM	81.9	67	84.9	66.4	81	73.3	80.5	73.1
7:30 AM	81.5	67.4	86.6	66	82.1	73.7	81.6	73.7
7:40 AM	81.2	68.6	87.4	66.8	81.4	74.4	81.8	74.1
7:50 AM	80.6	68.2	85.4	67.6	81.6	75.4	81.9	74.8
8:00 AM	80.1	68.1	84.8	68	82.6	75.7	82.6	76
8:10 AM	79.7	71	85.3	70	82.1	75.9	82.4	76.5
8:20 AM	79.5	70.2	85.3	69.6	83.2	76.5	82.9	76.5
8:30 AM	79.3	71.6	84.2	70.9	88.3	76.3	85	76.4
8:40 AM	79.1	71.9	83.8	70.9	92.4	77.1	87.7	77.1
8:50 AM	78.8	71.4	83.8	71.3	94.9	76.1	90.1	76.8
9:00 AM	78.7	74.4	82.8	73.1	97.2	77.3	92.4	77.4
9:10 AM	78.8	75.3	84	73.8	98.8	77.8	94.5	77.7
9:20 AM	78.8	76.7	83.6	75.1	99.8	80.5	96.1	77.8
9:30 AM	79	79.1	82.9	76.8	101	79.2	97.4	78.6
9:40 AM	79.2	78.3	82.9	77.1	102.2	81.8	98.7	80.9
9:50 AM	78.2	80.6	82.4	78.4	102.9	80.8	99.9	80.1
10:00 AM	78.3	81.8	81.7	80	103.4	82.3	100.8	81.8
10:10 AM	78.1	80.8	81.7	78.1	102.6	80.8	101.3	80.3
10:20 AM	78.5	80.9	83	80.7	101.6	81.6	100.9	81.1

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2	Arkansas Interior 1	Arkansas Exterior 1	Arkansas Interior 2	Arkansas Exterior 2
	5/22/15	**5/22/15**	**5/22/15**	**5/22/15**	**5/27/15**	**5/27/15**	**5/27/15**	**5/27/15**
10:30 AM	78.8	84.9	82.3	83.3	101.6	81.5	100.7	80.9
10:40 AM	79	81.5	83.2	81.7	101	81.6	100.5	81.9
10:50 AM	79.1	82.3	82.7	81.5	101.1	82.3	100.3	82.1
11:00 AM	79.2	87.5	81.5	85.1	101.8	83.7	100.7	83
11:10 AM	79.5	86.2	82.1	84.6	101.5	85.9	101.2	84.7
11:20 AM	79.9	82.4	82.8	83.2	100.8	85.1	101.1	83.8
11:30 AM	80.3	87.3	81.8	85.8	100.6	85	100.9	84.1
11:40 AM	80.3	82	80.9	79.8	101	84.2	101	83.5
11:50 AM	81	86.3	81.5	83.7	101.8	85.9	101.9	85.7
12:00 PM	81	85.9	81.4	87	102.5	87.4	102.8	86.9
12:10 PM	81.2	88	81.4	84.7	103.1	86.9	103.6	88.1
12:20 PM	81.2	84.8	81.5	83.1	103	86.4	104.2	86
12:30 PM	81.7	86.3	81.8	85.4	101.5	88.1	103.7	86.7
12:40 PM	82.2	88.4	82.2	89.4	101.5	86.9	103.5	87.9
12:50 PM	83.2	90.9	82.4	85.7	99.8	88.4	102.3	88.8
1:00 PM	83.7	87.4	83.5	87.3	100.2	91.2	102	91
1:10 PM	84.2	86.9	83.5	87.4	99.1	89.3	101	90.9
1:20 PM	84.8	88	84.2	87	97.1	96.1	101.3	96.5
1:30 PM	84.3	93.8	83.1	90.2	95.8	96.9	99.3	98.2
1:40 PM	85.5	89.3	83.4	87.8	93	94.7	97	95.6
1:50 PM	87.5	87.8	84.1	86.7	94.2	89.3	97.7	89.8
2:00 PM	88.8	85.3	84	82.4	93.4	87.6	99	88.1
Avg	80.9	80.0	83.0	78.7	95.6	82.4	95.3	82.3
Max	88.8	93.8	87.4	90.2	103.4	96.9	104.2	98.2
Min	78.1	64.5	74.3	64.4	78.2	71.5	77	71.5

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2
	6/2/15	**6/2/15**	**6/2/15**	**6/2/15**	**6/3/15**	**6/3/15**	**6/3/15**	**6/3/15**
7:00 AM	*	*	*	*	71.9	66.1	70.6	66.1
7:10 AM	*	*	*	*	71.9	66.4	71	66.3
7:20 AM	82.9	*	88.8	*	72.7	66.4	71.8	66.5
7:30 AM	85.8	*	92.9	*	72.8	66.3	72.1	66.2
7:40 AM	88.5	85.1	95.3	84	72.6	66.1	72	66.1
7:50 AM	90.6	84.7	98	83.7	72.4	65.7	71.7	65.7
8:00 AM	91.4	81.3	97.1	82.3	72	65.7	71.6	65.8
8:10 AM	90.2	81.3	93.8	80.7	71.9	65.7	71.4	66
8:20 AM	90.5	87.5	95.3	84.5	71.6	65.9	71.1	66
8:30 AM	92.1	86.7	98.3	85.2	71.3	66.1	70.9	66.1

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2
	6/2/15	**6/2/15**	**6/2/15**	**6/2/15**	**6/3/15**	**6/3/15**	**6/3/15**	**6/3/15**
8:40 AM	93.6	87.7	99.7	85.9	71.1	66.2	70.7	66.2
8:50 AM	93.5	82.6	98.5	83.3	70.9	66.4	70.5	66.5
9:00 AM	94	90.5	99.6	87.1	70.9	66.7	70.4	66.6
9:10 AM	95.1	87.2	100.6	85.6	70.8	66.9	70.4	66.8
9:20 AM	95.8	83.8	100.7	84.5	70.9	67	70.4	66.8
9:30 AM	95.9	88	101	86.5	71	67.4	70.6	67.2
9:40 AM	96.1	86.9	101	86.4	71.1	67.5	70.7	67.4
9:50 AM	96.3	86.4	101	87.1	71.2	67.9	71	67.8
10:00 AM	96.4	86.1	100.5	87.3	71.6	68.3	71.4	68
10:10 AM	96.8	89.2	100.9	91	72	68.9	71.8	68.3
10:20 AM	97.8	88.8	102.1	89.6	72.3	69.1	72.3	68.5
10:30 AM	99	88.7	103	91.8	72.6	69.3	72.7	68.8
10:40 AM	99.9	88.3	103.2	92.8	72.9	69.5	72.9	68.9
10:50 AM	100.5	88.9	103.6	93.3	73	69.5	73.1	69.2
11:00 AM	100	87.9	103.2	92.2	73.1	69.5	73.1	69
11:10 AM	100.4	90.4	103.6	92.1	73.2	69.5	73.1	69
11:20 AM	100	87.4	102.8	88.3	73.2	69.9	73.1	69.1
11:30 AM	100	89.1	102.6	93.6	73.3	70	73.3	69.3
11:40 AM	100.7	90.6	103	95.5	73.7	70.4	73.6	69.7
11:50 AM	101.8	90	103.8	95.6	73.8	70.4	73.7	69.6
12:00 PM	102.7	90.4	104.2	96.4	74.2	70.8	74	69.9
12:10 PM	102.9	88.9	104	94.4	74.7	71	74.7	70.2
12:20 PM	102.6	90	103.8	94	75.4	71.3	75.3	70.5
12:30 PM	102.6	90.8	103.5	95.6	75.6	71.1	75.7	70.7
12:40 PM	103.9	90.2	104.4	96.2	76	71.8	76	71.3
12:50 PM	103.9	87.5	103.8	91.7	76.7	71.8	76.6	71.4
1:00 PM	104.1	89.7	104	93.9	77.5	72.1	77.3	71.7
1:10 PM	102.1	83.6	102.7	81.7	77.1	71.2	77.2	71.4
1:20 PM	96.8	76.5	97.3	76.6	76.7	72	76.9	72.1
1:30 PM	85.9	75.8	87.9	76.1	68.5	71.2	72.4	71.7
1:40 PM	74.4	73.5	80.1	74	67.7	72.4	70.8	72.6
1:50 PM	79.3	70.2	82.5	70.2	73.2	71.5	74.7	72.1
2:00 PM	79.8	69.1	82.4	69.3	76	72.8	76.8	73.1
Avg	95.3	85.7	98.9	87.2	72.9	68.9	72.8	68.7
Max	104.1	90.8	104.4	96.4	77.5	72.8	77.3	73.1
Min	74.4	69.1	80.1	69.3	67.7	65.7	70.4	65.7

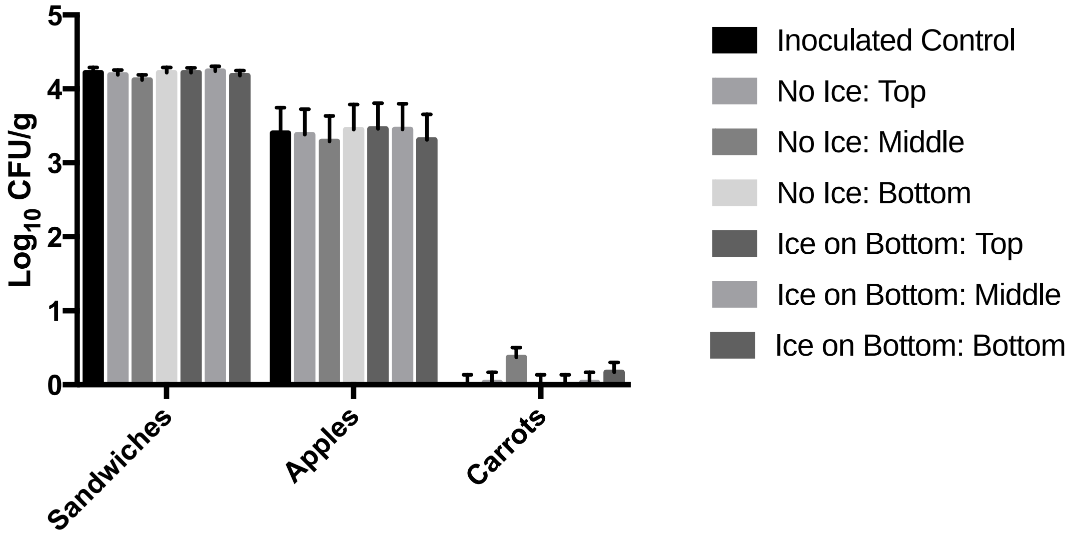
	North Carolina Interior 1 6/4/15	North Carolina Exterior 1 6/4/15	North Carolina Interior 2 6/4/15	North Carolina Exterior 2 6/4/15
7:00 AM	75.4	70.3	74.3	70.5
7:10 AM	73.5	70.5	72.7	70.7
7:20 AM	74	70.6	73	70.9
7:30 AM	74.4	70.7	73.3	70.9
7:40 AM	74.4	70.7	73.2	71
7:50 AM	74.4	71	73.2	71.1
8:00 AM	74.4	71.1	73.3	71.4
8:10 AM	74.8	72	73.7	71.9
8:20 AM	75.9	72.6	75.3	72.7
8:30 AM	76.6	72.1	76	72.9
8:40 AM	77.4	72.4	76.8	73.2
8:50 AM	78.6	72.9	78	74
9:00 AM	79.6	73.2	79	74.8
9:10 AM	80.2	73.5	79.7	74.9
9:20 AM	81	74.1	80.3	76.1
9:30 AM	81.6	75.3	80.9	75.6
9:40 AM	82.5	76	81.8	77
9:50 AM	83.5	77	83.1	79.1
10:00 AM	84.3	76.2	83.9	78.2
10:10 AM	84.5	76.6	84.1	77.3
10:20 AM	85.1	77.6	84.8	79
10:30 AM	85.9	78.6	85.8	80.3
10:40 AM	87	78.5	87	79.7
10:50 AM	88.3	80.5	88.4	80.8
11:00 AM	89.7	78.4	89.8	79.6
11:10 AM	89.6	77.5	89.5	78.7
11:20 AM	89.2	77.6	89.2	78.3
11:30 AM	88.6	76.7	88.6	78.7
11:40 AM	87.7	76.9	87.6	77.8
11:50 AM	88.2	78.4	88	80.1
12:00 PM	89.2	79.2	88.8	79.3
12:10 PM	90.1	79	89.5	80.8
12:20 PM	90.7	78.9	89.9	80.5
12:30 PM	91.5	81.5	90.4	82.5
12:40 PM	91.9	80	90.6	82.4
12:50 PM	91.4	79.6	90.1	80.7
1:00 PM	90.7	79	89.3	79.2
1:10 PM	89.8	78	88.4	78
1:20 PM	89.3	78.8	88.2	80.2
1:30 PM	69.2	79.6	77.5	79.2

	North Carolina Interior 1	North Carolina Exterior 1	North Carolina Interior 2	North Carolina Exterior 2
	6/4/15	6/4/15	6/4/15	6/4/15
1:40 PM	72.3	79.2	79.3	79
1:50 PM	79.8	80.8	83.6	80.4
2:00 PM	81.7	78.8	84.4	79.4
Avg	82.7	76.1	82.7	76.9
Max	91.9	81.5	90.6	82.5
Min	69.2	70.3	72.7	70.5

Appendix E:

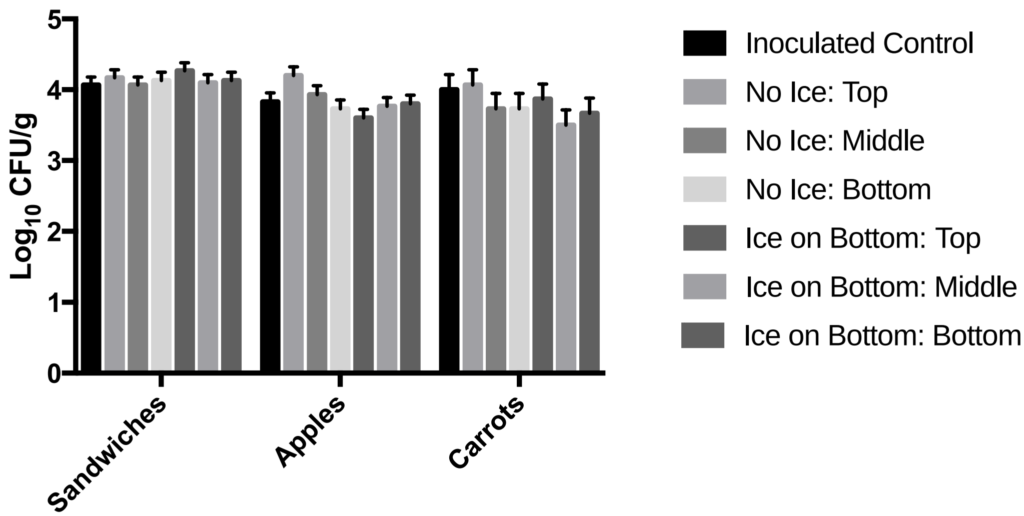
Compilation of Pathogen Data from Both Coolers and All Products

Compilation of Pathogen Data from Both Coolers and All Products



* Error bars represent the standard error of the mean.
** The use of ice x sack lunch location interaction was not significant (*P*>0.05). Data illustrate trends, not statistical significance.

Figure D 1. *Listeria monocytogenes* populations recovered from turkey sandwiches, baby carrots, and sliced apples packed in a cooler with no ice and a cooler with a layer of ice on the bottom.



* Error bars represent the standard error of the mean.
** The use of ice x sack lunch location interaction was not significant (P>0.05). Data illustrate trends, not statistical significance.

Figure D 2. *Salmonella* populations recovered from turkey sandwiches, baby carrots, and sliced apples packed into a cooler with no ice and a cooler with a layer of ice on the bottom.

Appendix F:

Statistical Significance of Main Effects and the Interaction for *Salmonella* and *Listeria monoctygoenes* on Carrots, Apples, and Sandwiches

Statistical Significance of Main Effects and the Interaction for *Salmonella* and *Listeria monoctygoenes* on Carrots, Apples, and Sandwiches

Salmonella			
Product	**Use of Ice**	**Sack Lunch Location**	**Use of Ice x Sack Lunch Location Interaction**
Carrots	p=0.0833	p =0.0022	p =0.5817
Apples	p =0.0625	p =0.7570	p =0.0648
Sandwich	p =0.5150	p =0.1894	p =0.8787

Listeria monocytogenes			
Product	**Use of Ice**	**Sack Lunch Location**	**Use of Ice x Sack Lunch Location Interaction**
Carrots	p =0.6048	p =0.4548	p =0.3409
Apples	p =0.6909	p =0.9398	p =0.4219
Sandwich	p =0.9504	p =0.9116	p =0.2744