Executive Summary

Title: Investigating the Development of Thermal Processing Tools to Improve the Safety of Ready-To-Eat Meat and Poultry Products

Principle Investigators: Jeffrey J. Sindelar[a], Kathleen Glass[a], Robert Hanson[b]

Research Institution: [a]University of Wisconsin-Madison and [b]HansonTech

Final Report Submitted on: March 1, 2016

Objectives

- Investigate the effect that certain thermal processing parameters and product characteristics have on the thermal inactivation of pathogenic *Salmonella* and *L. monocytogenes*;
- Develop scientifically based, regulatory supported, and industry useful thermal processing parameters to assure pathogen destruction and regulatory compliance in pre-cooked meats considering the vast array of products and thermal processing methods.

Conclusions:

This study investigated the validity of thermal processes designed to meet USDA, FSIS Appendix A lethality requirements for four different ready-to-eat, small dimension, fast-cook products (frankfurters, beef patties, chicken patties, chicken tenders). Results from this study confirmed that cooking temperatures and times that are currently being widely used in the meat and poultry industries following USDA, FSIS Appendix A for thermal lethality are sufficient to kill 1) *Salmonella* when the end point cooking temperatures meet or exceed 60.0°C (140°F) for non-impingement processes and 2) *L. monocytogenes* when the end point cooking temperatures meet or exceed 71.1°C (160°F) for non-impingement processes. Additionally, wet-bulb time/temperature target identified in this study (DB = 79.4°C (175°F, WB = 65.6°C (150°F), 15 minutes) may be a suitable replacement for Appendix A relative humidity requirements during smokehouse processing.

In contrast, *Salmonella* was found to be more thermotolerant than *L. monocytogenes* during rapid cook impingement processes (≤4.0 minutes). As product size and cook times decreased, a surface lethality step (SLS) was necessary to counter the protective effects of dehydration for pathogens — especially *Salmonella* — on the product surface. The protective effect of dehydration at the product surface was more pronounced for *Salmonella* than for *L. monocytogenes*, and therefore higher wet-bulb temperatures or SLS steps with longer dwell times may be necessary to assure 5-log kills of *Salmonella.* Understanding and instituting integrated lethality concepts are critical to ensuring the safety and efficacy of the lethality steps during these processes. The integrated process lethality profiles developed from this study will be useful tools for establishments to better evaluate overall pathogen lethality of their products and processes. Finally, the results of this study have also enabled the on-going development of new easy-to-use time-temperature tables for frankfurters, beef patties, chicken patties, and chicken tenders for validated reductions of both pathogens investigated.

Deliverable: Because of the wide variety of processed meat products, thermal process validations that encompass this wide range of products are important to confirm pathogen destruction during cooking. The results of this study will contribute to the on-going development of new easy-to-use time-temperature tables for frankfurters, beef patties, chicken patties, and chicken tenders for validated reduction of the pathogens investigated.

Investigating the Development of Thermal Processing Tools to Improve the Safety of Ready-To-Eat Meat and Poultry Products

Jeffrey J. Sindelar[a], Kathleen Glass[b], Robert Hanson[c], Principal Investigators

[ab]University of Wisconsin
[a]Meat Science and Muscle Biology Lab, 1805 Linden Drive, Madison, WI 53706
[b]Food Research Institute, 1550 Linden Drive, Madison, WI 53706
[c]HansonTech, 809 Third Street, Hudson, WI 54016
Phone: 608.262.0555 (JS); 608.263.6935 (KG); 913.709.7566 (RH)
E-mail: jsindelar@wisc.edu; kglass@wisc.edu; bob.hanson@hansontech.net

Technical Abstract: The objectives of this project were 1) to investigate the effect that certain thermal processing parameters and product characteristics have on the thermal inactivation of pathogenic *Salmonella* and *L. monocytogenes* and 2) to develop scientifically-based, regulatory-supported, and industry-useful thermal processing parameters to assure pathogen destruction and regulatory compliance in pre-cooked meats considering the vast array of products and thermal processing methods. Currently, U.S. establishments manufacturing RTE meat and poultry products have limited science-based supporting documentation to ensure and validate the thermal destruction of pathogenic microorganisms during cooking. As such, the existing documentation currently available is routinely applied to a wide array of products -- including some for which no scientific support was intended. Much of the available supporting documentation does not adequately address the impact of lethality effectiveness in products with varying compositional (*e.g.* fat, moisture, protein, etc.), physical (*e.g.* shape, size, etc.), or intrinsic (*e.g.* pH, water activity, etc.) properties. Furthermore, the documentation that provides recommendation for humidity requirements believed important for enhancing pathogenic destruction are similarly limited in scope and are often difficult for processors to measure and implement. Extrapolation of limited research is of concern since the heat resistance of microorganisms can be affected by the many factors described above as well as the bacterial properties of the pathogen of interest (cell concentration, phase of growth, strain/serotype). As a result, a comprehensive investigation of pathogen destruction considering several of these factors as well as an exploration of replacing relative humidity requirements with a wet bulb lethality approach is important to ensure that current thermal processing tools and guidelines are capable of reducing pathogenic bacteria to safe levels.

The scientific approach to reach the project objectives was to measure D- and z-values for *Salmonella* and *L. monocytogenes* in frankfurters (27% fat), beef patties (27% fat), chicken patties (15-17% fat), and chicken tenders (2-3% fat) following cooking temperatures and times commonly used in the meat industry. These D- and z- values could then be utilized in a number of ways, such as in pathogen modeling programs or to generate simple thermal processing tools such as time/temperature thermal processing tables valid for specific product/pathogen combinations. Validation of the log reduction was accomplished by comparing the actual pathogenic log reduction against the expected (via calculation) reduction according to the measured D- and Z-values. Validation of processes with varying wet-bulb/dry-bulb temperatures will improve thermal lethality of short-cook processes.

D- and z-values were determined using a ground meat mixture system. Frankfurter batter, beef patties, chicken patties, and chicken tenders were inoculated with 8-log CFU/g *L. monocytogenes* or *Salmonella enterica* (5-strain mix in each separate inoculum). All non-meat ingredient additions were based on the meat block weight. One gram portions (flattened to 0.5-1.0 mm in thick film in moisture-impermeable vacuum pouches) were heated at one of four temperatures, 54.4, 60.0, 65.6, or 71.1°C (130, 140, 150, or 160°F) in a water bath. Triplicate samples were removed and immediately chilled to ≤4°C when the inoculated samples reached target temperature and at seven additional times. Surviving *L. monocytogenes* or *Salmonella* were enumerated using Modified Oxford or XLD agar base, respectively,

with thin layer overlay of nonselective media to enhance recovery of injured cells. Linear regressions of the data were used to calculate D- and z-values for each treatment combination (4 product types x 2 pathogens x 4 temperatures).

D- and z- values were validated in products using the same formulations as above with physical dimensions that were designed to be representative of commercially-available products. Frankfurters and chicken and beef patties were inoculated with 8-log CFU/g of the designated pathogen cocktail and either stuffed into 26 mm cellulose casings (frankfurters) or chilled and formed into patties (chicken and beef patties). Chicken tenders were cut to the appropriate size and shape and then surface inoculated with 8-log CFU/g of the designated pathogen cocktail. Treatments were cooked using a wet bulb/dry bulb (WB/DB) thermal process in either a steam-convection oven (frankfurters) or a belt-fed impingement oven (chicken tenders, chicken and beef patties). Triplicate samples were removed for enumeration of surviving pathogens at pre-determined time-points during each thermal process, including prior to cooking as well as at the end of each of two cook stages. Surviving *L. monocytogenes* and *Salmonella* were enumerated as previously discussed. Frankfurters were cooked to an internal temperature of 71.1°C (160°F) following one of two cook schedules with varying relative humidity conditions. A control cycle maintained a relative humidity ≥50% for half the duration of the cook cycle (> 40 minutes) while a test cycle only applied steam for 15 minutes before the final step of the process. The test cycle (Surface Lethality Step) served to determine if target wet-bulb temperature/time requirements could produce similar or superior lethality to a cycle following the relative humidity requirements outlined in Appendix A. For both schedules, samples were removed when the product internal temperature reached 54.4, 62.8, and 71.1°C (130°F, 145°F, and 160°F). For impingement processes, chicken patties and tenders were cooked to a final internal temperature of either 71.1 or 79.4°C (160°F or 175°F) while beef patties were cooked to a final internal temperature of either 71.1 or 76.7°C (160°F or 170°F). The beef/chicken patties and tenders were cooked to both internal temperatures following a two-stage cook process either with or without application of steam to the cook chamber in the second stage. The cycle with steam application (Surface Lethality Step; SLS) was used to investigate the extent to which an increase in wet-bulb temperature would increase the lethality of pathogens on the surface of a product versus the Control cycle (no steam application).

The D-value studies revealed that the inactivation rates for *Salmonella* in all product types heated at 60, 65.6, or 71.1°C (140, 150, or 160°F), were comparable to or less (better) than times reported in Appendix A. Further, the thermotolerance was found to be greater for *L. monocytogenes* than for *Salmonella* under all conditions. Phase 2 studies using a commercial cook schedule confirmed that cooking to 71.1°C (160°F) was sufficient to kill >5.0 log of both *Salmonella* and *L. monocytogenes* in frankfurters. Both the Control and Surface Lethality Step (SLS) cycles yielded the same log reduction, suggesting that wet-bulb time/temperature control is a suitable replacement for relative humidity requirements during smokehouse processing of small-diameter frankfurter-type products.

Phase 2 validation studies with impingement processes revealed that increases in wet-bulb temperature during thermal processing became more important for lethality as product size and cooking time decreased. The data also suggested when the product surfaces were rapidly dehydrated, *Salmonella* became more thermotolerant than *L. monocytogenes*. For beef patties, cooking to 71.1°C (160°F) following either cook cycle (Control or SLS) was sufficient to kill >5.0 log of both *Salmonella* and *L. monocytogenes*. In chicken patties, a final temperature of 71.1°C (160°F) inactivated >5 log *L. monocytogenes* using both the Control and SLS cook cycles, but *Salmonella* required the additional humidity applied during the SLS cycle to achieve >5 log. In chicken tenders, (the smallest product with the shortest cooking time), a final temperature of 71.1°C (160°F) did not achieve a >5.0-log kill of *Salmonella* following either cook cycle; however, was eventually achieved with an internal temperature of 79.4°C (175°F) for *L. monocytogenes* however, the surface lethality step cycle was sufficient to kill >5.0-log at this temperature.

These data support the adequacy of current thermal processing procedures for the products investigated with the exceptions of 1) low temperature (54.4°C (130°F)) processing of high-fat frankfurter-like products and 2) rapid impingement processing of thin or small diameter products with a final internal temperature ≤ 71.1°C (160°F). They demonstrate that wet-bulb temperature/time control is comparable to existing Appendix A relative humidity requirements for ensuring adequate lethality during smokehouse processing. More importantly, these data suggest that incorporation of surface lethality steps may be necessary to ensure the safety of rapid cooking impingement processes. Finally, these results are being used for the on-going development and generation of new easy-to-use time-temperature tables for frankfurters, beef patties, chicken patties, and chicken tenders for valid reduction of pathogens.

Objectives:

1.) Investigate the effects of thermal processing parameters and product characteristics on the thermal inactivation of pathogenic *Salmonella* and *L. monocytogenes* in frankfurters, beef patties, chicken patties, and chicken tenders;
2) Develop scientifically based, regulatory supported, and industry useful thermal processing parameters to assure pathogen destruction and regulatory compliance in pre-cooked meats considering the vast array of products and thermal processing methods.

Materials and Methods:

This study was divided into two phases. Phase 1 determined D- and z-values for *L. monocytogenes* and *Salmonella* in ground meat mixtures. Phase 2 was designed to validate the D-values identified in Phase 1 using commercial products representing different product categories. Four products were selected for testing based on differences in meat species, fat content, physical dimensions, and typical cooking method. The formulas for each product represent the worst-case for thermal destruction in its category and would allow the thermal destruction data to be used as the basis for new Appendix A-style time-temperature tables for those product categories.

To accomplish these goals, the experimental plan included the following approaches:

1. Measure D- and z-values for *Salmonella* and *L. monocytogenes* in frankfurters, beef patties, chicken patties, and chicken tenders;
2. Validate the measured D-values in simulated commercial products and processes; and
3. Generate data for the on-going development of new Appendix A style time-temperature tables frankfurter, beef patty, chicken patty, and chicken tender product types/categories.

Phase 1: Determination of D- and z-values in model (ground) systems.

Four products were categorized by fat content, and physical dimensions and typical cooking method for a commercial product and included 1) frankfurters (high fat, small diameter/fast cook), chicken tenders (low fat, thin, fast cook), chicken patties (high fat, thin, fast cook) and beef patties (high fat, thin, fast cook). The formulas for each product represented the worst-case for thermal destruction in its category, and this thermal destruction data will be used as the basis for new Appendix A-style time-temperature tables for those product categories or other valuable thermal processing tools.

Frankfurter batter (containing 51.7% pork trimmings (42% Lean), 48.3% mechanically-separated turkey (MST), 1.77% salt, 0.4% sodium phosphates, 156 ppm sodium nitrite, 547 ppm sodium erythorbate, and 20% water), beef patties (73% lean), chicken patties (containing skin-on, boneless chicken thighs, 1.0% salt, 0.25% sodium phosphates, and 2.0% water), and chicken tenders (containing 1.3% salt, 0.35% sodium tripolyphosphate, and 5.0% water) were manufactured using typical industry protocols. All non-meat ingredient additions were based on the meat block weight. Products (n=4) were inoculated with a 1.0% inoculum to yield 8-log CFU/g meat of *L. monocytogenes* or *Salmonella enterica* (5-strain mix for

each separate inoculum). *L. monocytogenes* strains included: FSL-C1-109 (clinical isolate, processed deli-style turkey outbreak, serotype 4b), LM 101 (hard salami isolate, serotype 4b), LM 108 (hard salami isolate, serotype 1/2a), LM 310 (goat milk cheese isolate, serotype 4b), and V7 (raw milk isolate, serotype 1/2b). *Salmonella enterica* serotyptes included: Enteritidis 6424 (phage type 4, baked cheesecake isolate), Enteritidis E40 (chicken ovary isolate), Heidelberg S13 (clinical isolate), Typhimurium S9 (clinical isolate), and Typhimurium M-09-0001-A1 (peanut butter isolate). One-gram portions of inoculated meat were weighed into moisture-impermeable 16.51 cm x 20.32 cm (6.5 in x 8.0 in) vacuum pouches (3 mil high barrier EVOH pouches, Deli 1 material; oxygen transmission rate, 2.3 cm^3/cm^2; 24 h at 23°C; water transmission rate, 7.8 g/m^2; 24 h at 37.8°C; and 90% relative humidity; WinPak, Winnepeg, Manitoba, Canada) and flattened into a layer 0.5-1.0 mm thick. Pouches were then vacuum-sealed (Multivac AGW, Sepp Haggemuller KG, Wolfertschewenden, Germany) and held at 4.0°C (39.0°F) until used for testing (≤ 2 hours). For each trial, samples were heated to one of four temperatures (54.4, 60.0, 65.6, or 71.1°C; 130, 140, 150 and 160°F, respectively) by immersing all sample pouches simultaneously into a pre-heated water bath. *L. monocytogenes* samples were also heated at 62.8°C (145°F) to provide another temperature sampling point and improve z-value calculations. At each sampling point, triplicate sample pouches were removed and immediately chilled to ≤ 4.0°C (39.0°F) via submersion in an ice water bath. Initial samples were pulled as soon as the meat reached the target temperature (5-15 sec after immersion). The remaining samples were pulled at five to six intervals thereafter for a total of 18-21 samples for each pathogen/product/temperature combination. Chilled sample pouches were cut open using scissors sanitized with 70% ethanol. Samples were then diluted 1:10 with Butterfield's buffer (1 g meat: 9 ml buffer) and packages externally hand-massaged for approximately two minutes. Surviving *L. monocytogenes* or *Salmonella* were enumerated from the processed samples by plating serial dilutions onto Modified Oxford, or XLD agar plates, respectively, with thin layer overlay of nonselective media to enhance recovery of injured cells. Sample temperatures were monitored using a thermocouple with a k-probe (Fisher Scientific Traceable Thermometer and type K probe, Thermo Fisher Scientific, Waltham, MA) inserted into an uninoculated pouch through a rubber septum. This experiment was conducted three times for each product/pathogen combination.

Phase 2: Validation of D- and z-values in commercial-type products.

Phase 2 was conducted to validate the D-values generated in Phase I and document integrated lethality for the target internal temperature in each of the four products. Products in this phase were manufactured and thermally processed to mimic commercial manufacturing practices. In the original experimental design, the pathogen with the highest D-value for each of the four products was to be chosen for validation in this phase. However, to benefit the overall product applicability and industry impact of the study, the experimental design was modified to validate lethality for both pathogens in all four products. Additionally, each temperature/treatment combination was thermally processed using either a Control cycle (intended to replicate a standard industrial process) or a Surface Lethality Step (SLS) cycle. The SLS cycle was a rapid increase in wet-bulb temperature included towards the end of the cook process. These SLS cycles were designed to determine whether a high wet-bulb application towards the end (rather than early in heating as is done in validated jerky processing, for example) of cooking to an internal temperature (IT) of at least 71.1°C (160°F) can improve both surface and total process lethality in rapid cook processes (defined as those <8 minutes in length). For each product/temperature/cook cycle combination, samples were collected immediately prior to cooking as well as at two to three additional sampling points to enumerate pathogens. Frankfurters samples were removed when the internal temperature reached 54.4, 62.8, and 71.1°C (130°F, 145°F, and 160°F); beef patties, chicken patties, and chicken tenders samples were removed after the first and second passes through the impingement oven during the cook process. Each treatment/temperature/cook cycle combination listed in Table 1 was manufactured/tested in duplicate for a total of 56 separate experimental runs.

Frankfurter (Batch Oven Product) Manufacture

Frankfurter batter was prepared following the same formulation described above by forming an emulsion using a vertical cutter-mixer (Stephan Universal Machine, type UMC 5, Stephan Machinery GmbH., Hameln, Germany) with an end point chopping temperature of 18.3°C (65°F). When the batter reached 17.2°C (63°F), it was inoculated at 1.0% with 8-log CFU/g of either *Salmonella* or *L. monocytogenes* and chopped until the target end point temperature of 18.3°C (65°F) was reached. The batter was then manually stuffed into 26 mm cellulose casings and linked in 15 cm (approximately 6-inch) increments.

Cooking and Microbial Enumeration of Frankfurters (Batch Oven Product)

Inoculated frankfurter links were transferred to a combi-oven (Alto-Shaam Combi-therm, model 6.10 ESI SK, Alto-Shaam Inc., Menomonee Falls, WI) and thermally processed following one of two cook schedules (Tables 2 and 3) until a final internal temperature of 71.1°C (160°F) was achieved. The two cook cycles used different environmental wet bulb temperatures to investigate the effect wet-bulb temperature had on core/surface temperatures and the associated pathogen lethality. This data was intended to contribute to the development of guidelines for the use of specific wet-bulb temperatures (65.6°C) and durations to achieve the required log reductions for targeted pathogens. The Control cycle was designed to approximate a commercial process and maintained a relative humidity ≥50% for half the duration of the cook cycle to follow the humidity requirements of USDA Appendix A. The Test cycle introduced steam (relative humidity = 52.8%) only during the final step (approximately15 minutes) of the process to investigate the efficacy of a surface lethality step. Thermal profiles were recorded using calibrated data loggers (MadgeTech, HiTemp 140 data logger, MadgeTech, Inc., Warner, NH). Real-time temperature was monitored using a multi-channel thermocouple with k-probes measuring dry bulb and wet bulb temperatures as well as the internal temperature of two different, non-inoculated links (4-Channel Thermometer/Data logger; EXTECH Instruments) (Figure 1).

For both processes, triplicate links were removed when product internal temperatures reached 54.4, 62.8, and 71.1°C (130°F, 145°F, and 160°F). Links were immediately chilled to ≤ 4°C (40°F) by immersion in an ice water bath. Casings were then removed aseptically and 2.5 cm were removed from the end of each link and discarded. Twenty-five grams of sample from the central portion of each link was weighed into a sterile stomacher bag, diluted 1:1 with Butterfield's buffer, and stomached for two minutes. Samples were then enumerated for the survival of *Salmonella* and *L. monocytogenes* using the methods described above.

Impingement Oven Product Manufacture & Inoculation

The manufacturing procedures differed among the three remaining products types and are described below. Triplicate inoculated, raw samples were removed from each treatment for enumeration immediately prior to cooking and at appropriate times during cooking.

Chicken Tenders: Boneless, skinless chicken breasts (< 3.0% Fat) were obtained from a commercial supplier and stored at ≤ 4.0°C (39.0°F) until use (≤ 72 hours). Tenders were cut from the chicken breasts to an approximate size of 139.7 mm long x 36.8 mm wide x 11.43 thick (5.5 in x 1.45 in x 0.45 in) (Appendix 1). These product dimensions were determined to be representative of a typical commercial product after 1) analyses of similar commercially-available products and 2) input from discussions with local processors manufacturing a similar product. Following the same formulation previously described, the tenders were placed in vacuum tumblers (Lance Model LT-5, Koch Industries, Kansas City, KS) along with all non-meat ingredients and tumbled under vacuum continuously at ≤ 40°F on medium speed (18-20 rpm) for one hour to achieve adequate ingredient pick-up. The tenders were then vacuum-packaged in oxygen- and moisture-impermeable bags (3-mm high barrier pouches; oxygen transmission rate: 50 to 70 cm^3/m^2, 24 h at 25°C and 60% relative humidity; water transmission rate: 6 to 7.5 g/m^2, 24 h at 25°C and 90% relative humidity; UltraSource, Kansas City, MO) and stored at ≤ 4.0°C (39°F) for ≤ 72h prior to testing. For each trial, raw tenders were weighed prior to inoculation and tenders outside a

range of 37-43g were discarded in order to minimize variation in heating rate between samples. Weighed tenders were then inoculated to ~8 log CFU/g with a 5-strain cocktail of either *Salmonella* or *L. monocytogenes* using a 1.0% inoculum (v/w). Inoculum was spotted onto the surface of each tender using a sterile pipette and spread evenly over the entire surface of the tender. Inoculated tenders were air chilled to 4°C (40°F) and held for 60 minutes prior to testing.

Chicken Patties:
Boneless, skin-on chicken thighs (≤17% Fat) were purchased at a local retail store ≤ 72 hours prior to testing. The chicken was fine ground through a 4.8 mm (3/16") plate attached to a grinder (model 4732, Hobart Corp., Troy, OH), vacuum-packaged in oxygen- and moisture-impermeable bags (3-mm high barrier pouches; oxygen transmission rate: 50 to 70 cm^3/m^2, 24 h at 25°C and 60% relative humidity; water transmission rate: 6 to 7.5 g/m^2, 24 h at 25°C and 90% relative humidity; UltraSource, Kansas City, MO), and stored at ≤ 4°C (40°F) for ≤ 72h prior to testing. For each trial 1600 g of ground chicken was first mixed with all additional non-meat ingredients according to the formulation described above for 2 minutes in a Hobart Mixer **(**AS 200, Troy, Ohio) on medium speed. The mixture was then inoculated at 1.0% (v/w) to ~8 log CFU/g with a 5-strain cocktail of either *Salmonella* or *L. monocytogenes*. The treatments were mixed for 2 minutes using a sterile rubber spatula to scrape down the bowl after 1 minute of mixing. Seventy-one gram portions of inoculated meat were weighed onto a sanitized weigh boat and air chilled to an internal temperature of -3.0°C (26.7°F) during 60 minutes of storage in order to replicate the temperature at which patties are formed during a commercial process. Chilled sample portions were then formed into patties approximately 7.62 mm thick and 95.25 mm in diameter (0.3 in x 3.75 in) (Appendix 1) using a patty press sanitized with 70% ethyl alcohol prior to use. These product dimensions were determined to be representative of a typical commercial product after 1) analyses of similar commercially-available products and 2) input from discussions with local processors manufacturing a similar product.

Beef Patties:
Ground beef (73% Lean; 6.5 mm (0.25 in) grind) was purchased at a local retail store ≤ 72 hours prior to testing and vacuum packaged in oxygen- and moisture-impermeable bags (3-mm high barrier pouches; oxygen transmission rate: 50 to 70 cm^3/m^2, 24 h at 25°C and 60% relative humidity; water transmission rate: 6 to 7.5 g/m^2, 24 h at 25°C and 90% relative humidity; UltraSource, Kansas City, MO). The beef was stored at ≤4°C (40°F) until testing (< 72 hour storage). 1900 grams of ground beef were inoculated at 1% to ~8 log CFU/g with a 5-strain cocktail of either *Salmonella* or *L. monocytogenes*. Samples were mixed for 2 minutes in a Hobart Mixer **(**AS 200, Troy, Ohio) and a sterile rubber spatula was utilized to scrape the bowl after 1 minute of mixing to ensure uniform inoculum dispersion. For each trial, 90g portions of inoculated meat were weighed onto sanitized weigh boats and formed into patties approximately 7.62 mm thick and 101.6 mm in diameter (0.3 in x 4.0 in) (Appendix 1) using a patty press sanitized with 70% EtOH prior to use. These product dimensions were determined to be representative of a typical commercial product after 1) analyses of similar commercially-available products and 2) input from discussions with local processors manufacturing a similar product. Patties were then air chilled to an internal temperature of -3.0°C (26.7°F) during 60 minutes of storage in order to replicate the temperature at which patties are formed during a commercial process.

Cooking and Microbial Enumeration of Impingement Oven Products
Chicken tenders, chicken patties, and beef patties were thermally processed using a two-zone impingement oven (X3D-1855-S electric oven, XLT Inc., Wichita, KS) modified to allow for control of the wet-bulb temperature in each zone. Products were cooked to one of two target internal temperatures following either a Control cycle or an SLS cycle for a total of four different trials per product/pathogen combinations. Tables 4 and 5 list the cook cycle parameters and oven settings used. Figure 3 shows the physical layout of the ovens used for these validation experiments. For all three product types, the cook

cycle parameters were designed to approximate a commercial process as closely as possible. This was accomplished by using the processes employed by several local processors for the manufacture of similar products as models for a creating a typical commercial process. For the Control cycles, products were passed through the same oven twice to achieve the treatment required total cooking time. The Control cycle had no increase in wet-bulb temperature during the process to compare the pathogen lethality of a non-humidified process versus a humidified test process that included a Surface Lethality Step.

Dry-bulb and wet-bulb oven temperatures as well as surface and internal product temperatures were measured using calibrated data loggers (MadgeTech, HiTemp 140 Data logger, MadgeTech, Inc., Warner, NH). Data loggers were enclosed in protective Teflon casings to prevent damage to the logger batteries and ensure accurate readings. Internal and surface temperatures were measured using probes inserted into a non-inoculated patty/tender that was passed through the oven immediately prior to the inoculated samples.

Nine inoculated beef/chicken patties or chicken tenders were removed from refrigerated (chicken tenders)/frozen storage (chicken and beef patties) and placed on metal grates (triplicate samples per grate), 2-3 grates/trial) designed to allow for sufficient airflow across all sides of the samples. Non-inoculated samples used for temperature-logging during each trial were placed on a single, separate grate along with the wet-bulb and dry-bulb data loggers (Figure 2). Samples were first passed through the upper oven (DB = 204.4°C (400°F), No WB). Once the first grate passed completely through the oven chamber, triplicate samples were removed for enumeration (designated as "First Pass" samples); the remaining grates were then transferred either to the lower oven (DB = 204.4°C (400°F), WB = 71.1°C (160°F)) for the SLS cycle or ran again through the upper oven for completing the Control cycle. Once the grates had fully passed through the second oven chamber, triplicate samples were removed for enumeration. All samples removed for enumeration were placed into sterile Whirl-Pak bags and chilled to ≤ 4°C (39°F) via submersion in an ice water bath within 45 seconds of removal from the oven to rapidly cool the sample and stop any additional lethality caused by residual heat remaining in the product. Experiments were completed twice for each formulation-pathogen-process combination.

All samples were weighed and diluted 1:1 with Butterfield's buffer. Beef patty and chicken patty samples were homogenized in a benchtop stomacher for approximately 2 minutes. Chicken tender samples were hand massaged with buffer for approximately 2 minutes. Homogenization was not necessary for the chicken tenders because inoculum was only applied to the surface of the product. Approximately 15 ml of rinsate was then poured from each sample bag into sterile screw-cap tubes and the rest discarded. Samples were then enumerated for surviving *Salmonella* or *L. monocytogenes* as described above.

Proximate and chemical analysis: Moisture (5 h, 100°C, vacuum oven method AOAC 950.46), pH (10 g homogenized portion diluted in 90 ml distilled water, pH of slurry measured with Accumet Basic pH meter and Orion A111 combination electrode, Thermo Fisher Scientific, Waltham, MA), NaCl (measured as % Cl^-, $AgNO_3$ potentiometric titration, Mettler G20 food and beverage analyzer),water activity (Decagon AquaLab 4TE water activity meter, Pullman, WA), and crude fat (AOAC 991.36) were assayed in triplicate on raw, uninoculated samples from every trial with each product/pathogen combination.

Data analysis: The microbiological data reported are average values and standard deviations (log CFU/g) for triplicate samples per timepoint and with three separate trials for each product/pathogen/temperature combination in Phase 1 (n=88) and two separate trials for each product/pathogen/cook cycle combination in Phase 2 (n=56). Data was collected for calculation of the log reduction in pathogen concentration for all treatment combinations (4 product types x 2 pathogens; at 4 temperatures) over time to attain time-temperature-log reduction relationships. Further, data was analyzed using standard linear regression statistical methodology to generate linear regressions for each product type, pathogen and temperature

combination incorporating at least 4 time-points for each combination to allow for generation of D- and z-values.

Results and Discussion:

Phase 1: Determination of D- and z-values in model (ground) meat and poultry.

For D-value calculations, 31 graphs were developed (Figures 4 and 5) plotting pathogen log reduction over time using linear regression analysis. Each graph shows the linear regression of all trials for that pathogen/product combination at a given temperature. The "Frankfurter-*Salmonella*-60°C (140°F)" graph found in Figure 4a provides an example of a standard linear regression calculated for a frankfurter/*Salmonella* treatment combination at 60°C (140°F) where populations of *Salmonella* were determined at 0, 120, 240, 360, 480, and 600 seconds. To account for the initial come-up time (e.g. number of seconds for sample temperature to increase from 4°C to 60°C) to the first time-point (0 seconds at target temperature), time adjustments were made to all subsequent time-points. For example, in the "Frankfurter-*Salmonella*-60.0°C (140°F)" figure, "0" time was adjusted 15 seconds for replication 1 and 10 seconds for replication 2 to signify the actual time the internal sample temperature reached the pre-determined experiment temperature. This approach accounts for lethality that may occur during the initial heating and results in conservative D-values, particularly for higher temperatures with short D-values. From these linear regressions, the average D-value was determined to be 1.74 minutes, which is similar to the D- value of 1.72 minutes calculated from the USDA, FSIS Appendix A tables generated for roast beef at the same temperature. This result suggests that Appendix A is an adequate predictor of *Salmonella* reduction, even when raw materials sources were not the same. The D-values calculated for *Salmonella* in the other three products (beef patties, chicken patties, chicken tenders) at temperatures exceeding 60°C (140°F) were comparable to or less than the values identified in the USDA-FSIS Time-Temperature Tables for Cooking Ready-To-Eat Meat and Poultry Products. The calculated D-values for all product types tested with *Salmonella* and *L. monocytogenes* are listed in Tables 6 and 7, respectively. Z-values were calculated (Table 8) for *Salmonella* and *Listeria monocytogenes* by plotting the D- values for each treatment combination against temperature.

As in the previous NAMIF thermal inactivation study, *L. monocytogenes* showed greater thermotolerance than *Salmonella* under all conditions *(24)*. For example, at 140°F (60°C), >5-log reduction of *L. monocytogenes* required 5 and 14 minutes in beef and chicken patties, respectively, as compared to < 0.5 and < 1.3 minutes for *Salmonella*. The D-values for *Salmonella* in beef patties, chicken patties, and chicken tenders at the lowest temperature tested (130°F/54.4°C) predicted less time would be needed to achieve a 6.5/7.0 log reduction than times reported in USDA, FSIS Appendix A. In frankfurter batter, however, the calculated D-value suggested that Appendix A recommendations may not be adequate. This exceptional tolerance to high heat may be may be due to either the induvial or combined effects of the high fat content, high pH, and small particle size of this product. Despite the same high fat level as frankfurters, the beef patty formulation produced the shortest D-values for *Salmonella* at this temperature. One hypothesis for this difference in thermal lethality may be that the size of fat particles (e.g. emulsion vs. distinct particle), created a difference in thermo-protection, perhaps by encapsulating the microbe during the creation of the emulsion. However, the low pH of the beef patties (5.5) likely accelerated thermal inactivation compared to the higher pH (6.5-6.6) in the frankfurters, chicken patties or chicken tenders. More research is needed to better understand the relationship between the two factors and how their combined effects contribute to the thermal tolerance of food borne pathogens.

D-values for *L. monocytogenes* in frankfurter batter were not able to be accurately calculated at any temperature due to a significant tailing effect that was observed at all 4 temperatures which became more severe as the temperature increased. At temperatures ≤ 140°F (60°C), *L. monocytogenes* populations persisted in samples at concentrations exceeding 4.0 log CFU/g, well above the minimum detectable limit

for the methodology utilized in these trials. The resulting death curves did not achieve the minimum 5-log reduction and were poorly modeled by linear regression. Figure 6 shows an example of this tailing effect that was observed with *L. monocytogenes* in frankfurter batter cooked to 65.6°C (150°F). The D-value calculated from this graph (4.05 minutes) was not implausibly long but a linear death rate was not an accurate descriptor for the behavior of *L. monocytogenes* in this product. However, Phase 2 validations with frankfurters suggested that that these D-values may still be accurate, as an endpoint temperature of 160°F (71.1°C) was sufficient for achieving ≥ 5 log reduction for both cook cycles utilized and the death curves for *L. monocytogenes* did not appear to follow a biphasic pattern. This then suggests that the tailing observed was either exaggerated by or an artifact of the standard "cook-in-bag" methodology used for isothermal testing with *L. monocytogenes*. This tailing effect was also observed to a lesser degree with *L. monocytogenes* in the other three products tested. As with the *Salmonella* at low temperatures in frankfurter batter, it is possible that this tailing effect may also be the result of an interaction effect between the high fat and high pH. More research is necessary to determine if this methodology is still appropriate for testing with frankfurter-type products (i.e. finely comminuted, high fat, high pH).

Results from Phase I support the USDA Appendix A as an acceptable tool for *Salmonella* lethality in a wider range of products at final internal temperatures exceeding 60.0°C (140°F). As expected, *L. monocytogenes* was considerably more thermo-tolerant than *Salmonella* in all products tested. Since Phase I utilized one-gram meat samples with short come-up times, by design, only immediate lethality was measured. It is important, however, to consider expected differences with integrated lethality because significantly different thermal processing conditions exist in a 1 g sample (Phase I sample size) versus a larger mass, thickness, or diameter. Due to these physical differences, slower temperature increases and longer come-up times naturally exist. Generally, longer come-up times create higher lethality processes because the longer times at lethal temperatures (e.g., >135°F) promote more pathogen destruction. In contrast, fast cooking processes at low relative humidity potentially allow bacterial survival due to the potential protective effect of dehydration, which dramatically increases the heat resistance of the pathogens — resulting in decreased pathogen lethality for the process even if the product is cooked to the same or a higher internal temperature. Therefore, care must be taken when interpreting and utilizing Phase I data to predict lethality under various heating rates and relative humidities. As such, it is important to validate D-values against actual log reductions achieved using commercial meat product processes.

Phase 2: Validation of D- and z-values in commercial-type products.

Batch Oven Product (Frankfurters)

Figures 8 and 9 show the average integrated thermal process/pathogen reduction profiles for frankfurter batter inoculated with *L. monocytogenes* heated to 71.1°C (160°F) using the Control and SLS cycles, respectively. Figures 10-11 report the average integrated thermal process/pathogen reduction profiles for frankfurter batter inoculated with *Salmonella* heated to 71.1°C (160°F) using the Control and SLS cycles, respectively. A target internal temperature of 71.1°C (160°F) was sufficient to achieve ≥ 5-log reduction of *Salmonella* for both cook cycles. These results support the D-values determined for *Salmonella* in Phase 1. They also demonstrate the efficacy of a wet-bulb time/temperature target versus the current Appendix A relative humidity requirements. The Control process followed the Appendix A requirement for a continuous steam injection into the oven for ≥50% of the cook process, but no less than 1 hour, while the SLS process only applied steam during a surface-lethality step of 15 minutes at a 65.6°C (150°F). The humidity guidelines provided in Appendix A are designed to minimize evaporative cooling, dehydration, and concentration of solute on the surface of the product throughout the cooking process, all of which can lead to increased thermotolerance in *Salmonella*. The 5-log kill achieved with the SLS cycle shows that the negatives effects of any thermoprotective surface conditions which may exist during

cooking are mitigated when minimum target wet-bulb temperatures are maintained at the end of the process for a designated amount of time to ensure surface lethality. Further validation studies will be necessary to establish appropriate wet-bulb time/temperature targets for a wider range of product types and evaluate their safety and efficacy. The potential for product-specific wet-bulb time/temperature targets and the ease of precisely monitoring and verifying these two variables could be a significant improvement over the current Appendix A relative humidity requirements.

Phase 2 data also confirmed that cooking to an internal temperature of 71.1°C (160°F) using either the Control or the SLS cycles was sufficient to achieve a 5-log reduction of *L. monocytogenes*. Although Phase 1 tests observed a biphasic death curve characterized by a rapid decline in initial pathogen load followed by a markedly slower death rate, neither of the cook cycles for Phase 2 produced similar death curves. Biphasic death curves have been observed previously for both *Salmonella* and *L. monocytogenes,* particularly in products where dry surface conditions exist during processing *(5, 30)*. However, such conditions were not found in Phase 2 experiments with frankfurters.

D-value calculated from the Phase 1 trials with *L. monocytogenes* (D=4.05 min) in frankfurters cooked to 65.6°C (150°F) (Figure 6) appeared to be an adequate, conservative predictor for pathogen reduction in both cook cycles tested in Phase 2. Because we were unable to calculate accurate Z-values for *Listeria monocytogenes* in frankfurters, the chicken patty z-value was chosen for comparison because it was the experimentally-derived value that would yield the lowest predicted lethality (worst case scenario). Using the values of D=4.05 minutes, a reference temperature of 65.6°C (150°F) and the z-value for *L. monocytogenes* calculated from chicken patty experiments (8.80°C (15.7°F)), the predicted total lethality for the SLS cycle was 12.42 log kill. Given the initial inoculum of 8.12 log CFU/g and the detectable limit for direct plating methodology of 1.0 log, the observable kill was 7.12 log. Using these same input values, the predicted lethality at the 62.8°C (145°F) internal temperature sampling point during the SLS cycle was 1.46 log, a full log less than the 2.45 log reduction that was actually observed. These finding show that the use of linear regression for calculation of D-values from non-linear curves can yield results that may be useful for conservative lethality prediction. These results also support general finding from Phase 1 as well as from previous experiments that *L. monocytogenes* is significantly more thermotolerant than *Salmonella* for products where evaporative cooling is not a confounding factor

Impingement Oven Products (Chicken Tenders, Chicken Patties, and Beef Patties)

Tables 10 and 11 provide a summary of the lethality observed for each product and temperature/cook cycle combination for *L. monocytogenes* and *Salmonella*, respectively, during Phase 2 validation studies. Figures 12-23 show the average integrated thermal process/pathogen reduction profiles for both *Salmonella* and *L. monocytogenes* for each product and temperature/cook cycle combination. A 5-log reduction line is included in these figures to maintain consistency with Phase 1 trials and to show a limitation of these experiments. While USDA, FSIS Appendix A prescribes a 6.5-7 log reduction, this amount of kill cannot be accurately measured using the methodology described above. The minimum detectable limit for these experiments was 1.0 log CFU/g. This was the concentration that was calculated if each of the duplicate plates at the lowest possible dilution had a single colony on them. Lower concentrations could exist in a sample however no calculations could be made if no observable bacteria were recovered. With an average starting concentration of ~8.0 log CFU/g, the greatest log kill that could be measured in these experiments was 7.0 log. As populations approach the limit of detection however, each individual colony begins to have much larger impact on the log CFU calculation. With this methodology, the difference between a 6.5 and 7.0 log kill is only two additional colonies per plate at the lowest dilution. A greater starting concentration would allow for more accurate observation of larger kills, however, it also introduces new problems. As the pathogen concentration level is artificially increased it becomes less representative of a real-world situation. Pathogens are usually detected in raw materials at levels far lower than the initial concentration used for these experiments. As an example, one study attempting to determine the prevalence of *Salmonella* on the carcasses of retail broiler chickens

found cells via direct enumeration at only log 3.8-4.5 CFU/carcass*(6)*. The unnaturally high cell density used for these studies may itself become a confounding variable, increasing or decreasing the thermal tolerance of the cells. At the same time, decreasing the initial pathogen load results in shorter death curves that are less suitable for linear regression. The 5-log reduction target was chosen because it can be reliably detected using this methodology while minimizing the ingoing pathogen concentration needed.

In beef patties, cooking to an internal temperature of 71.1°C (160°F) was sufficient to achieve ≥ 5-log reduction of both pathogens tested with either process (Control or SLS). This result was expected as the beef patty formulation yielded the shortest D-values in Phase 1 and had the longest dwell time in the impingement ovens. For chicken patties, cooking to an internal temperature of 71.1°C (160°F) was also sufficient to achieve a ≥ 5-log reduction of *L. monocytogenes* in both the Control and SLS cook cycles. For *Salmonella*, cooking chicken patties to 71.1°C (160°F) following the SLS cycle consistently reduced populations of *Salmonella* by ≥ 5-log, however, A 5-log reduction was not achieved when cooking to the same temperature using the Control cycle. Furthermore, in chicken tenders, a final internal temperature of 71.1°C (160°F) did not achieve a ≥ 5-log reduction of *Salmonella* or *L. monocytogenes* using the Control cycle. The SLS cycle achieved a ≥ 5-log reduction for *L. monocytogenes* at this temperature but did not achieve a 5-log reduction for *Salmonella*.

These results indicate that the wet-bulb application in the SLS cycle becomes more important for achieving the required process lethality for small products with fast cooking times, and for high dry-bulb processes that have the potential for rapid surface dehydration. The SLS cycle did not achieve a 5-log kill of *Salmonella* in chicken tenders even at the higher internal temperature of 79.4°C (175°F). Rapid desiccation of the product surface during the first half of both the Control and the SLS cycle is the most likely explanation for these results. Low water activity substrate and dry cook processes are both associated with decreased lethality of *Salmonella* (and *L. monocytogenes* to a lesser degree) during cooking *(2, 4, 10, 22, 29)* . The wet-bulb application in the SLS cycle enhanced lethality by eliminating these dry conditions at the surface of the product. The failure to achieve a consistent 5-log reduction during these experiments could mean that the amount of moisture introduced inside the second oven was insufficient to overcome any protective effects of dehydration created in the first oven. The impingement ovens used in this study were only capable of raising the wet-bulb temperature to ~71.1°C (160°F). Commercial impingement ovens with humidity controls are likely able to apply significantly more moisture and achieve a higher wet bulb temperature than the equipment used for this study. Future experiments will need to determine if there is a protective effect due to dehydration and utilize a range of wet-bulb temperatures during surface lethality steps to test the effect of moist heat on desiccated pathogens. Also, for *Salmonella*, it is possible that the moist-heat SLS step may have to be applied to the entire process, not just the second half to achieve desired lethality.

Conclusions:

This study investigated the validity of thermal processes designed to meet USDA, FSIS Appendix A lethality requirements for four different ready-to-eat, small dimension, fast-cook products (frankfurters, beef patties, chicken patties, chicken tenders). Results from this study confirmed that cooking temperatures and times that are currently being widely used in the meat and poultry industries following USDA, FSIS Appendix A for thermal lethality are sufficient to kill 1) *Salmonella* when the end point cooking temperatures meet or exceed 60.0°C (140°F) for non-impingement processes and 2) *L. monocytogenes* when the end point cooking temperatures meet or exceed 71.1°C (160°F) for non-impingement processes. Additionally, wet-bulb time/temperature target identified in this study (DB = 79.4°C (175°F, WB = 65.6°C (150°F), 15 minutes) may be a suitable replacement for Appendix A relative humidity requirements during smokehouse processing

In contrast, *Salmonella* was found to be more thermotolerant than *L. monocytogenes* during rapid cook impingement processes (≤4.0 minutes). As product size and cook times decreased, a surface lethality step (SLS) was necessary to counter the protective effects of dehydration for pathogens — especially *Salmonella* — on the product surface. The protective effect of dehydration at the product surface was more pronounced for *Salmonella* than for *L. monocytogenes*, and therefore higher wet-bulb temperatures or SLS steps with longer dwell times may be necessary to assure 5-log kills of *Salmonella.* Understanding and instituting integrated lethality concepts are critical to ensuring the safety and efficacy of the lethality steps during these processes. The integrated process lethality profiles developed from this study will be useful tools for establishments to better evaluate overall pathogen lethality of their products and processes. Finally, the results of this study have also enabled the on-going development of new easy-to-use time-temperature tables for frankfurters, beef patties, chicken patties, and chicken tenders for validated reductions of both pathogens investigated.

Recommendations for Future Research:

This study investigated the validity of thermal processes designed to meet Appendix A requirements for four different products and two different pathogenic bacteria. Isothermal model experiments suggested that high fat content and high pH may enhance the thermotolerance of both *Salmonella* and *L. monocytogenes*. Future experiments should examine the individual and combined effects of these two characteristics on thermal tolerance of pathogens at varying levels within a single, standardized meat system While this study demonstrated the efficacy of a surface lethality step used during impingement cooking, more research is needed to fully develop this concept to incorporate it into a new set of thermal processing tools. Finally, rapid desiccation of product surfaces was found to dramatically increase the thermotolerance of the pathogens — especially *Salmonella*. Further research is needed to understand the protective effects of dehydration and desiccation, and to adjust the application of moist heat SLS cycles to assure adequate destruction of desiccated pathogens.

Acknowledgements

The authors would like to acknowledge Mr. Russ McMinn who led all experimental work, data collection, and analyses for this project as part of his doctor of philosophy degree program. The authors also wish to thank Dr. Andrew Milkowski for his helpful insights and collaboration, and Dr. Amanda King, Robert Weyker, William Shazer, Katina Fisher, Adam Bartling, Madisen Potratz, Brandon Wanless, and Max Golden for their technical assistance. Funding was provided by the North American Meat Institute Foundation.

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Figure 1: Batch oven temperature probe placement and sample layout for frankfurters in Phase 2 validation s

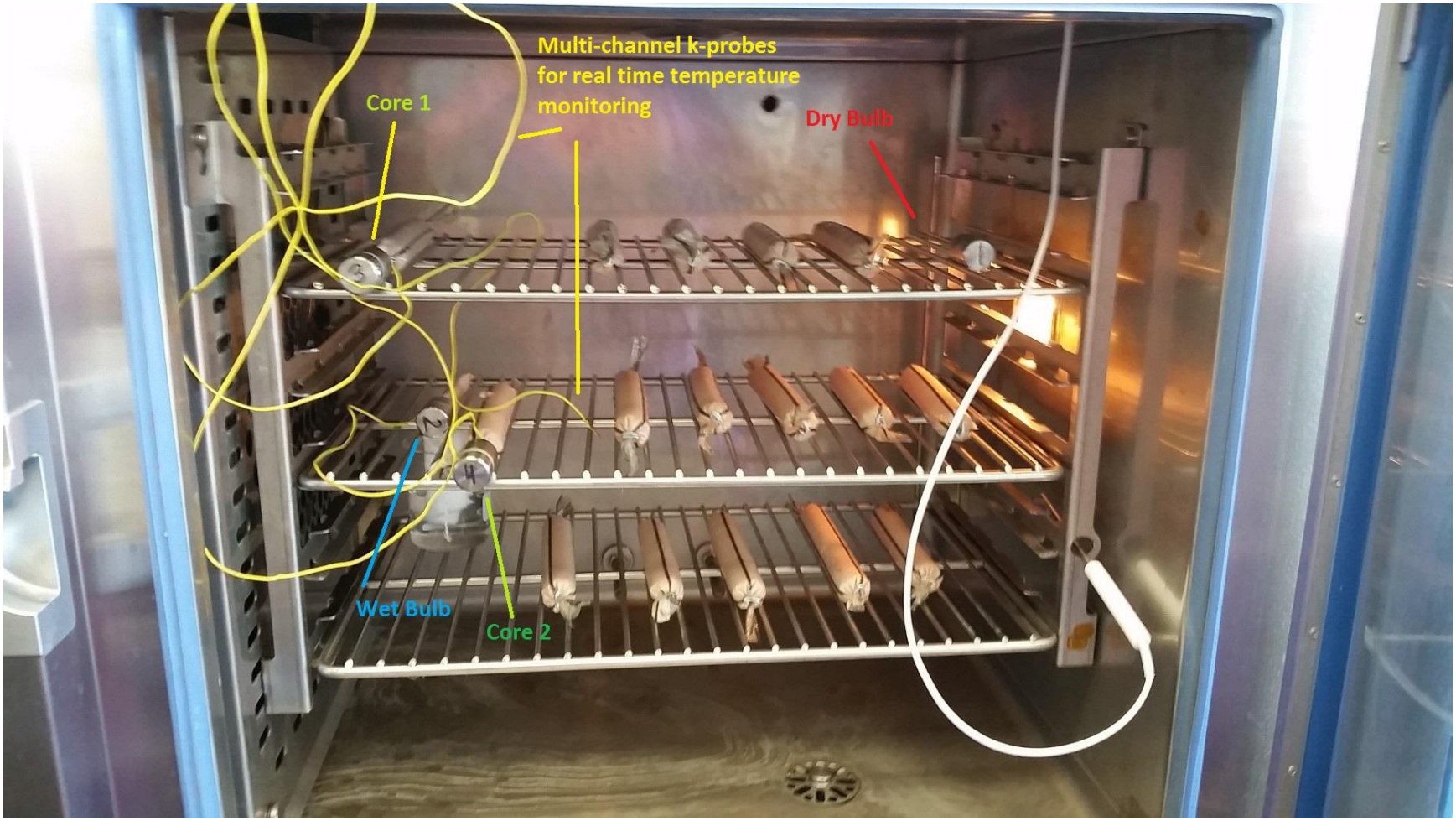


Figure 2: Temperature probe placement and sample layout for Phase 2 validation studies in impingement oven. Chicken tender probe placement shown below; placement was similar for other two products.

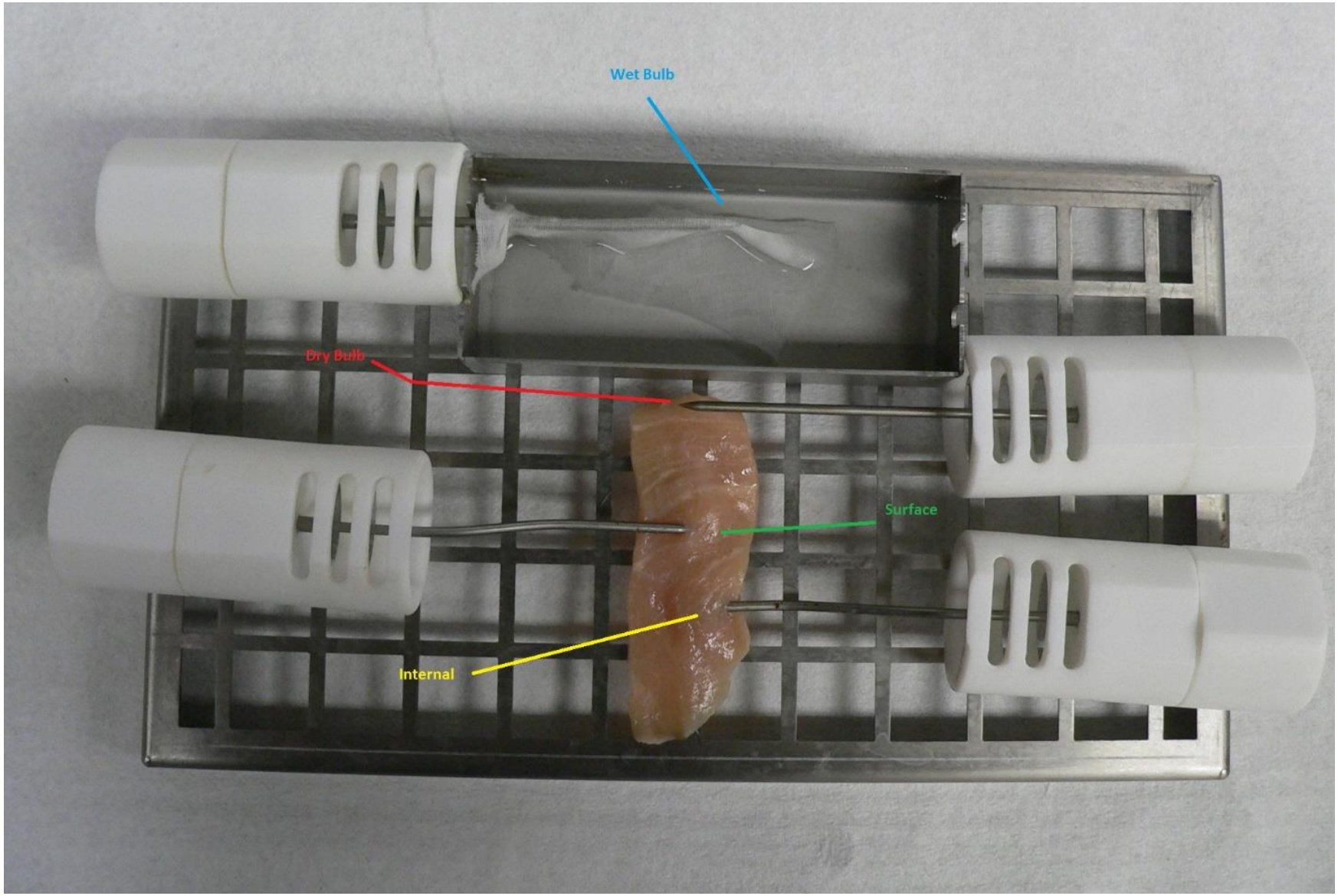


Figure 3: Physical layout of two-zone impingement ovens with relative humidity controls.

Table 1: The product/pathogen/internal temperature/cook cycle combinations tested for Phase 2 validation studies

Product	Pathogen	Internal Temperature	Cook Cycle[1]
Frankfurter	*Salmonella*	71.1°C (160°F)	Control
Frankfurter	*Salmonella*	71.1°C (160°F)	SLS
Frankfurter	*L. monocytogenes*	71.1°C (160°F)	Control
Frankfurter	*L. monocytogenes*	71.1°C (160°F)	SLS
Beef Patty	*Salmonella*	71.1°C (160°F)	Control
Beef Patty	*Salmonella*	71.1°C (160°F)	SLS
Beef Patty	*L. monocytogenes*	71.1°C (160°F)	Control
Beef Patty	*L. monocytogenes*	71.1°C (160°F)	SLS
Beef Patty	*Salmonella*	76.7°C (170°F)	Control
Beef Patty	*Salmonella*	76.7°C (170°F)	SLS
Beef Patty	*L. monocytogenes*	76.7°C (170°F)	Control
Beef Patty	*L. monocytogenes*	76.7°C (170°F)	SLS
Chicken Patty	*Salmonella*	71.1°C (160°F)	Control
Chicken Patty	*Salmonella*	71.1°C (160°F)	SLS
Chicken Patty	*L. monocytogenes*	71.1°C (160°F)	Control
Chicken Patty	*L. monocytogenes*	71.1°C (160°F)	SLS
Chicken Patty	*Salmonella*	79.4°C (175°F)	Control
Chicken Patty	*Salmonella*	79.4°C (175°F)	SLS
Chicken Patty	*L. monocytogenes*	79.4°C (175°F)	Control
Chicken Patty	*L. monocytogenes*	79.4°C (175°F)	SLS
Chicken Tender	*Salmonella*	71.1°C (160°F)	Control
Chicken Tender	*Salmonella*	71.1°C (160°F)	SLS
Chicken Tender	*L. monocytogenes*	71.1°C (160°F)	Control
Chicken Tender	*L. monocytogenes*	71.1°C (160°F)	SLS
Chicken Tender	*Salmonella*	79.4°C (175°F)	Control
Chicken Tender	*Salmonella*	79.4°C (175°F)	SLS
Chicken Tender	*L. monocytogenes*	79.4°C (175°F)	Control
Chicken Tender	*L. monocytogenes*	79.4°C (175°F)	SLS

[1] SLS = Surface Lethality Step

Table 2: Control thermal processing schedule utilized for cooking frankfurter batter

Control Process

Step/Process	Time	Dry Bulb (°C (°F))	Wet Bulb (°C (°F))	Relative Humidity (%)
1: Pre-Dry	00:25	54.4 (130)	-	0
2: Cook	00:10	54.4 (130)	-	0
3: Cook	00:10	60.0 (140)	54.4 (130)	74.8
4: Cook	00:15	65.6 (150)	60.0 (140)	76
5: Cook	00:10	73.9 (165)	-	0
6: Cook	00:15	79.4 (175)	65.6 (150)	52.8
7: Cook	IT= 71.1°C (160°F)	79.4 (175)	76.7 (170)	88.7

Table 3: Test thermal processing schedule utilized for cooking frankfurter batter including a surface lethality step (SLS)

Test Process

Step/Process	Time	Dry Bulb (°C (°F))	Wet Bulb (°C (°F))	Relative Humidity (%)
1: Pre-Dry	00:25	54.4 (130)	-	0
2: Cook	00:10	54.4 (130)	-	0
3: Cook	00:10	60.0 (140)	-	0
4: Cook	00:15	65.6 (150)	-	0
5: Cook	00:10	73.9 (165)	-	0
6: Lethality	00:15	79.4 (175)	65.6 (150)	52.8
7: Final Cook	IT= 71.1°C (160°F)	79.4 (175)	-	0

Table 4: Impingement oven schedules for **Control** cook cycle.

Product	Target IT[1] (°C(°F))	Upper Oven DB[2] (°C(°F))	Upper Oven WB[3] (°C(°F))	Upper Dwell[4] (min:sec)	Total Cook Time[5] (min:sec)
Beef Patty	71.1 (160)	204.4 (400)	OFF	3:30	7:00
	76.7 (170)	204.4 (400)	OFF	4:00	8:00
Chicken Strip	71.1 (160)	204.4 (400)	OFF	1:40	3:20
	79.4 (175)	204.4 (400)	OFF	2:00	4:00
Chicken Patty	71.1 (160)	204.4 (400)	OFF	2:10	4:20
	79.4 (175)	204.4 (400)	OFF	2:35	5:10

Table 5: Impingement oven schedules for **Surface Lethality Step (SLS)** cook cycle.

Product	Target IT[1] (°C(°F))	Upper Oven DB[2] (°C(°F))	Upper Oven WB[3] (°C(°F))	Upper Dwell[4] (min:sec)	Lower Oven DB (°C(°F))	Lower Oven WB (°C(°F))	Lower Dwell[6] (min:sec)	Total Cook Time[5] (min:sec)
Beef Patty	71.1 (160)	204.4 (400)	OFF	3:30	204.4 (400)	71.1 (160)	3:30	7:00
	76.7 (170)	204.4 (400)	OFF	4:00	204.4 (400)	71.1 (160)	4:00	8:00
Chicken Strip	71.1 (160)	204.4 (400)	OFF	1:40	204.4 (400)	71.1 (160)	1:40	3:20
	79.4 (175)	204.4 (400)	OFF	2:00	204.4 (400)	71.1 (160)	2:00	4:00
Chicken Patty	71.1 (160)	204.4 (400)	OFF	2:10	204.4 (400)	71.1 (160)	2:10	4:20
	79.4 (175)	204.4 (400)	OFF	2:35	204.4 (400)	71.1 (160)	2:35	5:10

[1] IT = Internal Temperature
[2] DB = Dry Bulb temperature
[3] WB = Wet Bulb temperature
[4] Amount of time a sample spent passing through a the upper oven
[5] Total amount of time the sample spent passing through both ovens
[6] Amount of time a sample spent passing through the bottom oven

Table 6: D-values for *Salmonella* in frankfurter batter, beef patties, chicken patties, and chicken tenders[1]

D-values (min) for *Salmonella*							
	Current Project				**Prior NAMIF study[2]**		
Temperature °C(°F)	Frankfurter Batter	Beef Patty	Chicken Patty	Chicken Tender	Lean Roast Beef	Turkey Breast	Ham
54.4 (130)	20.48 ± 6.55	7.48 ± 1.55	16.32 ± 3.82	14.08 ± 3.27	11.90	20.83	16.67
60.0 (140)	1.74 ± 0.10	0.48 ± 0.10	1.36 ± 0.24	0.83 ± 0.17	0.72	2.42	1.50
65.6 (150)	0.24 ± 0.07	0.2 ± 0.10	0.15 ± 0.01	0.30 ± 0.04	0.18	0.24	0.25
71.1 (160)	0.06 ± 0.0	0.07 ± 0.04	0.08 ± 0.03	0.05 ± 0.00	0.02	0.03	0.02

Table 7: D-values for *Listeria monocytogenes* in beef patties, chicken patties, and chicken tenders[8]

D-values (min) for *L. monocytogenes*						
	Current Project			**Prior NAMIF study[2]**		
Temperature (°F)	Beef Patty	Chicken Patty	Chicken Tender	Lean Roast Beef	Turkey Breast	Ham
54.4 (130)	48.15 ± 30.60	83.33 ± 0	83.33 ± 0	48.13	50.58	66.22
60.0 (140)	4.95 ± 0.78	13.95 ± 1.17	14.15 ± 3.88	7.25	5.98	8.57
62.8 (145)	2.37 ± 0.60	7.50 ± 1.18	5.97 ± 0.30	-	-	-
65.6 (150)	0.77 ± 0.30	4.63 ± 0.60	3.67 ± 1.08	1.72	37.2	64.8
71.1 (160)	11 (n=1)	1.18 ± 0.53	44 (n=1)	0.33	0.07	0.27

Table 8: Z-values for *Salmonella* and *L. monocytogenes* in frankfurters, beef patties, chicken patties, and chicken tenders

	Z-value (°C (°F))			
Pathogen	Frankfurter	Beef Patty	Chicken Patty	Chicken Tender
Salmonella	6.42 (11.15)	8.68 (15.60)	7.00 (12.58)	7.18 (12.90)
Listeria	-[3]	6.31 (11.27)	8.80 (15.70)	8.06 (14.39)

[1] D-values are the average of triplicate experiments ± S.D
[2] Sindelar, J. J., K. A. Glass, and R. Hanson. 2013. Developing Validated Time-Temperature Thermal Processing Guidelines for Ready-To-Eat Deli Meat and Poultry Products. *In*, North American Meat Institute Foundation
[3] D-values not determined for *L. monocytogenes* in Frankfurter batter

Table 9: Proximate composition of raw frankfurters, beef patties, chicken patties, and chicken tenders[1].

Product	Moisture[2] (%)	a_w[3]	pH[4]	NaCl[5] (%)	Crude Fat[6] (%)
Frankfurter	57.71 ± 0.92	0.977 ± 0.003	6.62 ± 0.04	1.79 ± 0.07	26.84 ± 1.4
Beef Patty	58.04 ± 0.84	0.983 ± 0.003	5.52 ± 0.08	0.22 ± 0.08	26.52 ± 1.1
Chicken Patty	69.43 ± 0.63	0.975 ± 0.004	6.63 ± 0.07	1.08 ± 0.03	15.8 ± 1.3
Chicken Tender	74.58 ± 0.42	0.978 ± 0.002	6.49 ± 0.08	1.32 ± 0.12	2.3 ± 0.4

[1] Values are mean ± S.D. from all replications. For each rep triplicate samples were analyzed for proximate composition.
[2] Vacuum oven method, 5 hour, 100°C; AOAC 950.46, 1990
[3] Measured using a Decagon Aqua lab 4TE water activity meter.
[4] pH of slurry measured with Accumet Basic pH meter and Orion A111 combination electrode, Thermo Fisher Scientific.
[5] Measured as % Cl^-, $AgNO_3$ potentiometric titration, Mettler G20 food and beverage analyzer
[6] Crude fat method, Soxhlet ether extraction; AOAC 991.36, 1990.

Table 10: Average microbial populations of *Listeria monocytogenes* after Phase 2 validation testing with Control and Surface Lethality Step (SLS)[1] cycles (n=2). Populations are listed as average log CFU/g ± S.D.[2]

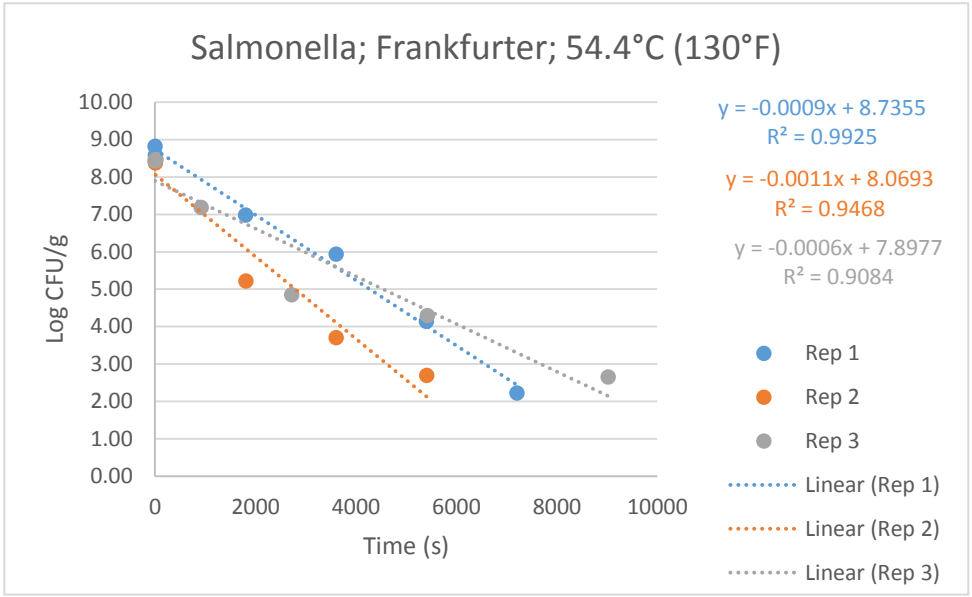
	L. monocytogenes											
Formulation	Chicken Tenders				Chicken Patties				Beef Patties			
Target Temperature (°C(°F))	71.1 (160)		**79.4 (175)**		71.1 (160)		**79.4 (175)**		71.1 (160)		**76.7 (170)**	
Treatment →	Control	SLS	Control	SLS	Control	SLS	Control	SLS	Control	SLS	Control	SLS
Timepoint												
Raw	7.71 ± 0.10	7.71 ± 0.10	7.71 ± 0.10	7.71 ± 0.10	7.48 ± 0.04	7.48 ± 0.04	7.48 ± 0.04	7.48 ± 0.04	7.34 ± 0.03	7.34 ± 0.03	7.34 ± 0.03	7.34 ± 0.03
First Pass	7.27 ± 0.15	7.27 ± 0.15	6.76 ± 0.04	6.76 ± 0.04	7.31 ± 0.07	7.31 ± 0.07	7.06 ± 0.26	7.06 ± 0.26	7.10 ± 0.15	7.1 ± 0.15	6.86 ± 0.18	6.86 ± 0.18
Target IT	3.5 ± 2.22	2.18 ± 0.06	2.97 ± 1.02	1.85 ± 0.93	2.30 ± 0.18	1.62 ± 0.14	1.42 ± 0.11	1.03 ± 0.04	≤1.00	1.08 ± 0.39	≤1.00	≤1.00

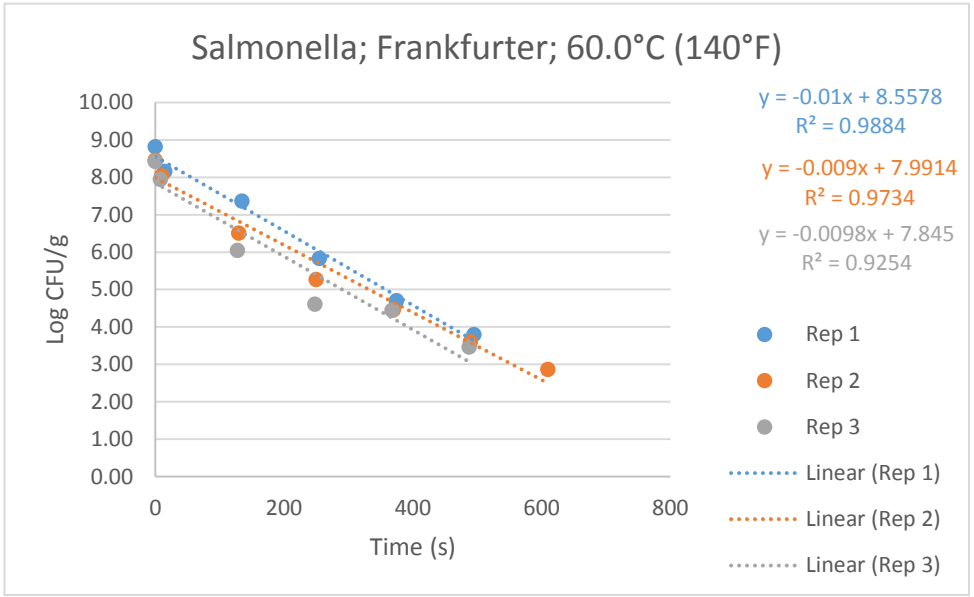
Table 11: Average microbial populations of *Salmonella* after Phase 2 validation testing with Control and Surface Lethality Step (SLS) cycles. (n=2). Populations are listed as average log CFU/g ± S.D.

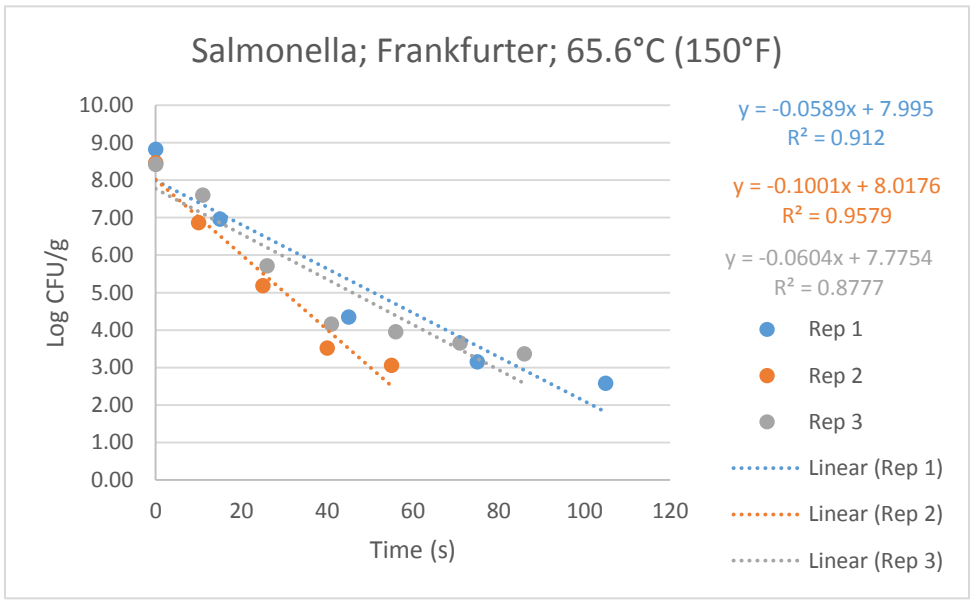
	Salmonella											
Formulation	Chicken Tenders				Chicken Patties				Beef Patties			
Target Temperature (°C(°F))	71.1 (160)		**79.4 (175)**		71.1 (160)		**79.4 (175)**		71.1 (160)		**76.7 (170)**	
Treatment →	Control	SLS	Control	SLS	Control	SLS	Control	SLS	Control	SLS	Control	SLS
Timepoint												
Raw	7.63 ± 0.32	7.63	7.63 ± 0.32	7.63 ± 0.32	7.92 ± 0.02	7.92 ± 0.02	7.92 ± 0.02	7.92 ± 0.02	7.78 ± 0.10	7.78 ± 0.10	7.78 ± 0.10	7.78 ± 0.10
First Pass	6.87 ± 0.53	6.87 ± 0.53	6.88 ± 0.22	6.88 ± 0.22	7.42 ± 0.11	7.42 ± 0.11	7.43 ± 0.10	7.43 ± 0.10	7.28 ± 0.30	7.28 ± 0.30	7.13 ± 0.07	7.13 ± 0.07
Target	3.62 ± 1.76	3.67 ± 0.25	1.83 ± 0.72	3.39 ± 0.36	3.65 ± 2.26	2.36 ± 0.89	1.99 ± 1.07	1.67 ± 0.94	1.47 ± 0.15	1.59 ± 0.84	≤1.00	≤1.00

[1] Surface Lethality Step cycles incorporated an increase in wet-bulb temperature to 71.1°C(160°F) for the second half of the cook cycle
[2] Red squares indicate a treatment where a 5-log reduction was not achieved. Yellow squares indicate a treatment within 1 S.D. of the 5-log reduction target

Figure 4a: Determination of D-values for *Salmonella* in frankfurter by plotting Log CFU/g pathogen levels against temperature (n=3).







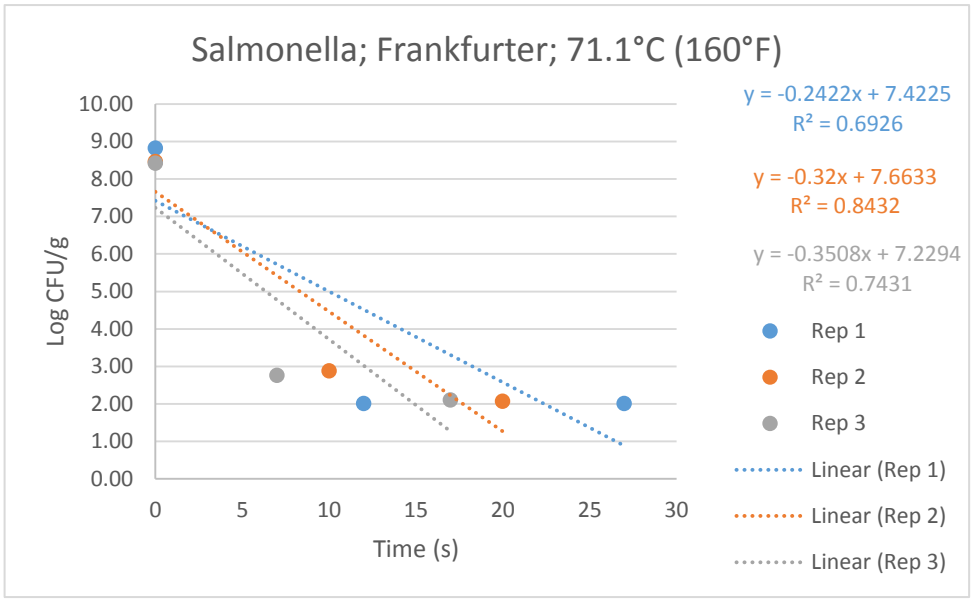
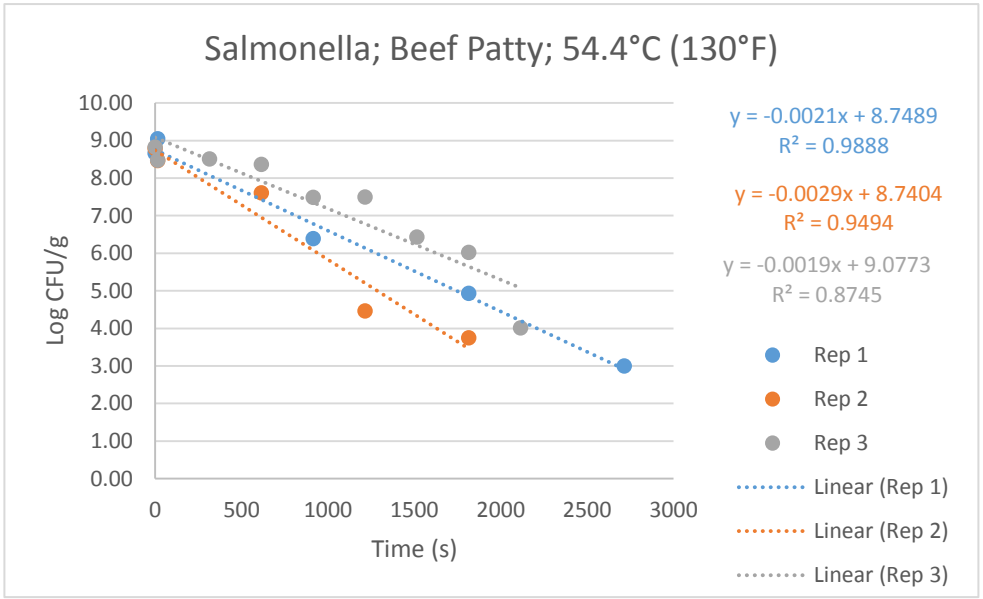
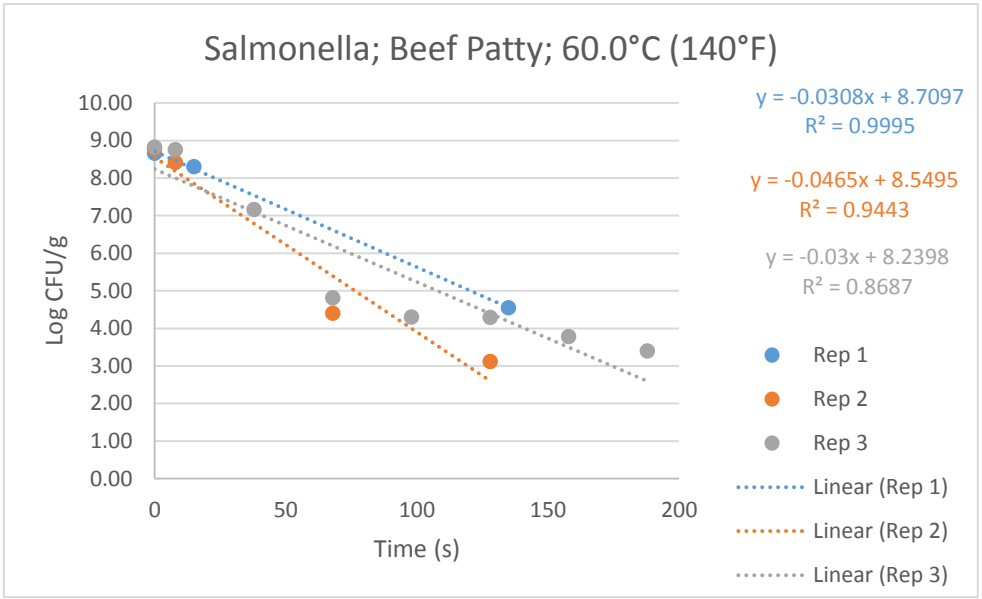
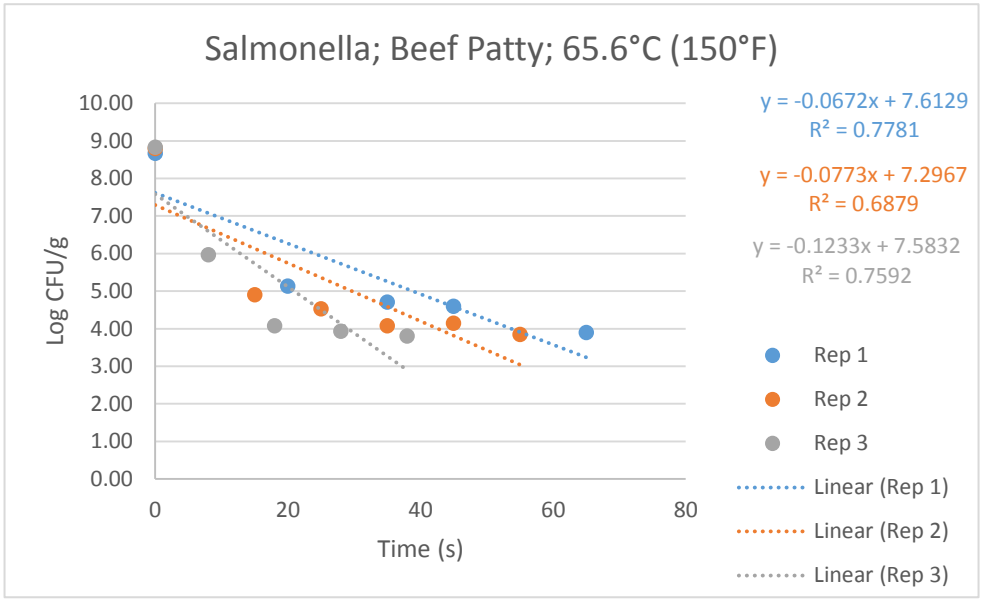


Figure 4b: Determination of D-values for *Salmonella* in beef patty by plotting Log CFU/g pathogen levels against temperature (n=3).







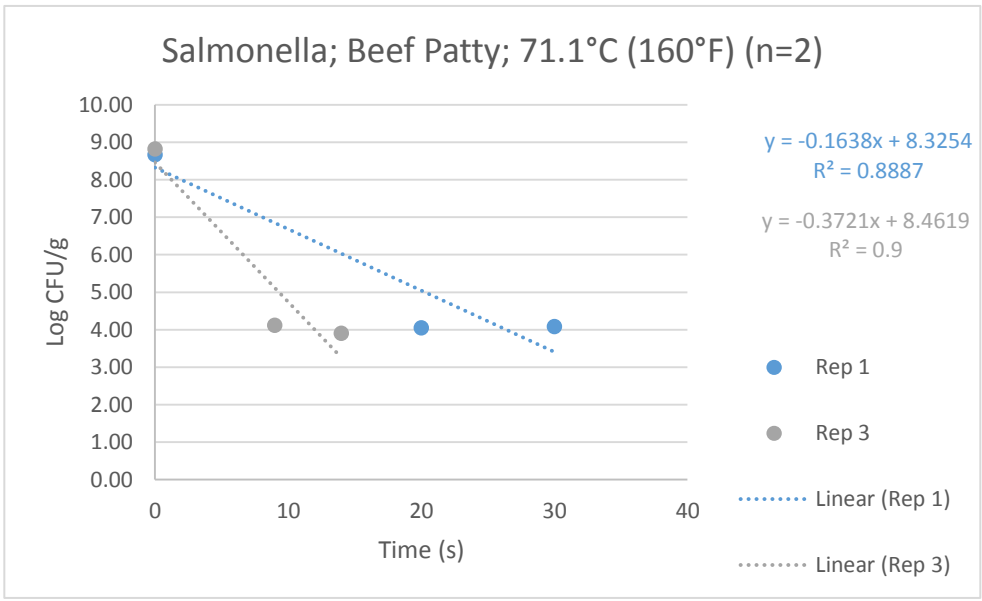
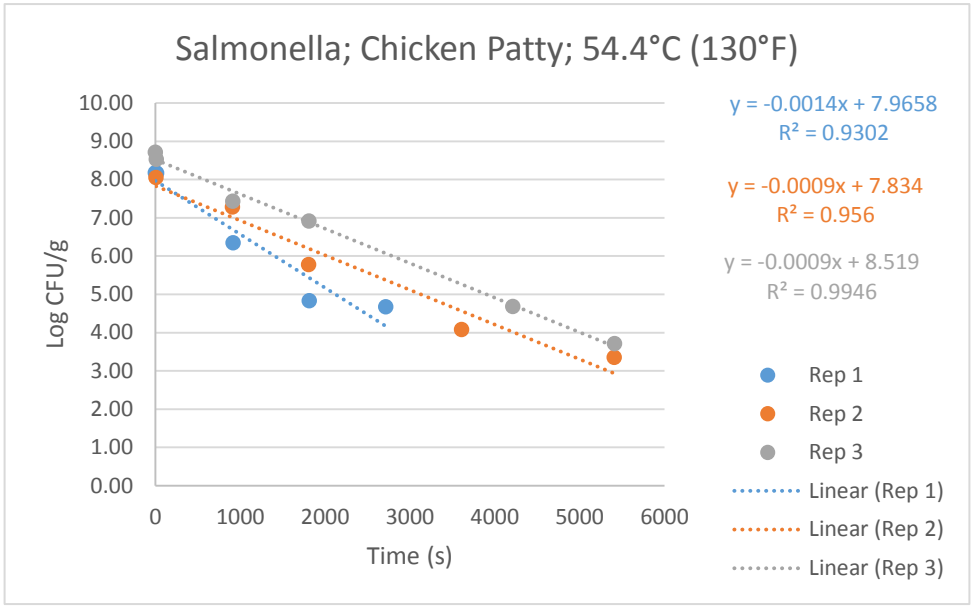
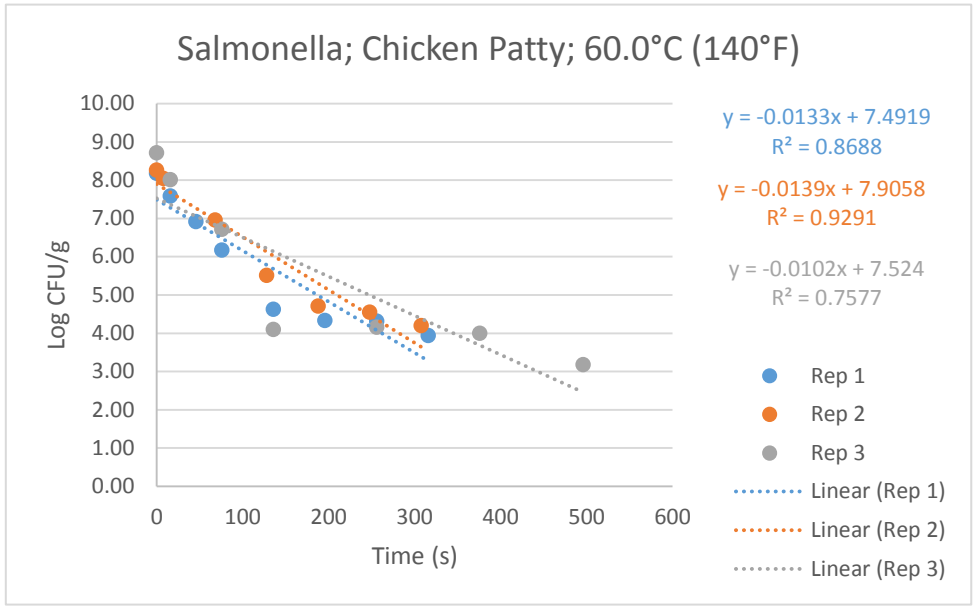
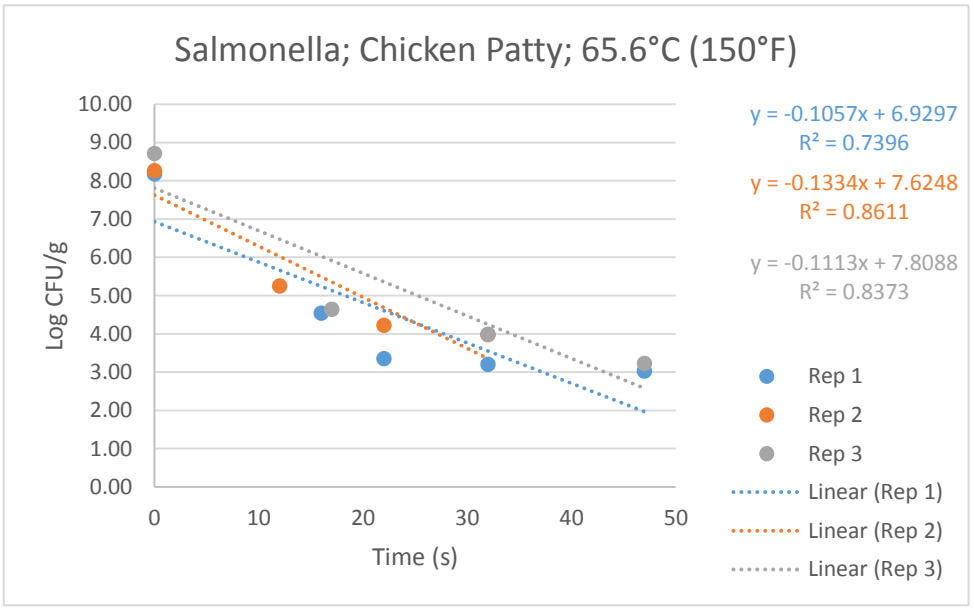


Figure 4c: Determination of D-values for *Salmonella* in chicken patty by plotting Log CFU/g pathogen levels against temperature (n=3).







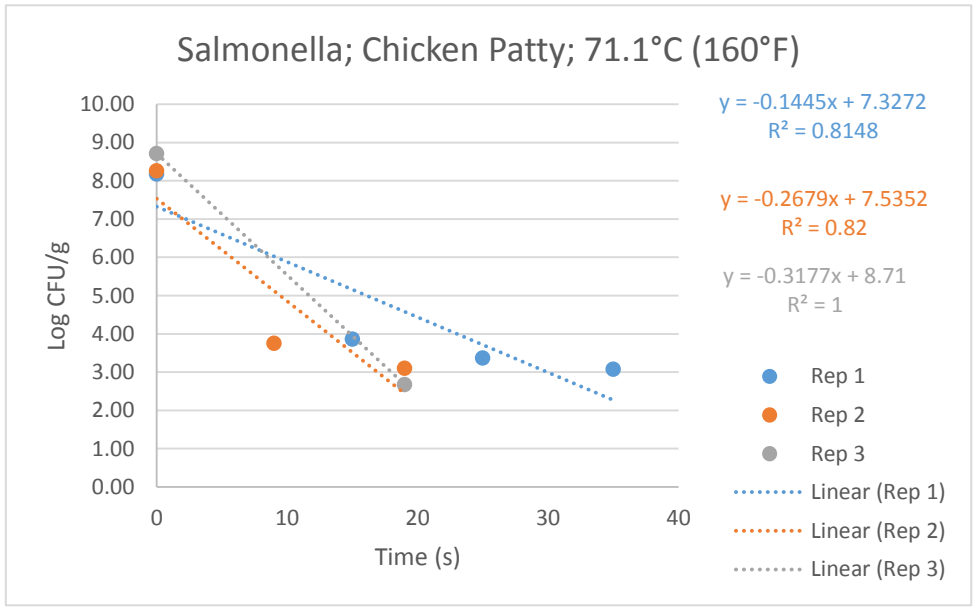
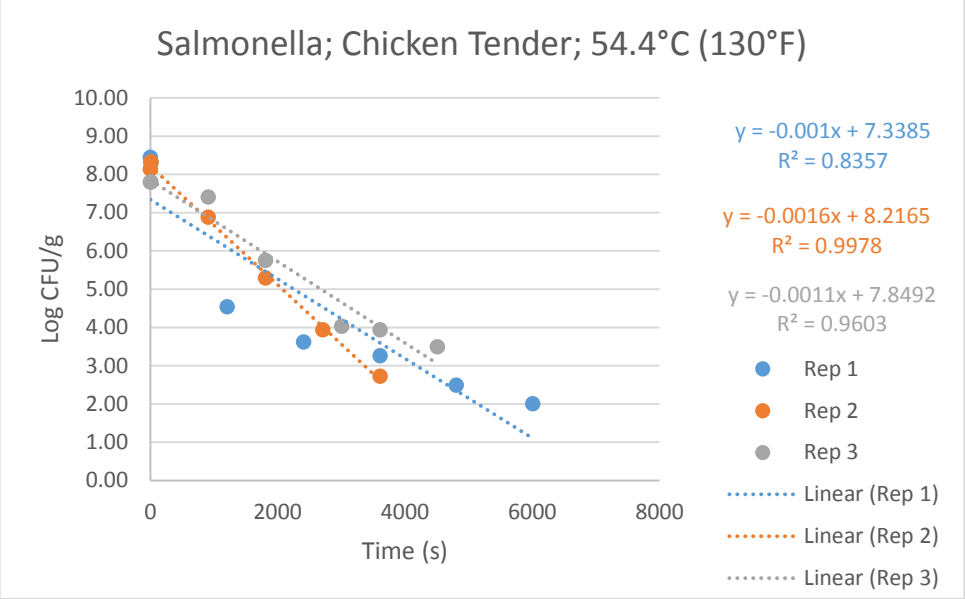


Figure 4d: Determination of D-values for *Salmonella* in chicken tenders by plotting Log CFU/g pathogen levels against temperature (n=3).



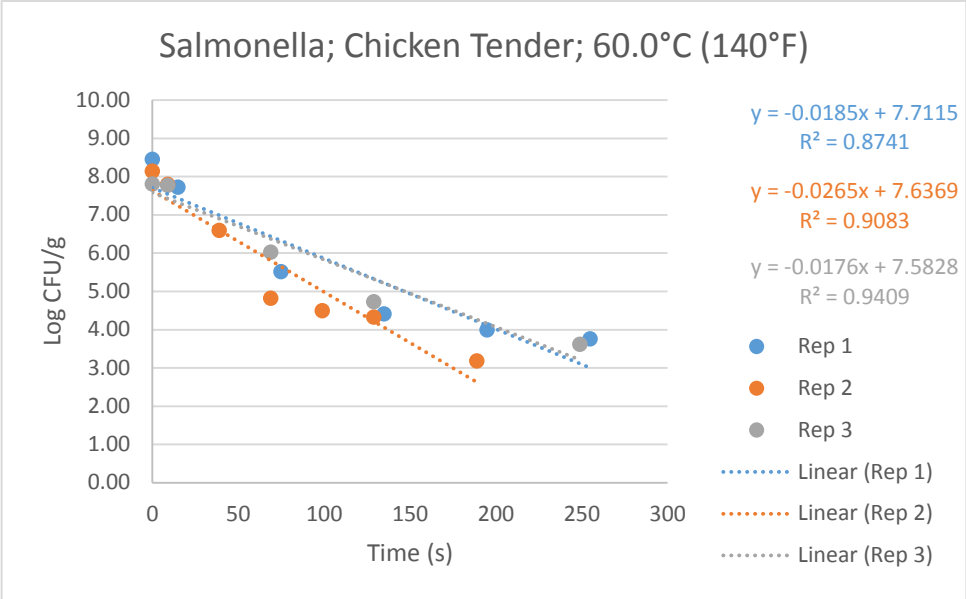
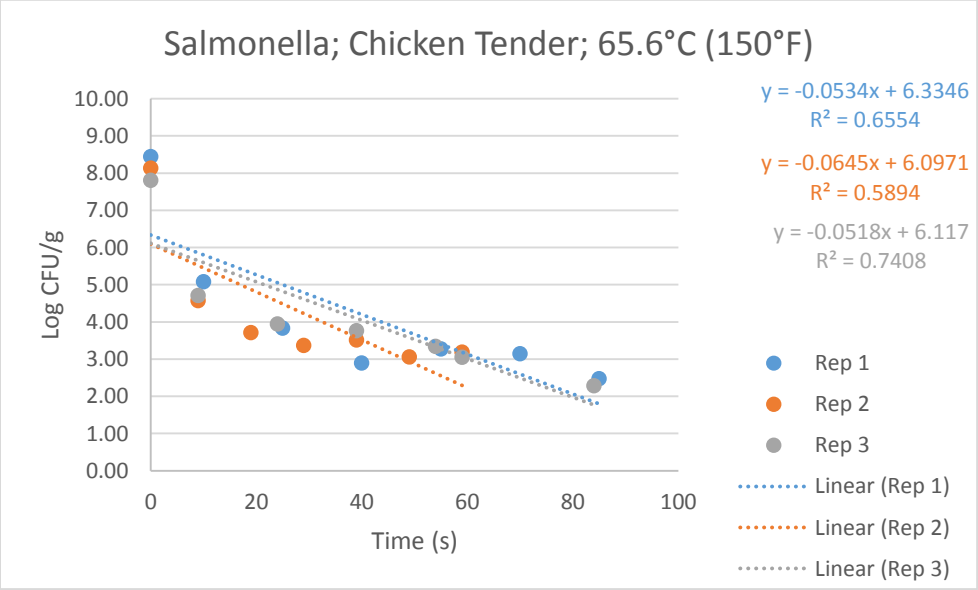
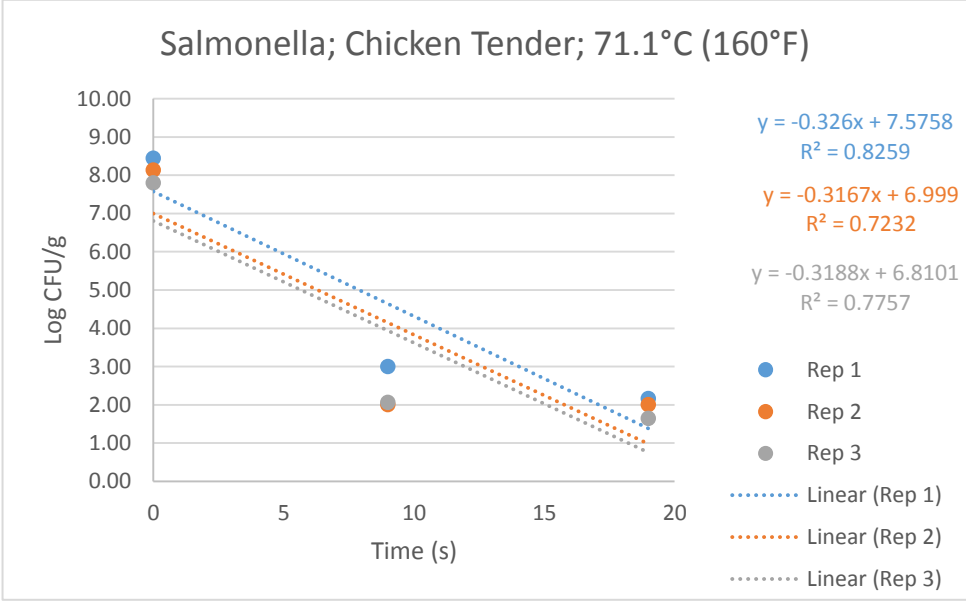
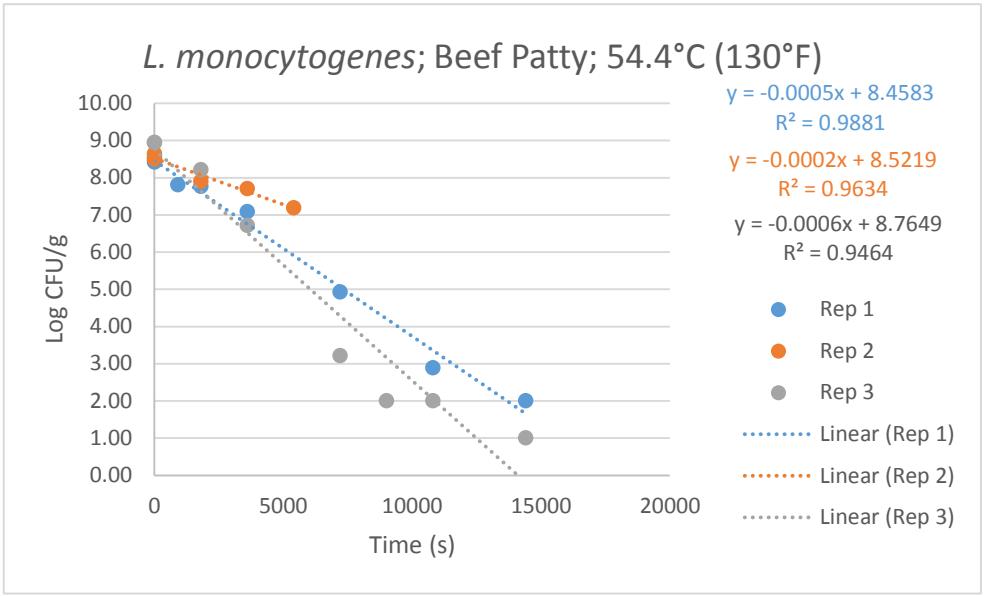
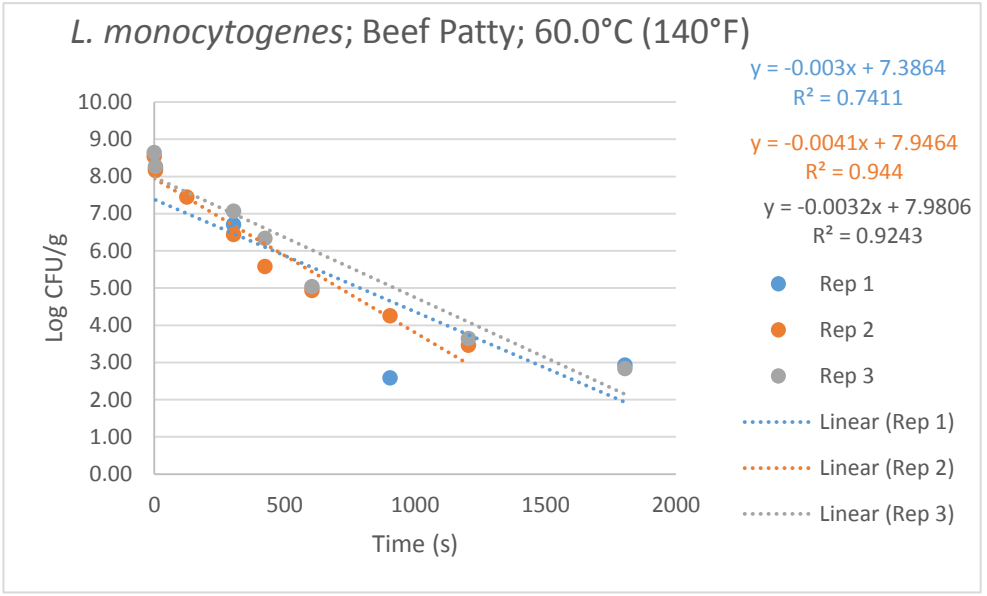


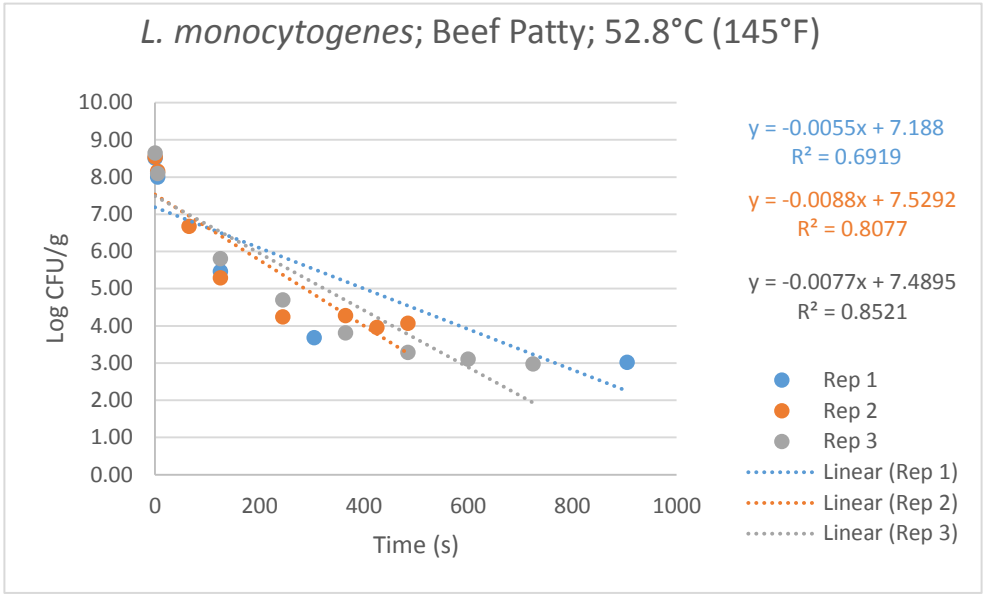
Figure 5a: Determination of D-values for *Listeria monocytogenes* in beef patty by plotting Log CFU/g pathogen levels against temperature (n=3).






L. monocytogenes; Beef Patty; 54.4°C (130°F)
Log CFU/g
Time (s)
y = -0.0005x + 8.4583
R² = 0.9881
y = -0.0002x + 8.5219
R² = 0.9634
y = -0.0006x + 8.7649
R² = 0.9464
Rep 1
Rep 2
Rep 3
Linear (Rep 1)
Linear (Rep 2)
Linear (Rep 3)


L. monocytogenes; Beef Patty; 60.0°C (140°F)
Log CFU/g
Time (s)
y = -0.003x + 7.3864
R² = 0.7411
y = -0.0041x + 7.9464
R² = 0.944
y = -0.0032x + 7.9806
R² = 0.9243
Rep 1
Rep 2
Rep 3
Linear (Rep 1)
Linear (Rep 2)
Linear (Rep 3)


L. monocytogenes; Beef Patty; 52.8°C (145°F)
Log CFU/g
Time (s)
y = -0.0055x + 7.188
R² = 0.6919
y = -0.0088x + 7.5292
R² = 0.8077
y = -0.0077x + 7.4895
R² = 0.8521
Rep 1
Rep 2
Rep 3
Linear (Rep 1)
Linear (Rep 2)
Linear (Rep 3)

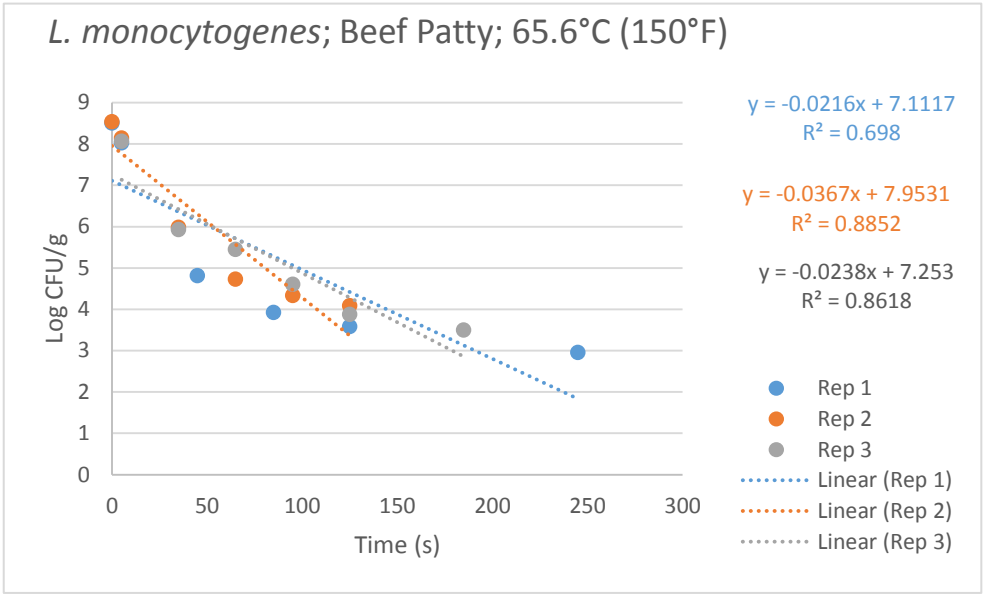
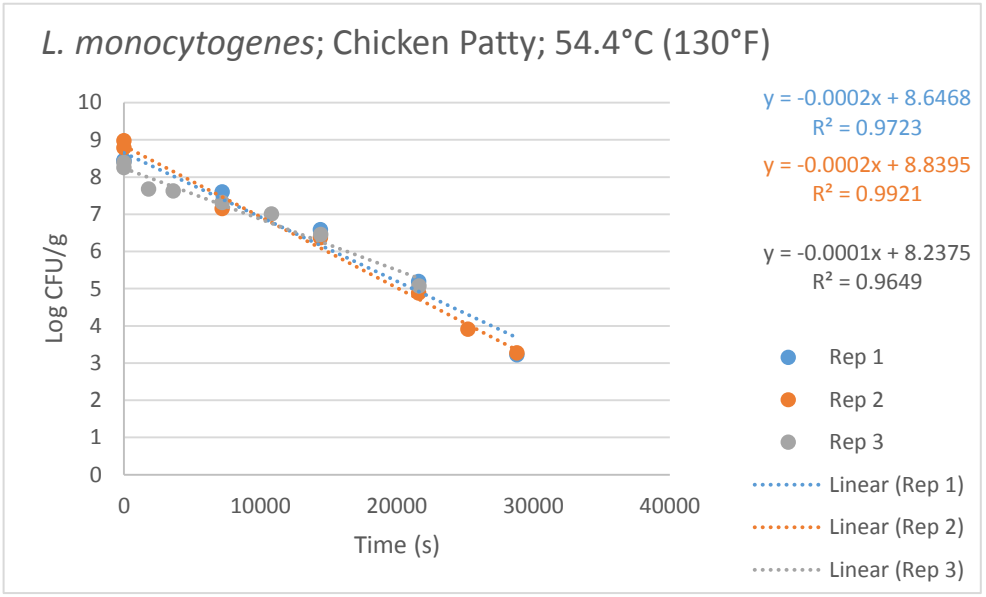
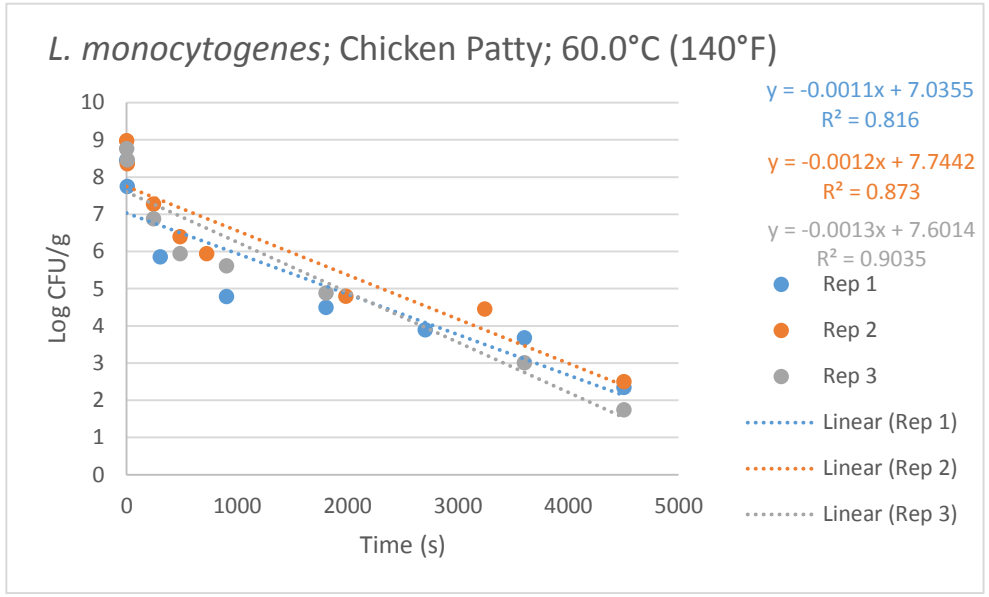
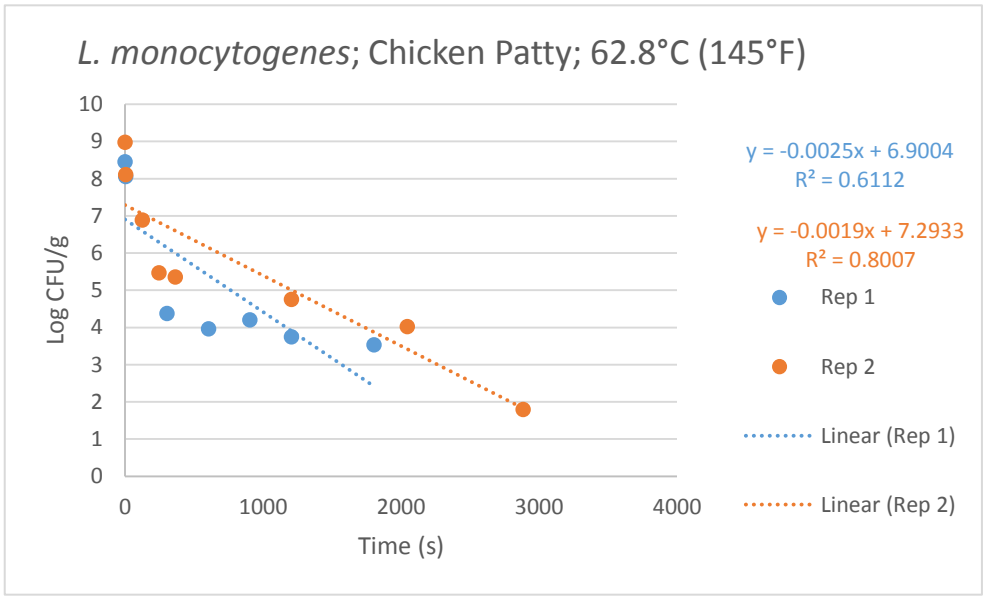

L. monocytogenes; Beef Patty; 65.6°C (150°F)
Log CFU/g
Time (s)
y = -0.0216x + 7.1117
R² = 0.698
y = -0.0367x + 7.9531
R² = 0.8852
y = -0.0238x + 7.253
R² = 0.8618
Rep 1
Rep 2
Rep 3
Linear (Rep 1)
Linear (Rep 2)
Linear (Rep 3)

Figure 5b: Determination of D-values for *Listeria monocytogenes* in chicken patty by plotting Log CFU/g pathogen levels against temperature (n=3).







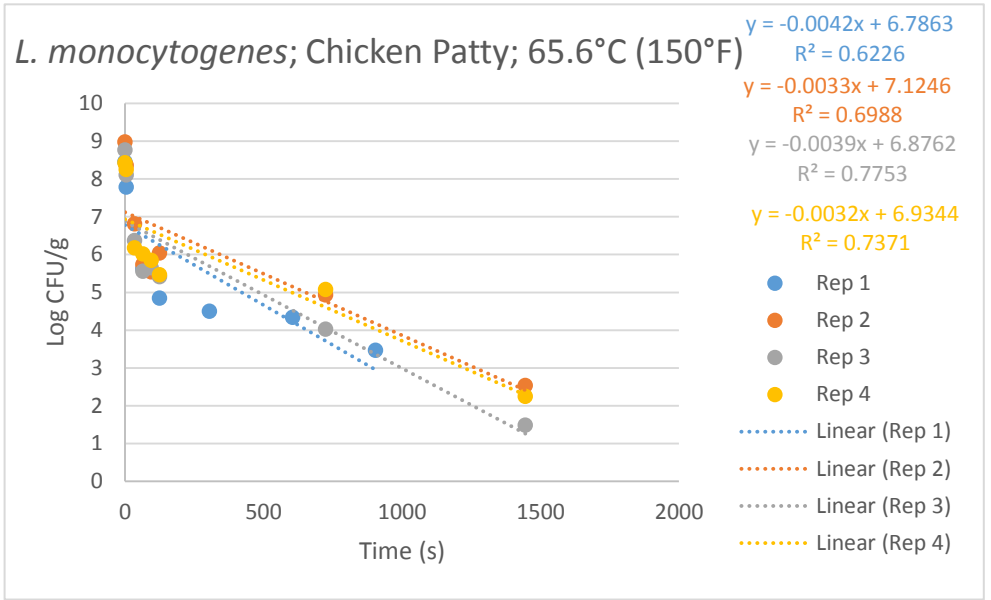
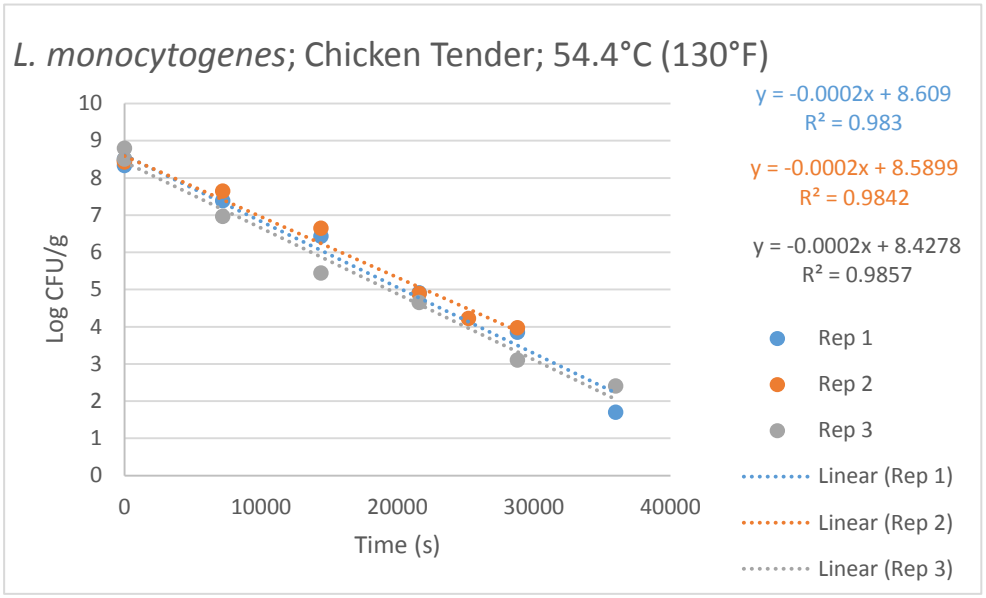
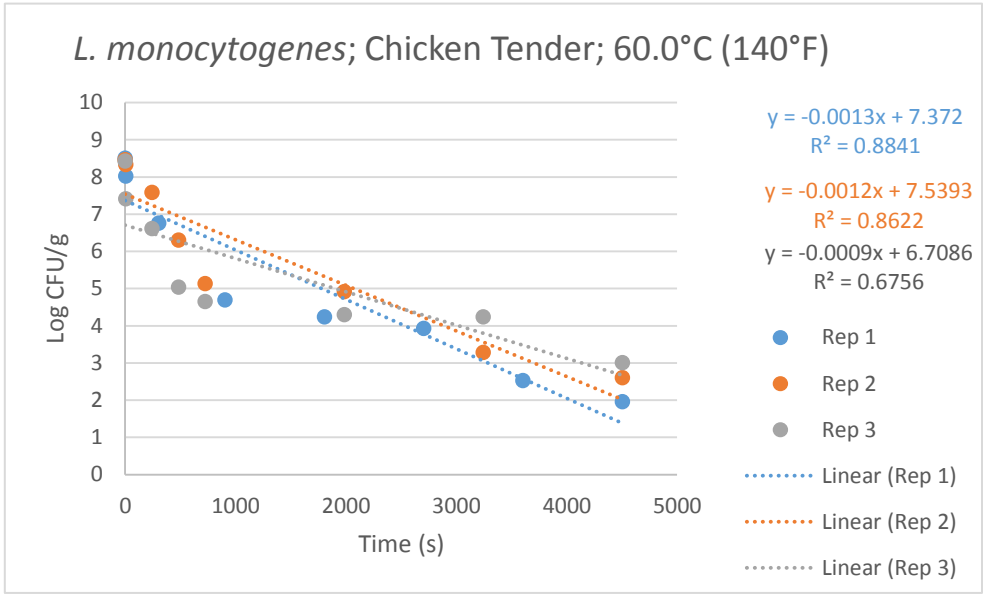
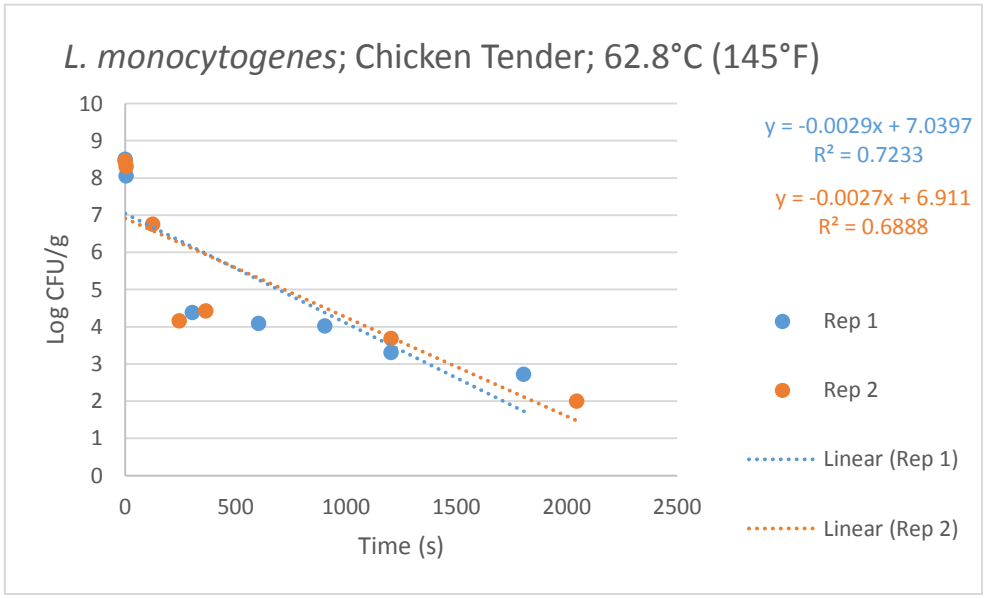
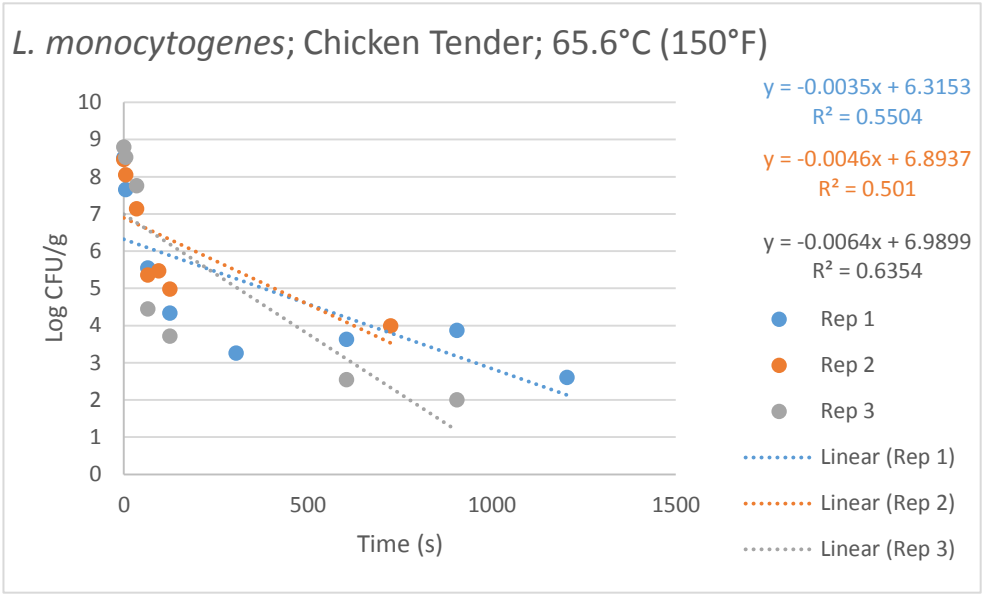


Figure 5c: Determination of D-values for *Listeria monocytogenes* in chicken tender by plotting Log CFU/g pathogen levels against temperature



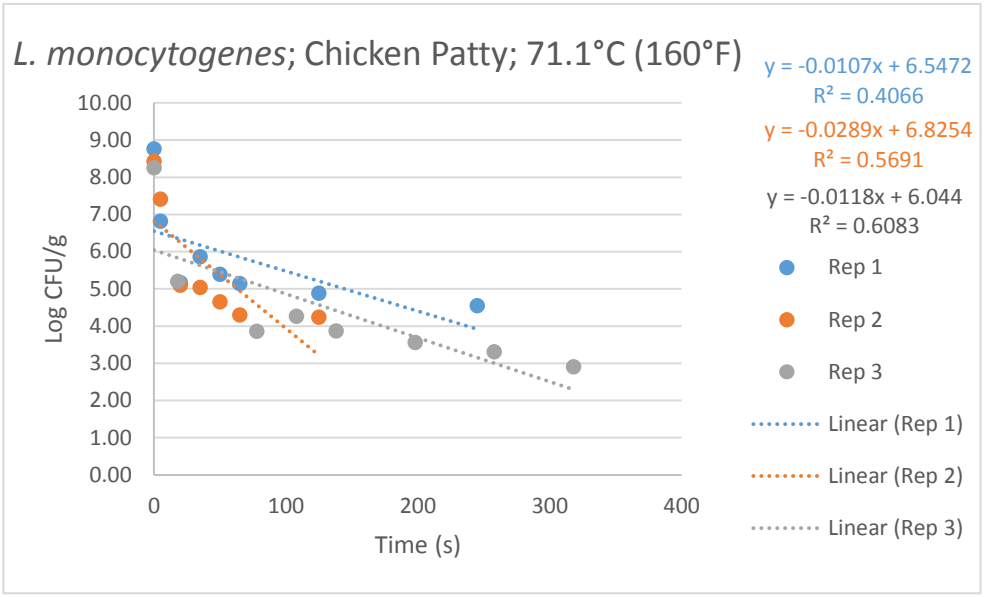


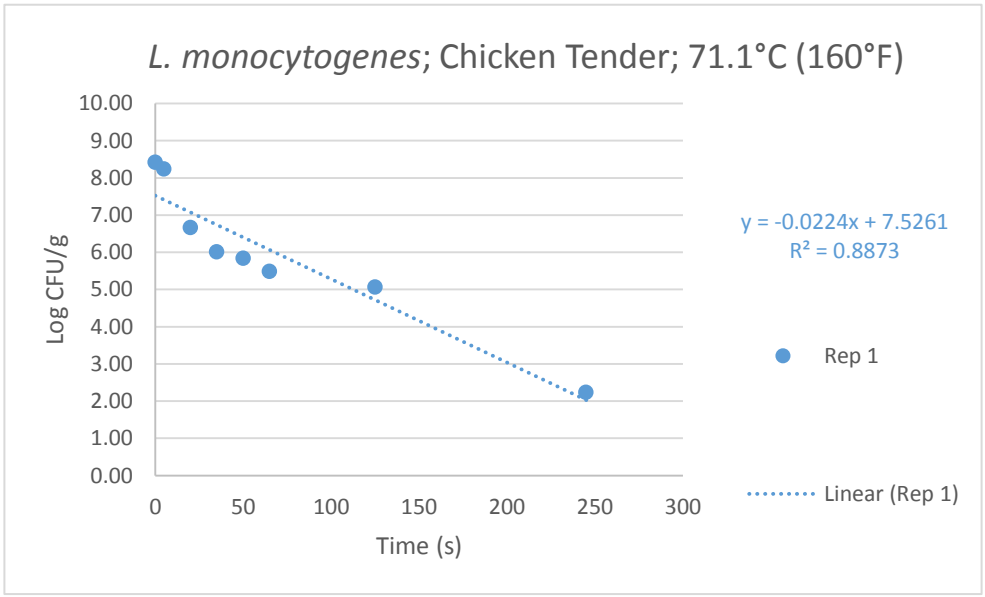




(n=3).

Figure 5d: Determination of D-values for *Listeria monocytogenes* in chicken tenders, chicken patties, and beef patties cooked to 160°F (71.1°C) by plotting Log CFU/g pathogen levels against temperature (n=3).





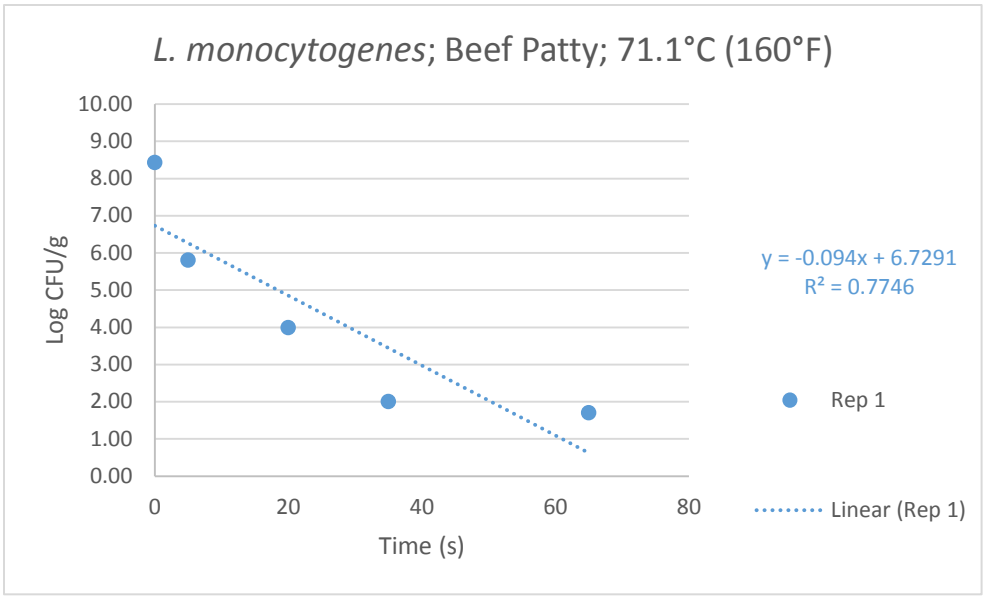


Figure 6: Example graph for determination of D-value for *Listeria monocytogenes* in frankfurters cooked to 150°F (71.1°C) demonstrating extreme tailing effect. (n=2). The D-value calculated from this graph was 4.05 minutes.

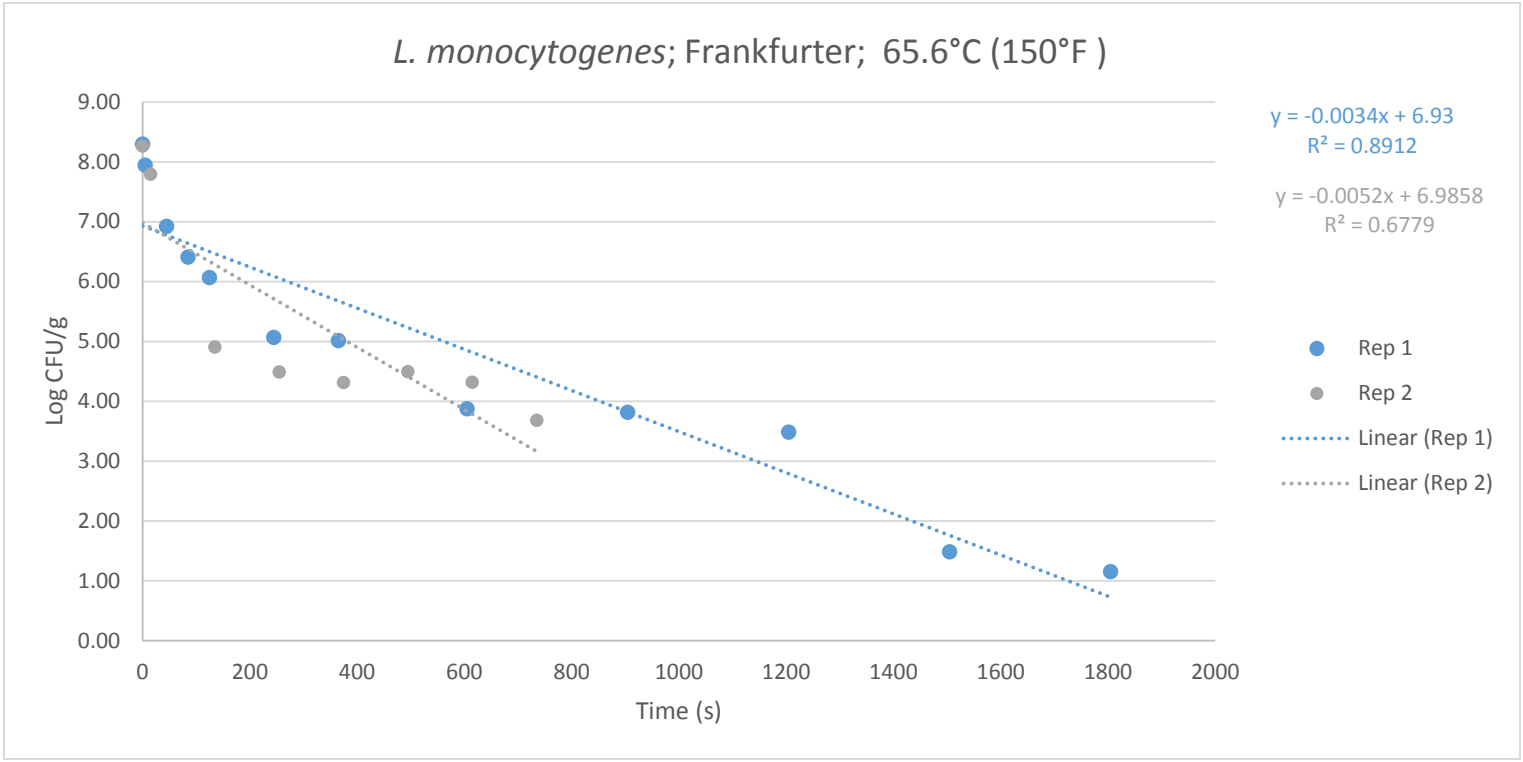


Figure 7a: Determination of z-values for *Salmonella* in frankfurter, beef patty, chicken patty, and chicken tender. Log D-values (y-axis) against temperature (°C) (x-axis).

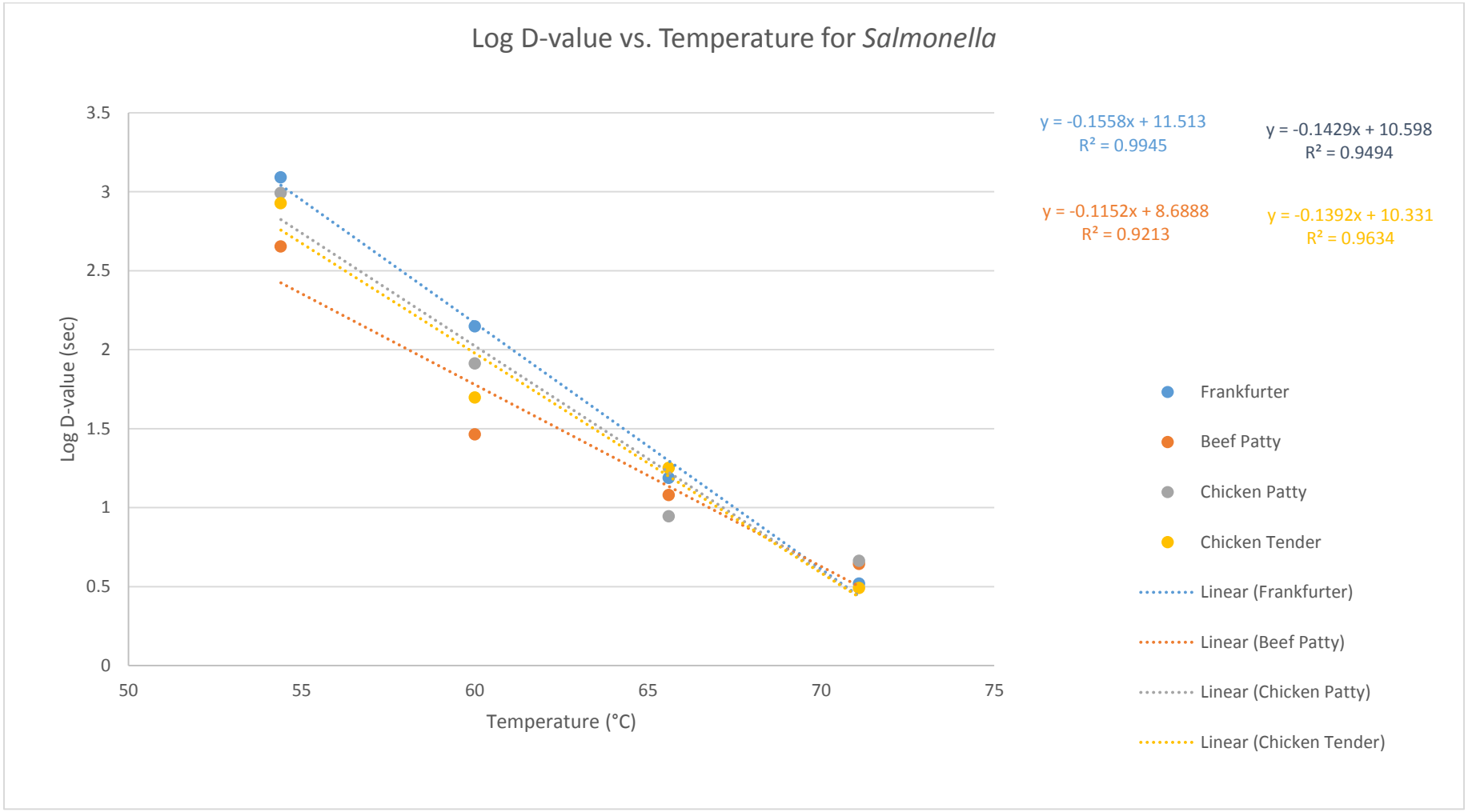


Figure 7b: Determination of z-values for *Listeria monocytogenes* in beef patty, chicken patty, and chicken tender. Log D-values (y-axis) against temperature (°C) (x-axis).

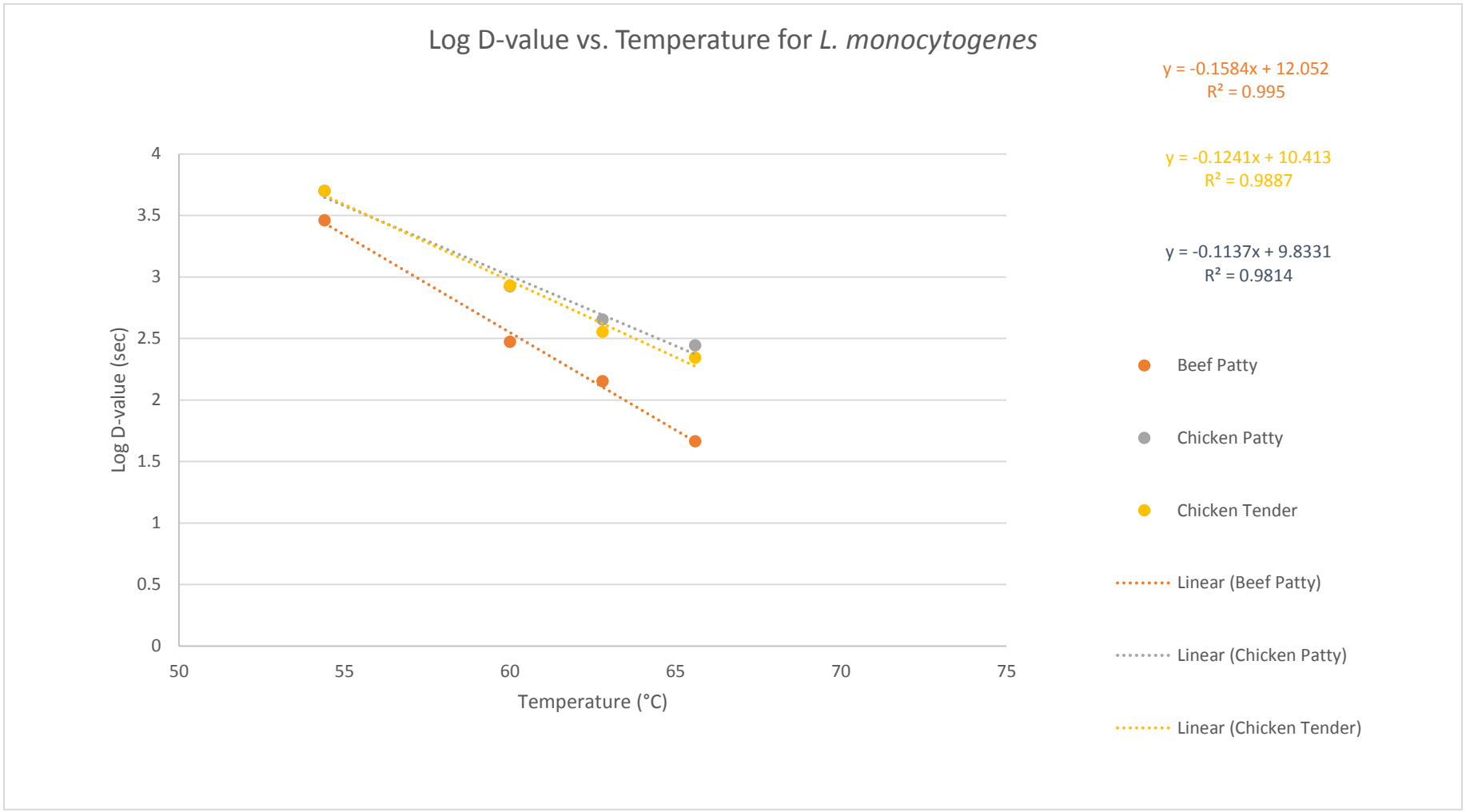
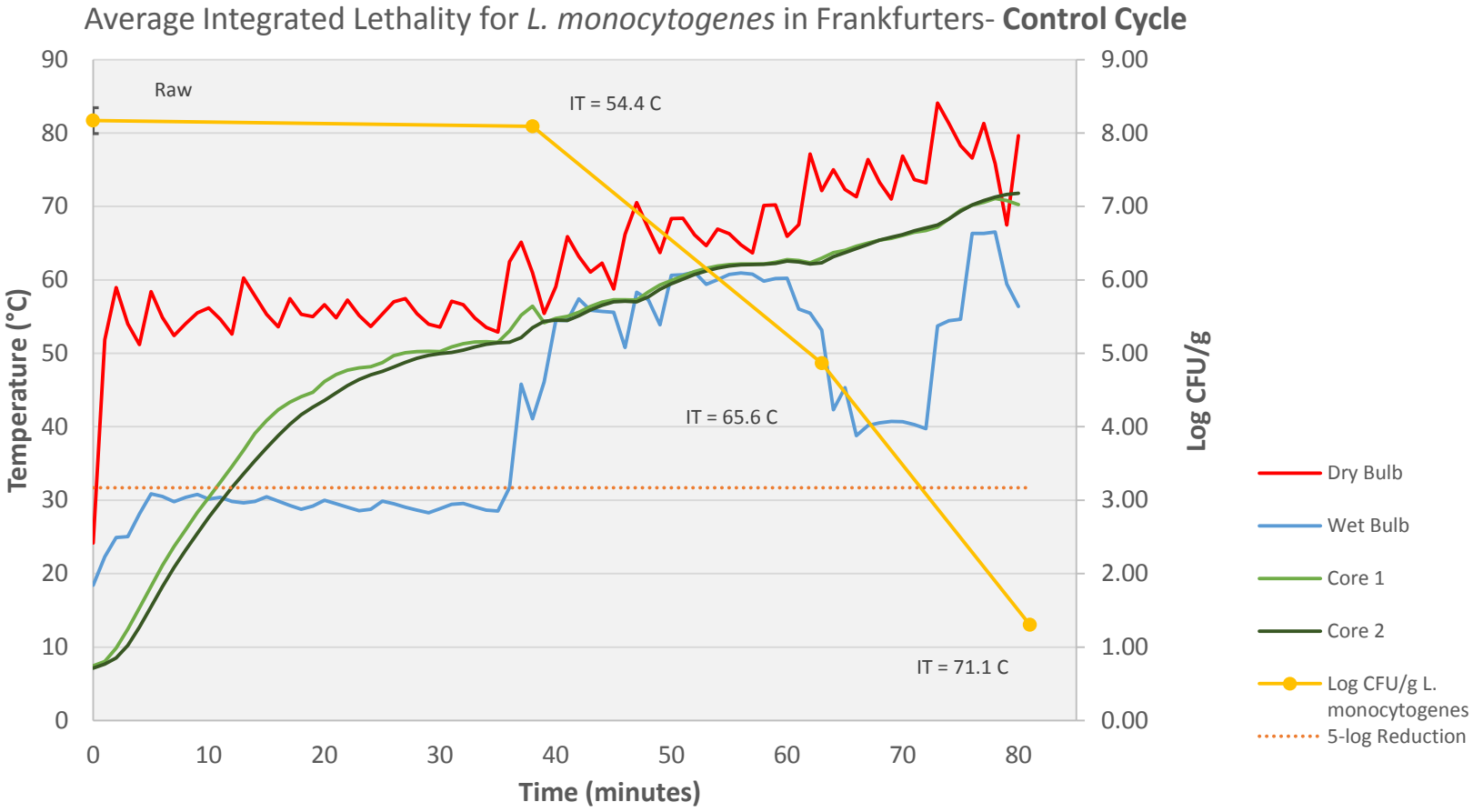
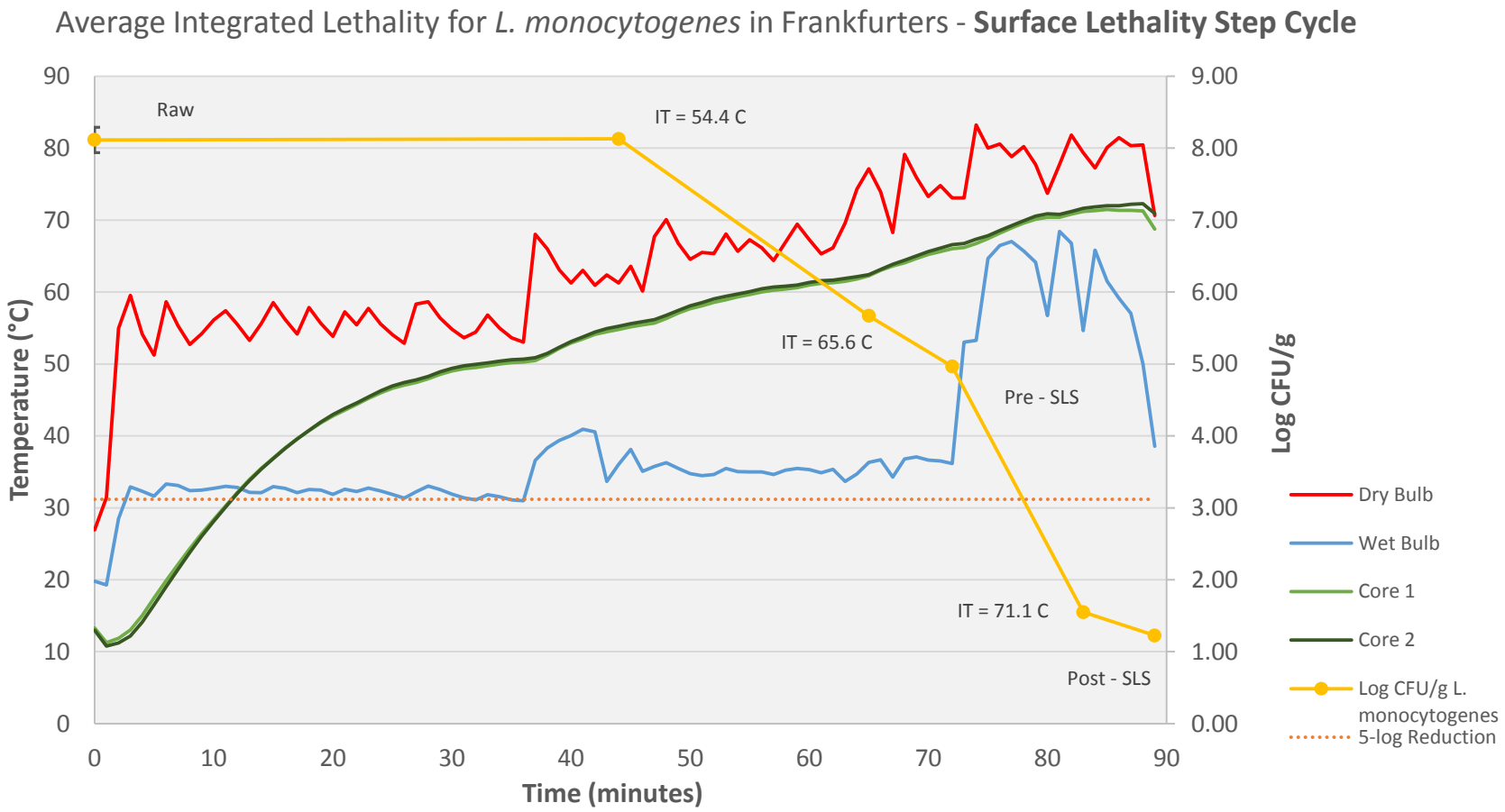


Figure 8: Average integrated thermal process/pathogen reduction profile for frankfurter batter inoculated with *Listeria monocytogenes* and heated to 71.1°C (160°F) – control cycle[1].



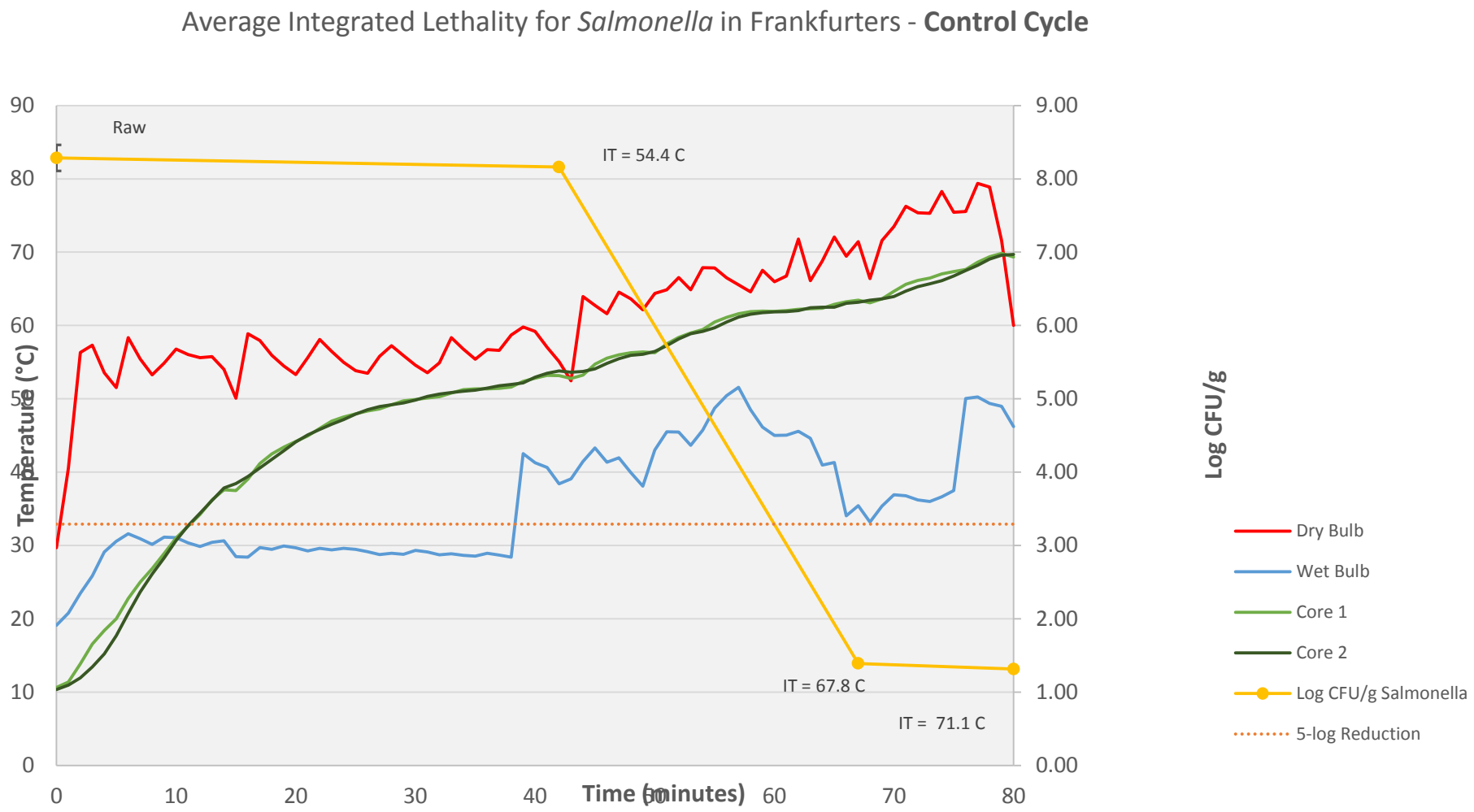
[1] 5-log reduction was the target for Phase 1 D-value determination experiments. The 5-log reduction line is included only as a reference point.

Figure 9: Average integrated thermal process/pathogen reduction profile for frankfurter batter inoculated with *L. monocytogenes* and heated to 71.1°C (160°F) – Surface Lethality Step cycle[1].



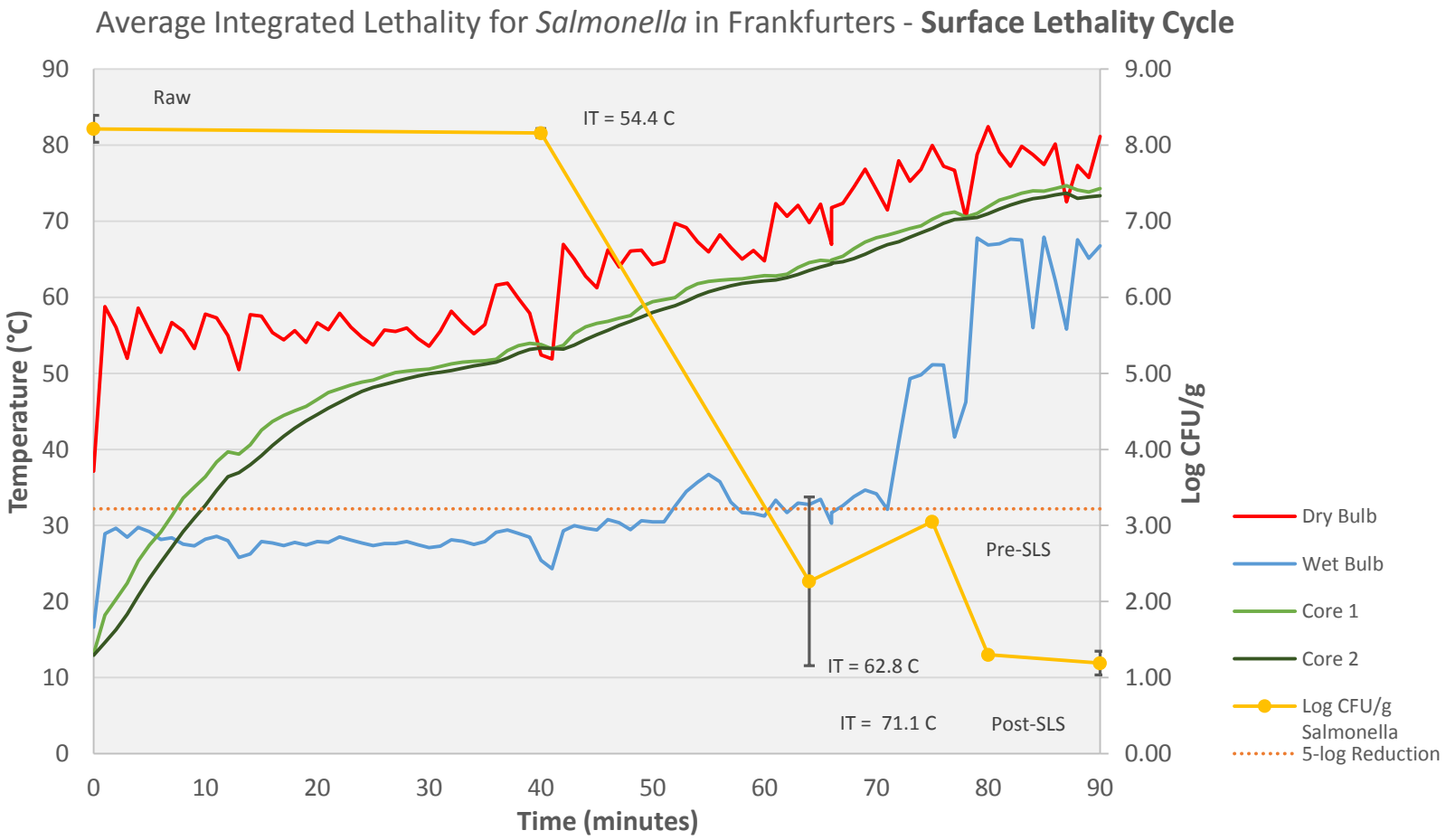
[1] 5-log reduction was the target for Phase 1 D-value determination experiments. The 5-log reduction line is included only as a reference point.

Figure 10: Average integrated thermal process/pathogen reduction profile for frankfurter batter inoculated with *Salmonella* and heated to 71.1°C (160°F) – control cycle[1].



[1] 5-log reduction was the target for Phase 1 D-value determination experiments. The 5-log reduction line is included only as a reference point.

Figure 11: Integrated thermal process/pathogen reduction profile for frankfurter batter inoculated with *Salmonella* and heated to 71.1°C (160°F) – Surface Lethality Step cycle[1]



[1] 5-log reduction was the target for Phase 1 D-value determination experiments. The 5-log reduction line is included only as a reference point.

Figure 12: Average integrated thermal process/pathogen lethality profile for chicken tenders inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 71.1°C (160°F) - **Control Cycle** (n=2)

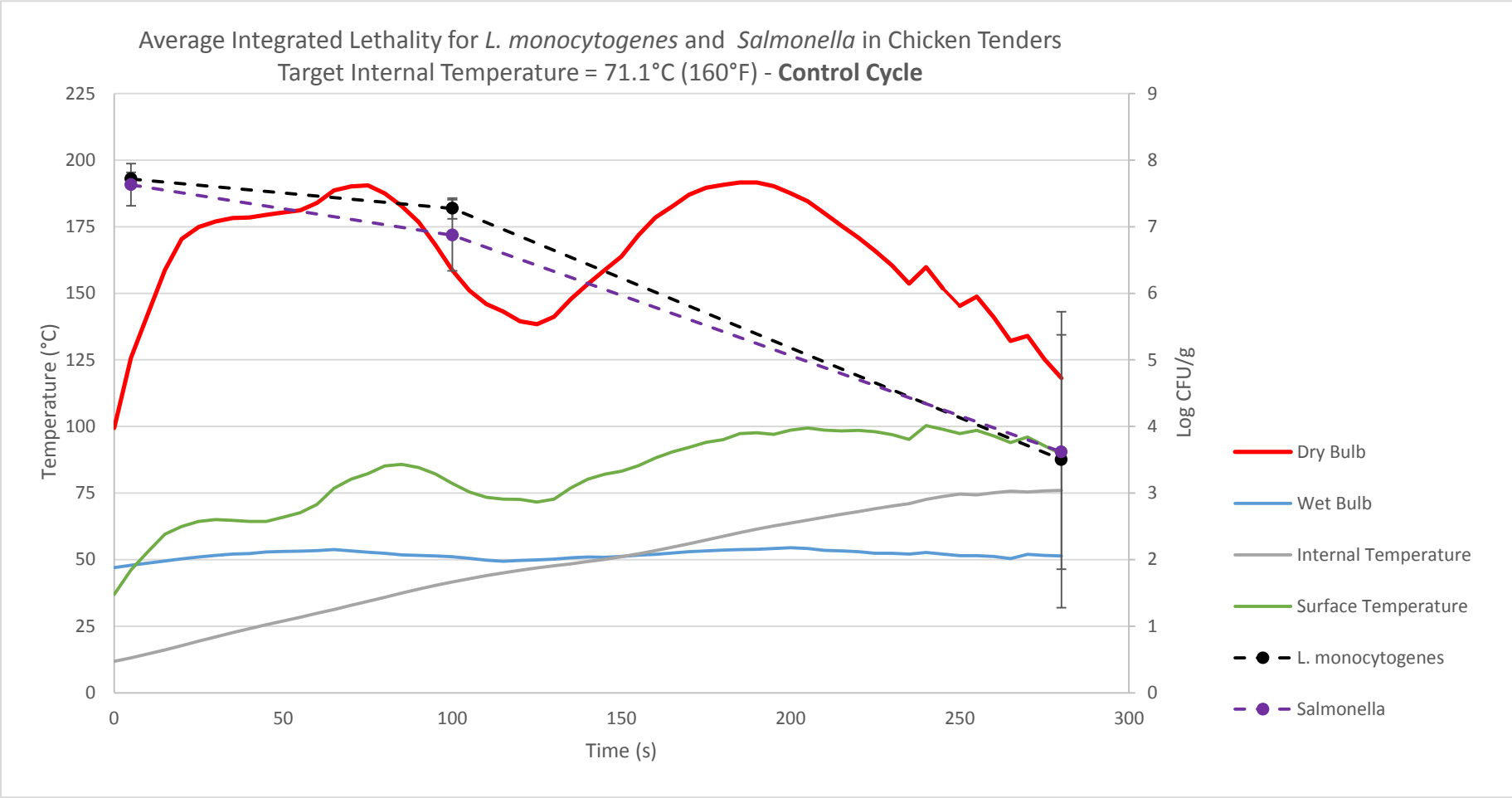


Figure 13: Average integrated thermal process/pathogen lethality profile for chicken tenders inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 71.1°C (160°F) – **Surface Lethality Step Cycle**

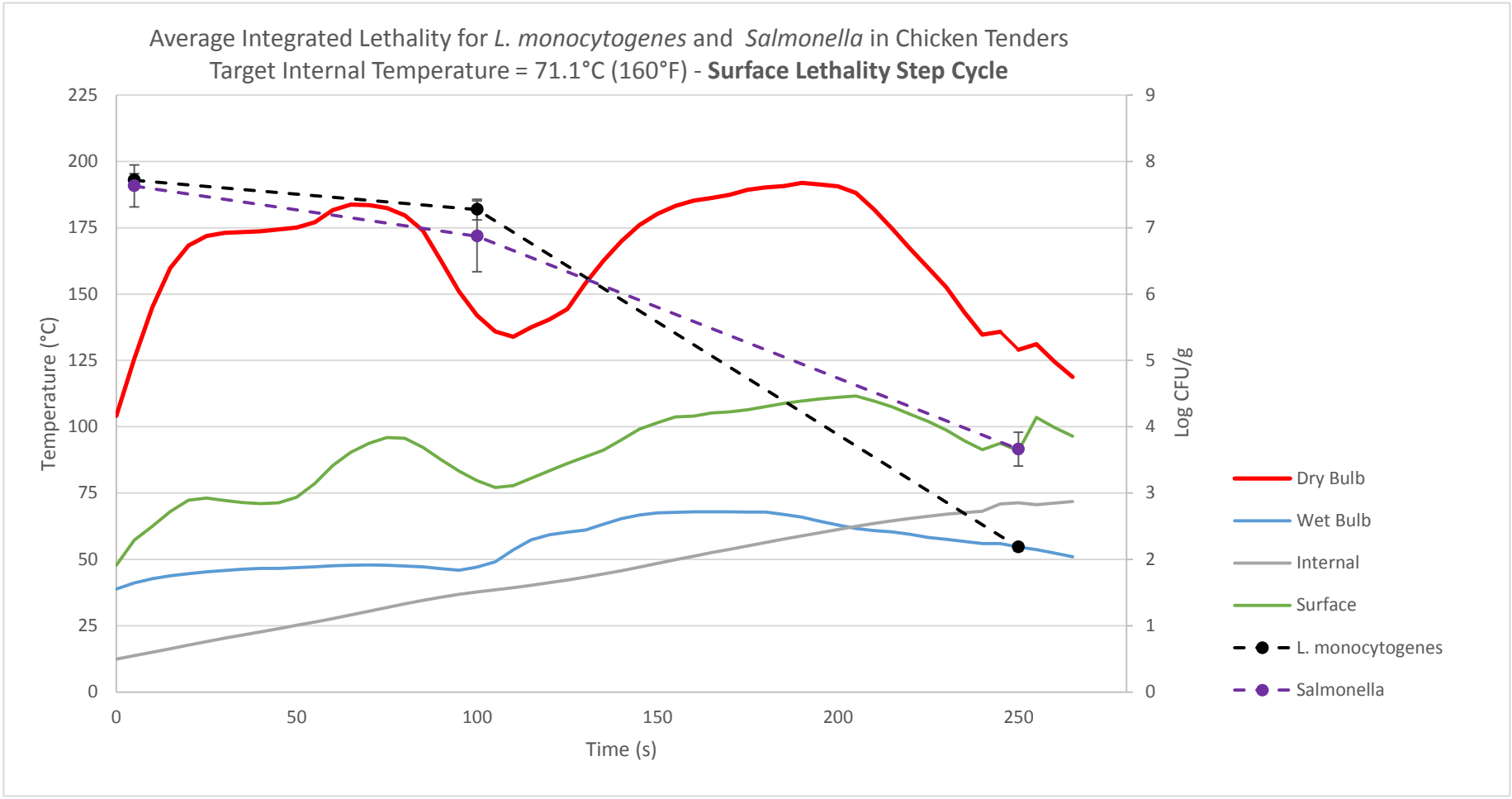


Figure 14: Average integrated thermal process/pathogen lethality profile for chicken tenders inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 79.4°C (175°F) - **Control Cycle** (n=2)

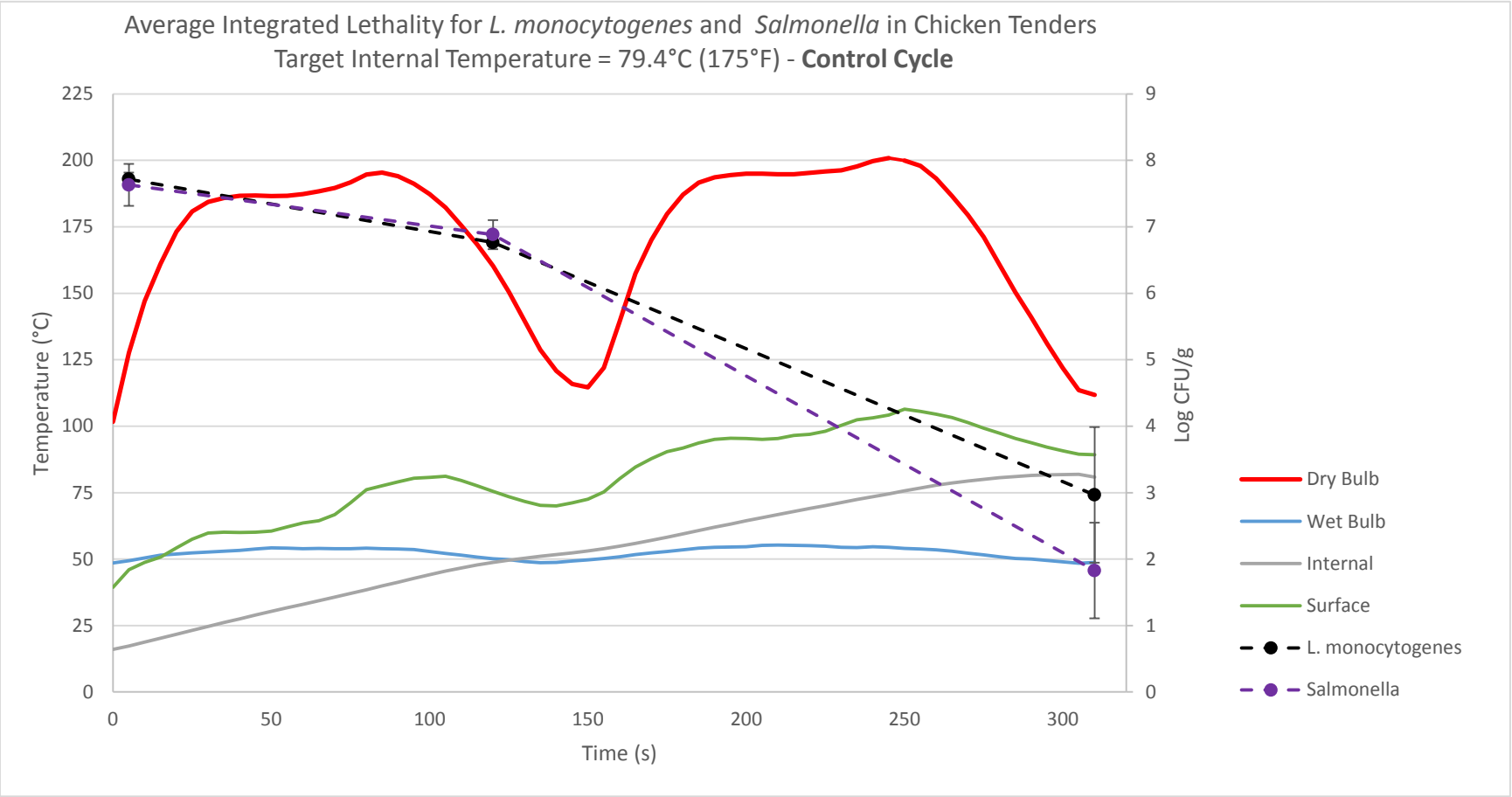


Figure 15: Average integrated thermal process/pathogen lethality profile for chicken tenders inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 79.4°C (175°F) – **Surface Lethality Step Cycle** (n=2)

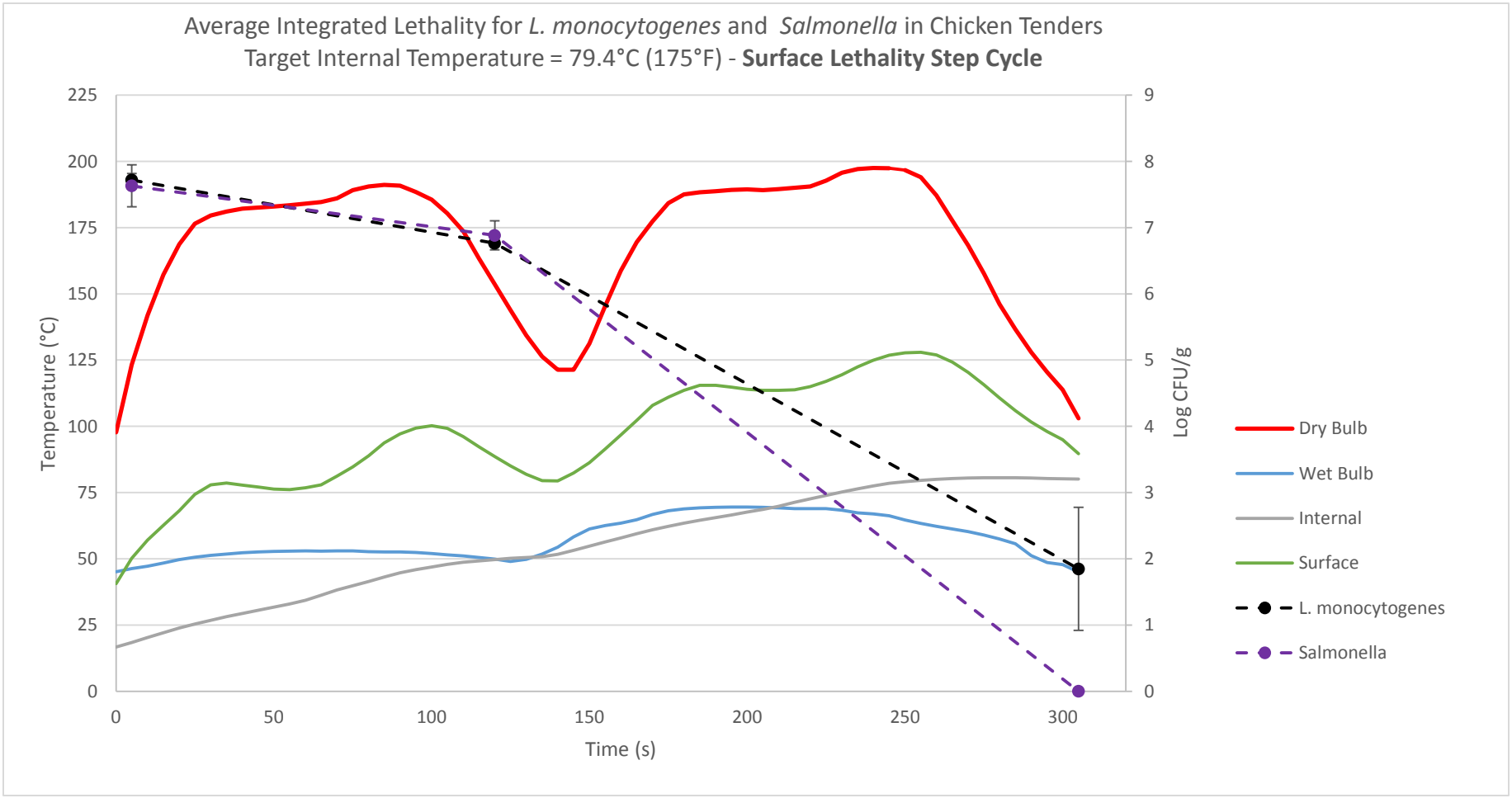


Figure 16: Average integrated thermal process/pathogen lethality profile for chicken patties inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 71.1°C (160°F) - **Control Cycle** (n=2)

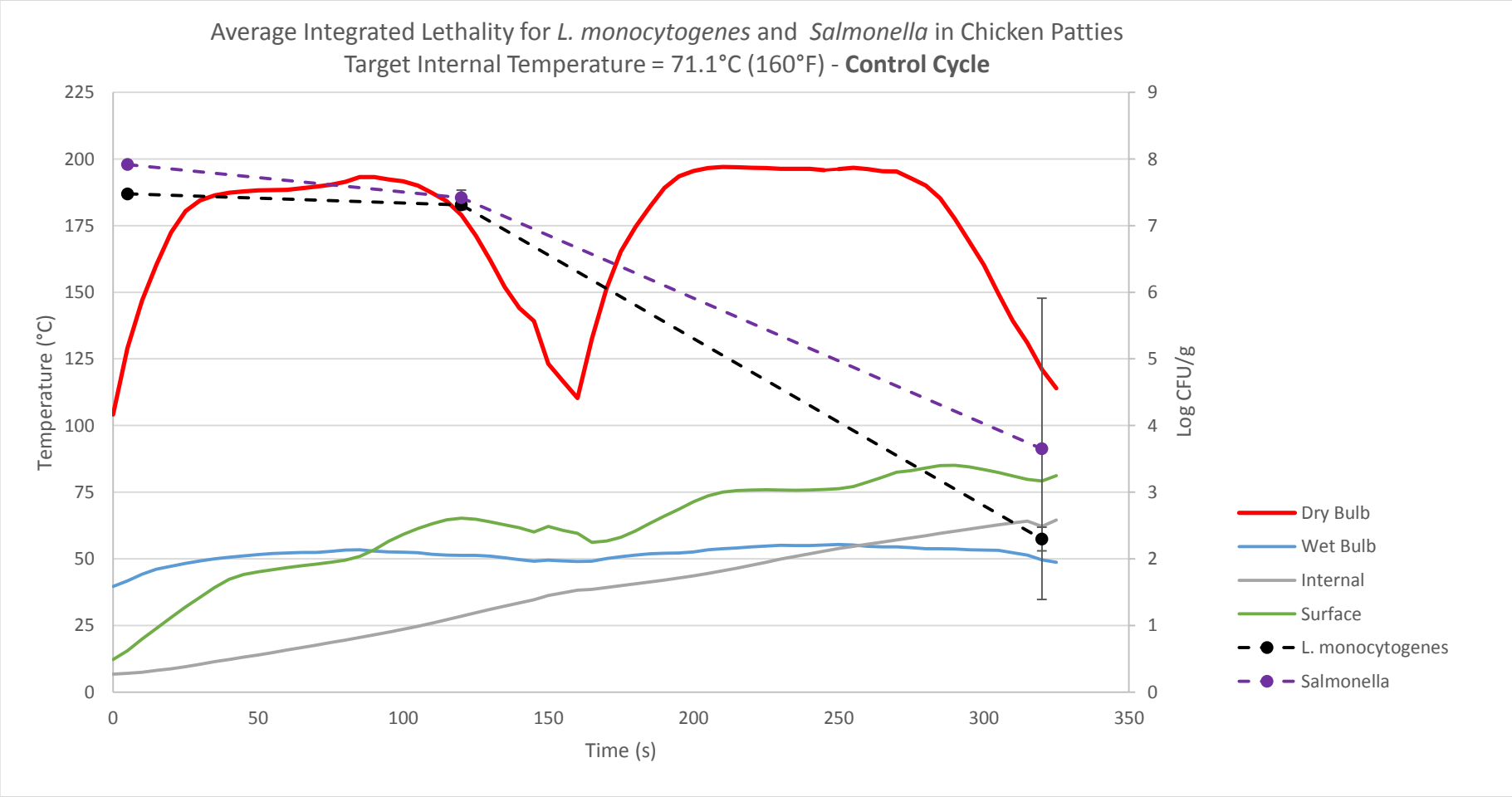


Figure 17: Average integrated thermal process/pathogen lethality profile for chicken patties inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 71.1°C (160°F) – **Surface Lethality Step Cycle** (n=2)

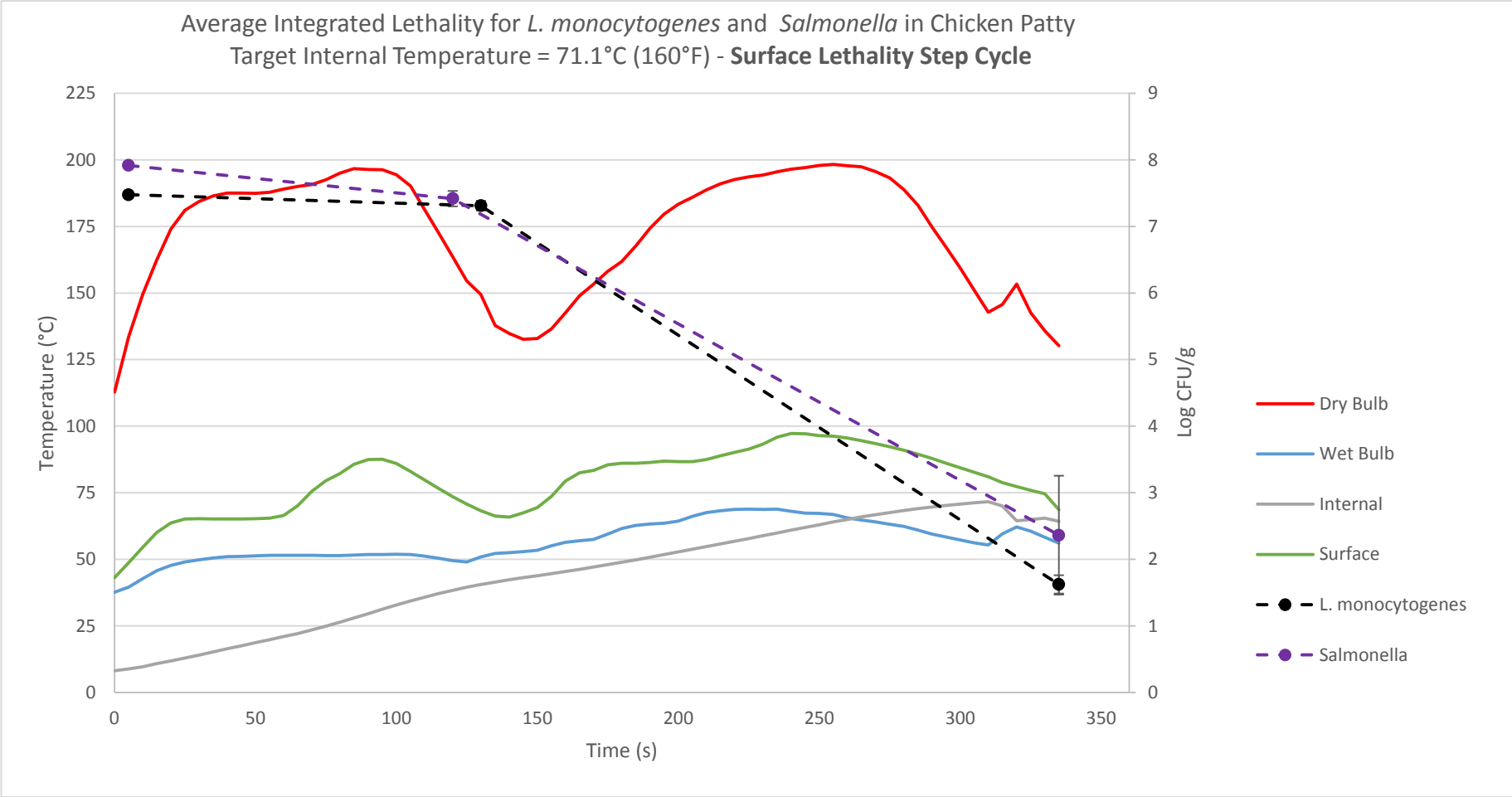


Figure 18: Average integrated thermal process/pathogen lethality profile for chicken patties inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 79.4°C (175°F) - **Control Cycle** (n=2)

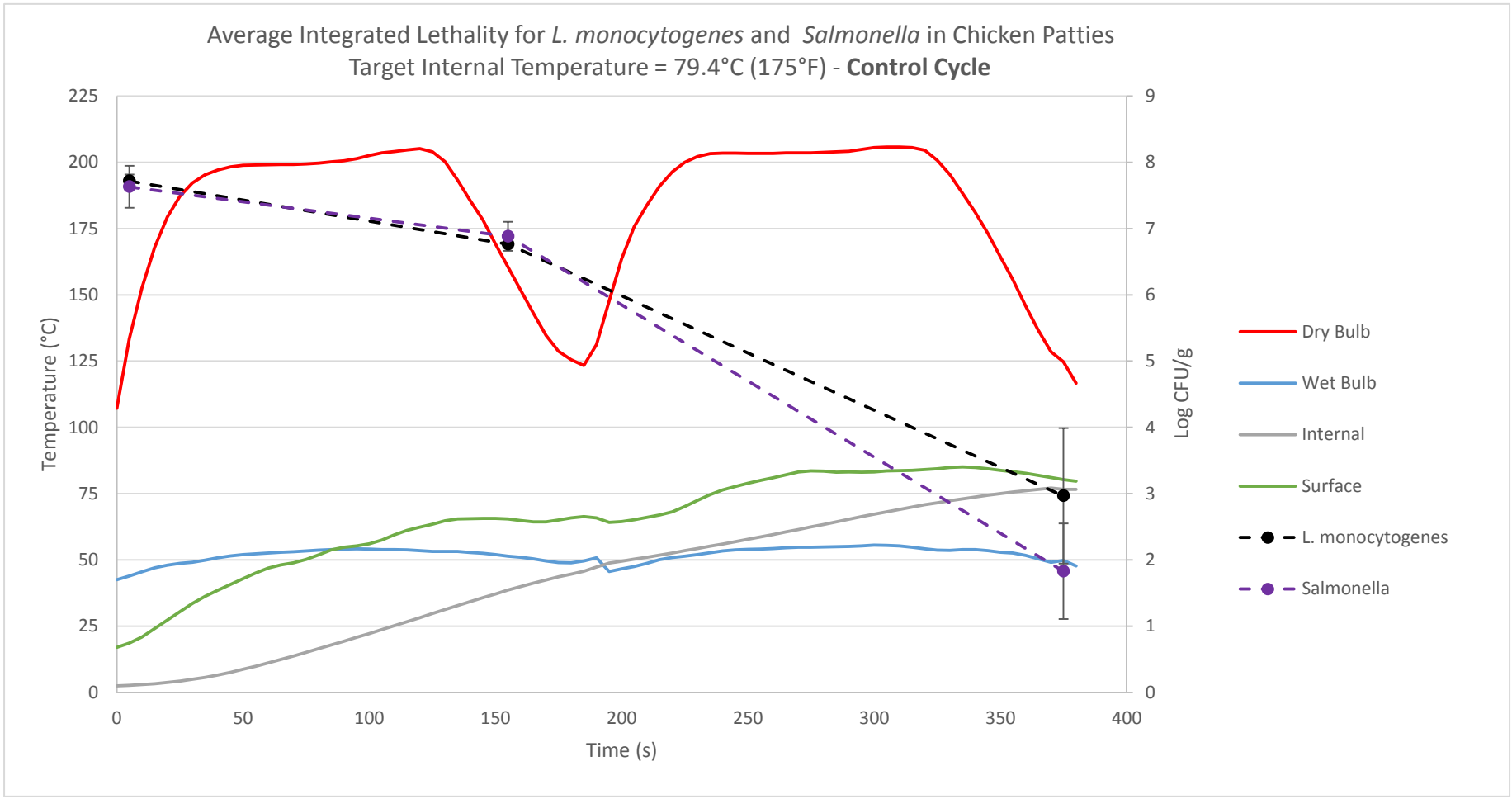


Figure 19: Average integrated thermal process/pathogen lethality profile for chicken patties inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 79.4°C (175°F) – **Surface Lethality Step Cycle** (n=2)

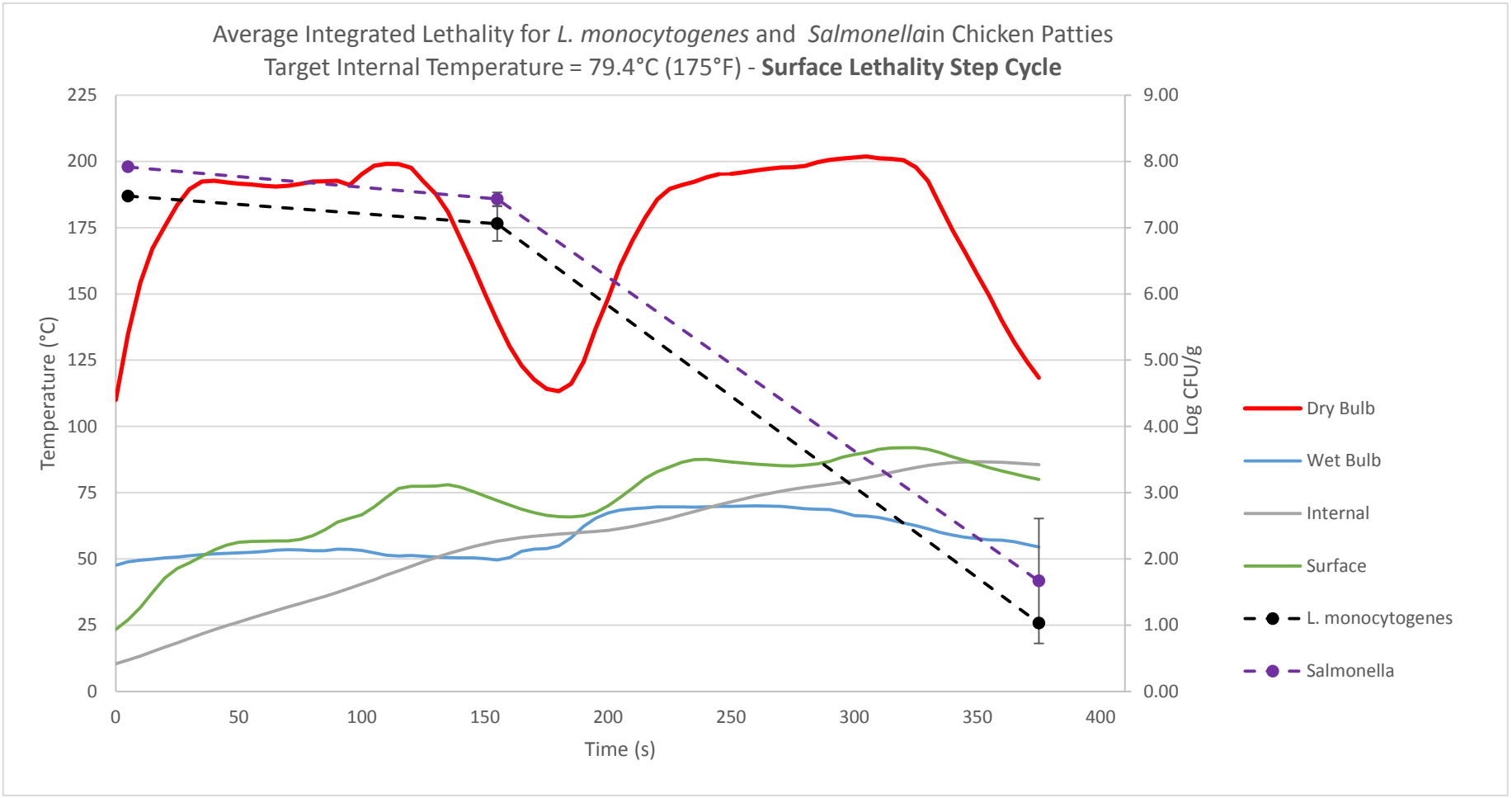


Figure 20: Average integrated thermal process/pathogen lethality profile for beef patties inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 71.1°C (160°F) – **Control Cycle** (n=2)

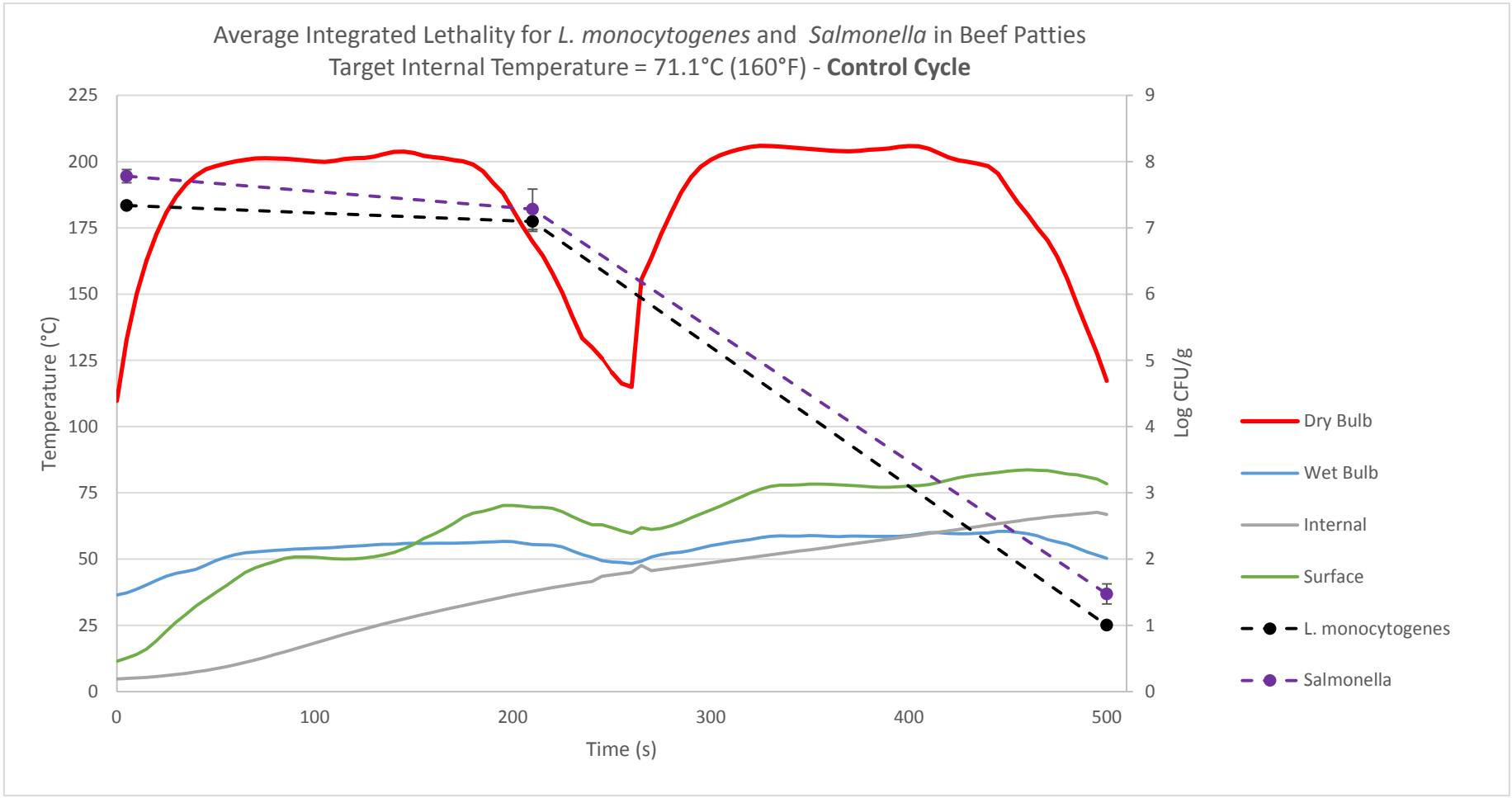


Figure 21: Average integrated thermal process/pathogen lethality profile for beef patties inoculated with *Salmonella* and *Listeria*. *monocytogenes* cooked in an impingement oven to a final internal temperature of 71.1°C (160°F) – **Surface Lethality Step Cycle** (n=2)

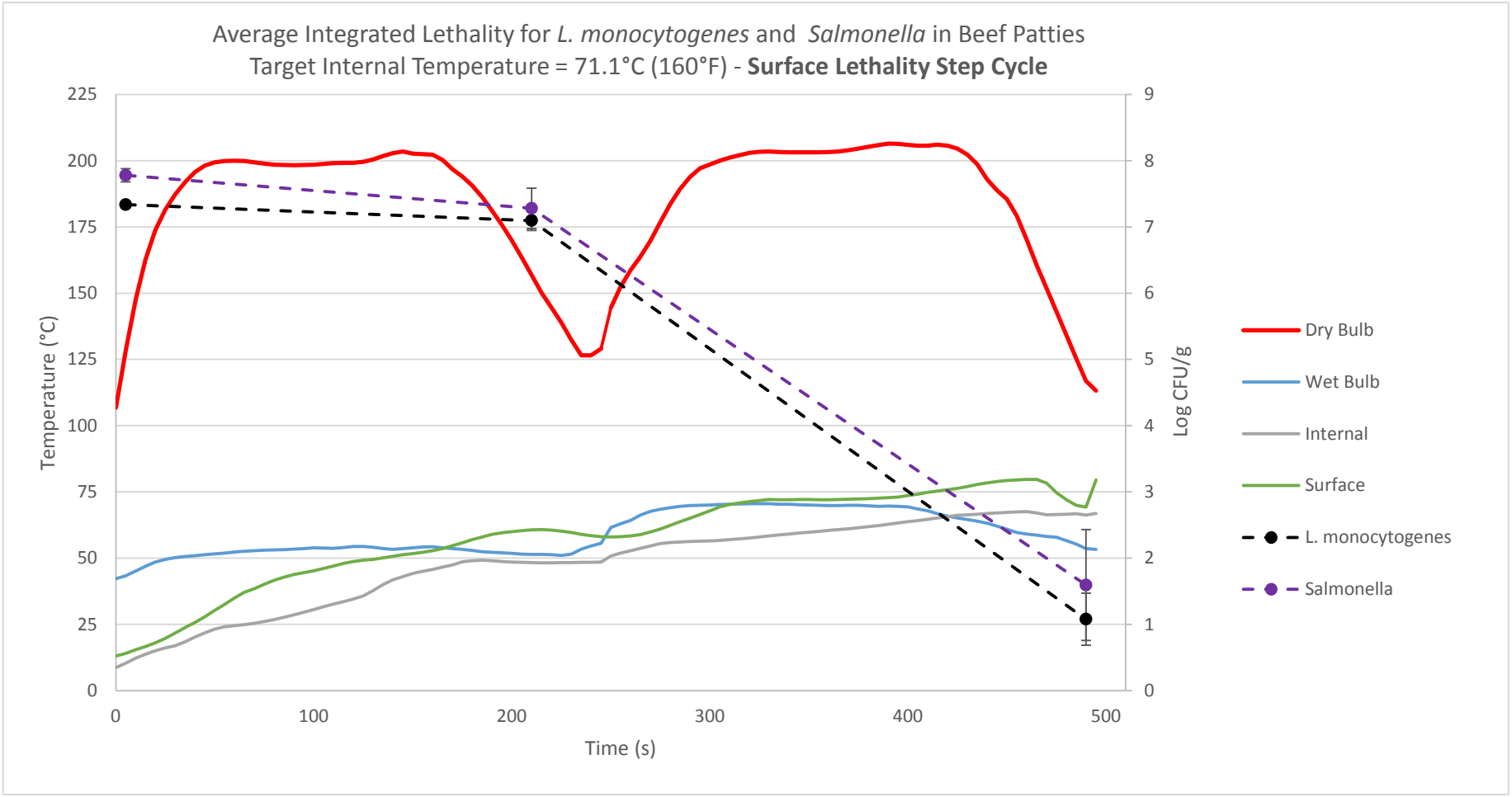


Figure 22: Average integrated thermal process/pathogen lethality profile for beef patties inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 76.7°C (170°F) – **Control Cycle** (n=2)

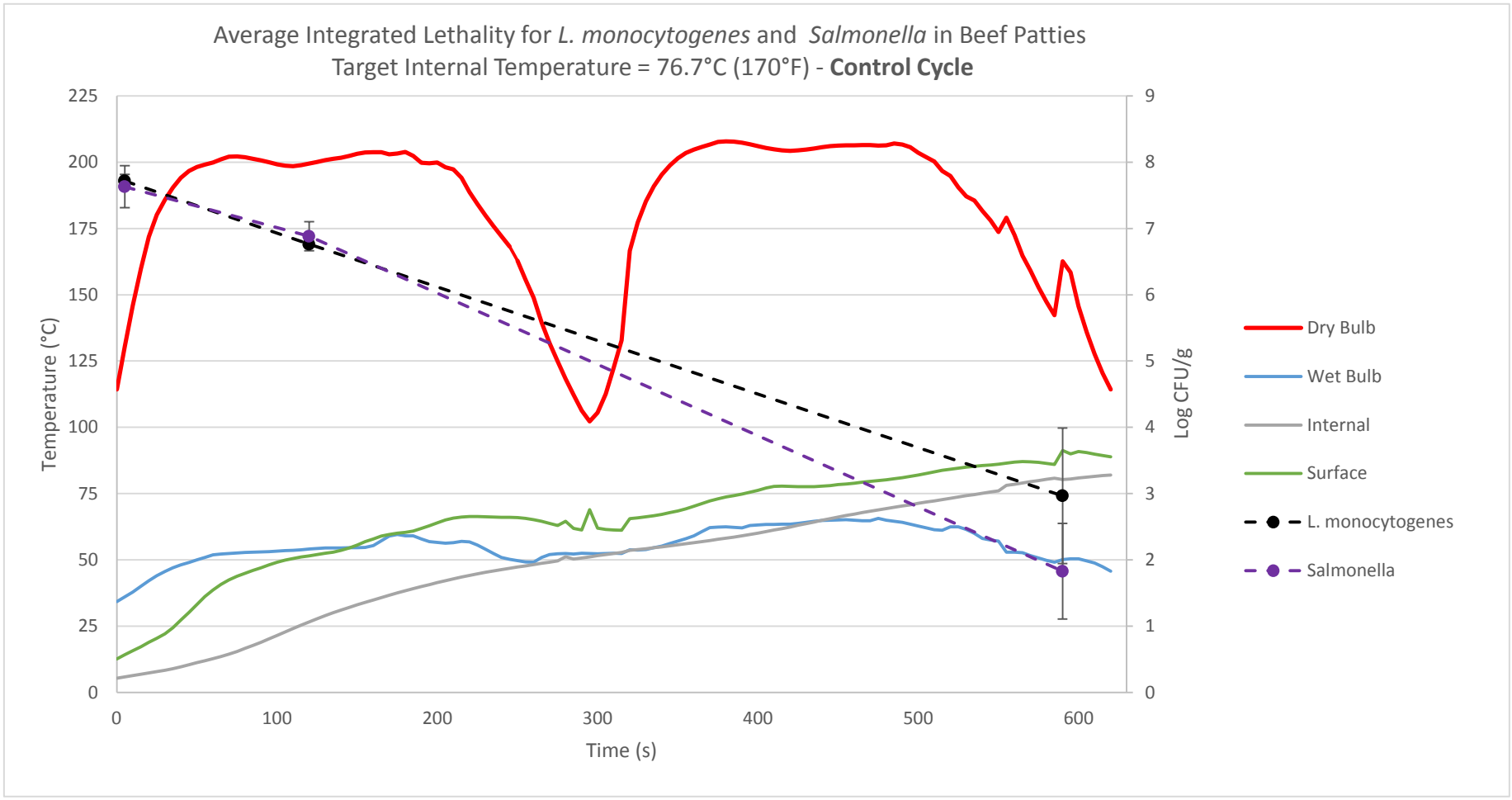
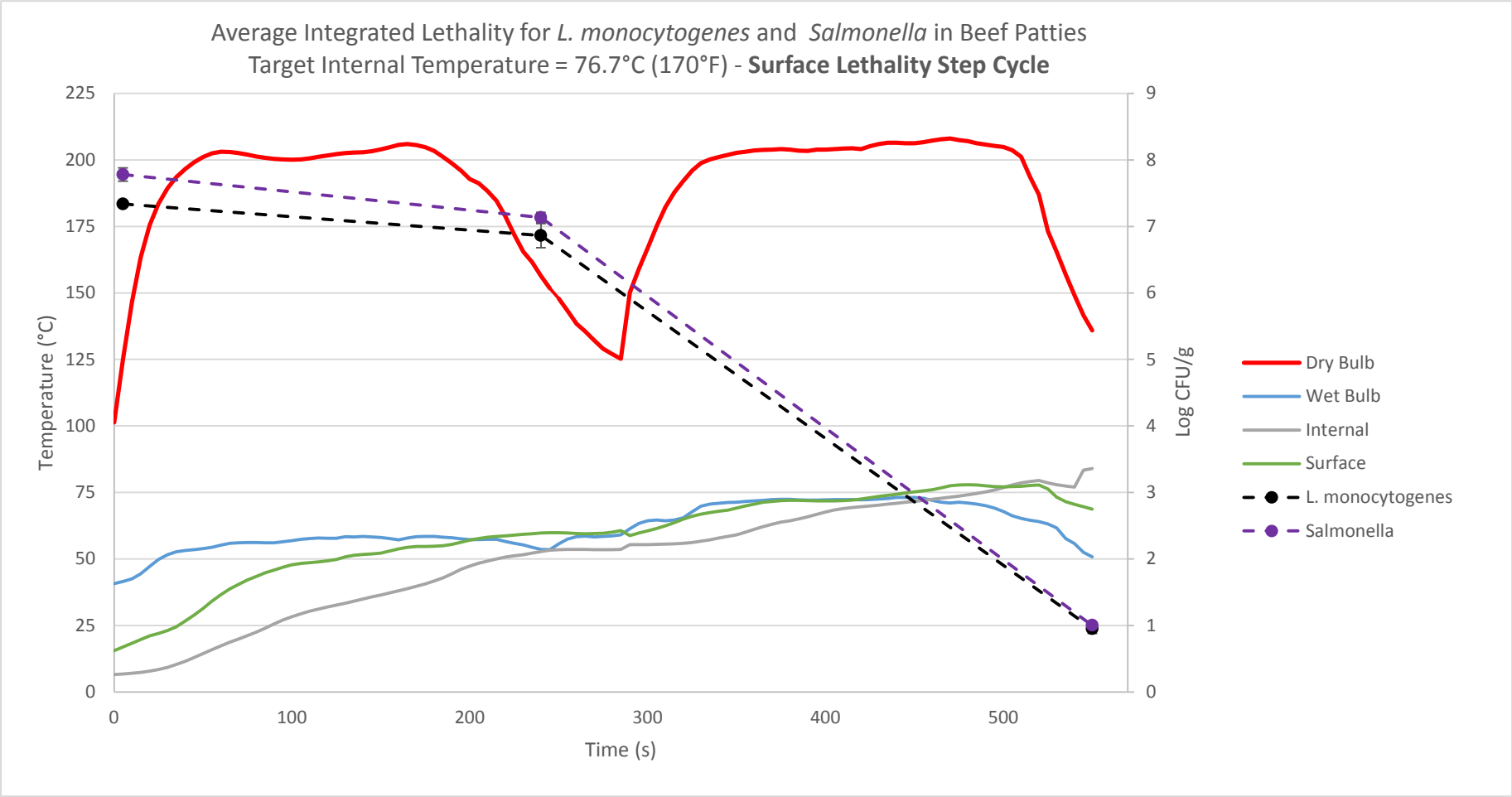


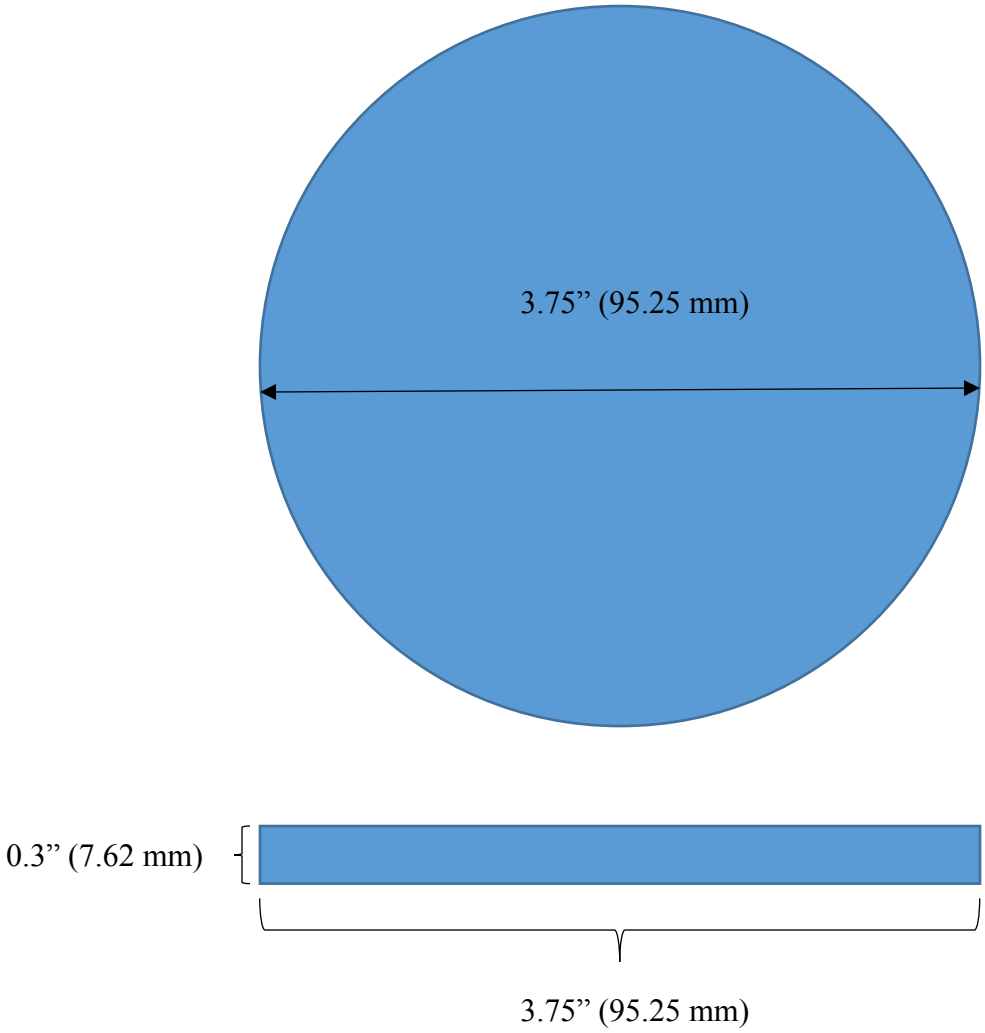
Figure 23: Average integrated thermal process/pathogen lethality profile for beef patties inoculated with *Salmonella* and *Listeria monocytogenes* cooked in an impingement oven to a final internal temperature of 76.7°C (170°F) – **Surface Lethality Step Cycle** (n=2)



Appendix 1: Product Dimensions

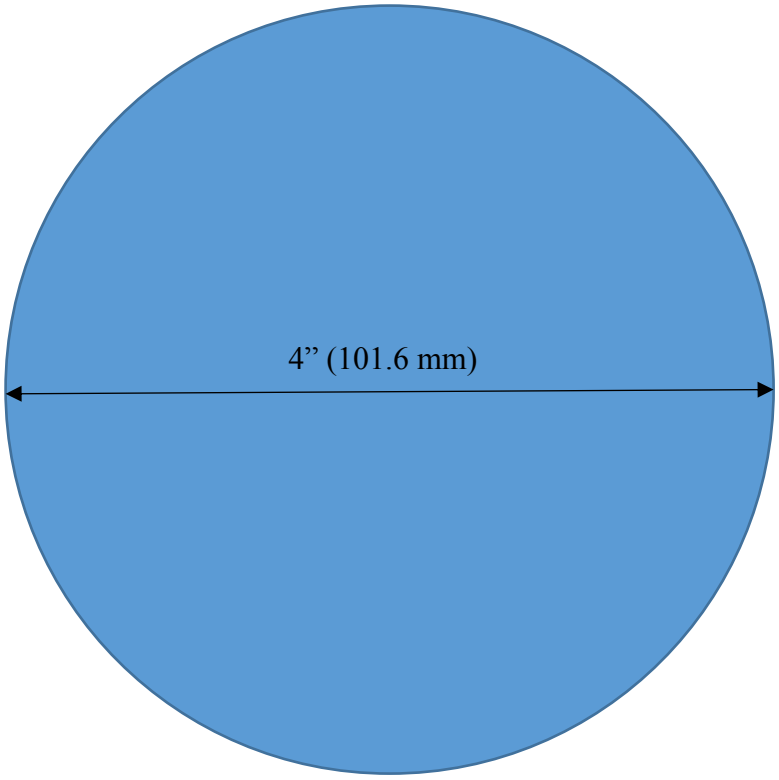
Chicken Patty

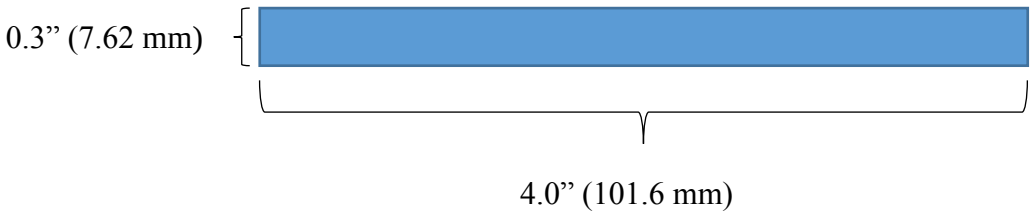
	US	Metric
Patty Weight	2.5 oz.	71 g
Diameter	3.75 in.	95.25 mm
Thickness	0.3 in.	7.62 mm



Beef Patty

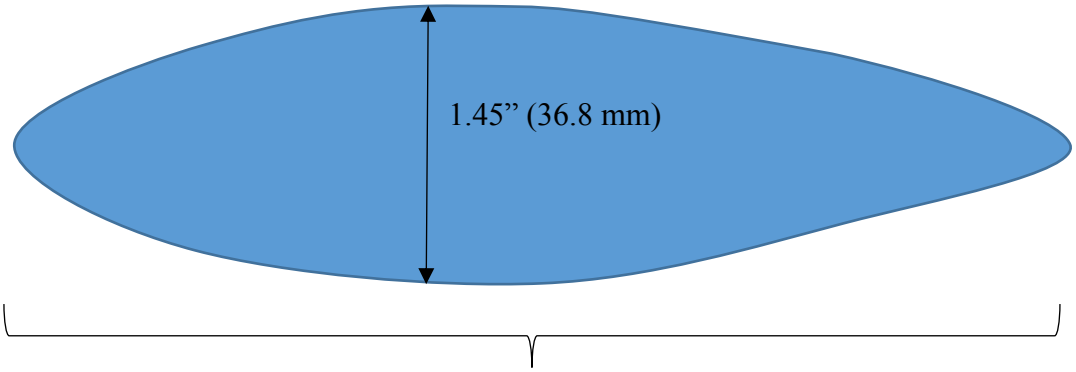
	US	Metric
Patty Weight	3.17 oz.	90 g
Diameter	4 in.	101.60 mm
Thickness	0.3 in.	7.62 mm



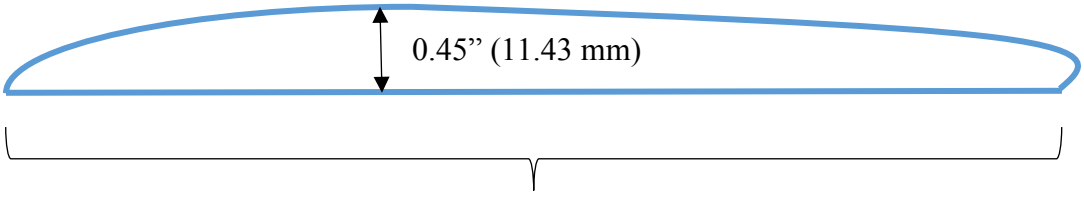


Chicken Tender

	US	Metric
Tender Length	5.5 in.	139.7 mm
Maximum Width	1.45 in.	36.8 mm
Maximum Thickness	0.45 in.	11.43 mm
Target Weight	1.23 oz.	35 g



5.5” (139.7 mm)



5.5” (139.7 mm)