CPS 2023 RFP – FINAL PROJECT REPORT

Project Title:
Evaluation of sanitation protocols for non-conventional food-contact surface materials used in produce packinghouses

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Objectives:

1. Utilize a mixed methods approach to confirm types of porous food-contact surfaces in produce packing areas.
2. Evaluate the effectiveness of cleaning and sanitizing of porous food-contact surfaces in produce packing areas.

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FINAL REPORT

Summary of Findings and Recommendations

This project evaluated pathogen persistence and sanitation effectiveness on common food-contact surfaces in produce packinghouses. Interviews with produce safety educators and packinghouse operators identified key barriers to effective sanitation, including limited financial resources, legacy equipment not designed for hygienic cleaning, language and training gaps, and reliance on visual inspection rather than validation tools. Laboratory studies showed that surface material strongly influences pathogen survival. High-density foam consistently supported the longest persistence of *Listeria monocytogenes*, *Salmonella*, and Shiga toxin–producing *Escherichia coli* (STEC), while stainless steel and rubber showed intermediate survival. Pathogens were capable of surviving for several days, particularly under dry contamination conditions. Virus persistence was influenced primarily by virus type and temperature, with longer survival on dirty surfaces and at cooler temperatures. Cleaning followed by sanitizing consistently produced the greatest pathogen reductions.

Packinghouse operations should prioritize the use of hygienically designed, nonporous food-contact surfaces whenever possible, as porous materials such as high-density foam can harbor microorganisms and support prolonged pathogen survival. When replacement of these materials is not feasible, operations should implement more frequent and thorough cleaning and sanitation procedures and consider alternative sanitizers or equipment modifications that improve cleanability. Effective sanitation programs should emphasize the importance of **cleaning prior to sanitizer application**, since the removal of organic matter and debris is critical for sanitizers to work effectively. In addition, packinghouses should strengthen sanitation standard operating procedures (SOPs) and provide clear training to employees on proper sanitizer preparation, application methods, and required contact times to ensure consistent implementation of sanitation practices. Packinghouses should also move beyond relying solely on visual inspection to evaluate cleanliness and instead adopt sanitation verification approaches such as environmental monitoring programs or other validation tools to confirm that cleaning procedures are effective. Finally, maintaining clean packing environments by minimizing organic debris, managing moisture, and controlling environmental conditions can help reduce both bacterial and viral persistence on surfaces and lower the risk of cross-contamination during produce handling and packing.

Abstract

This study investigated the persistence of foodborne pathogens on packinghouse food-contact surfaces (FCS) and evaluated the efficacy of cleaning and sanitation (C&S) protocols. Qualitative interviews with produce safety educators (n = 21) and packinghouse operators (n = 15) identified persistent implementation challenges, including limited financial and labor resources, outdated equipment, language barriers, knowledge gaps, and reluctance or inability to invest in improved infrastructure. Common FCS included stainless steel, wood, rubber, and high-density foam, as well as brushes and rollers. Bacteria and virus persistence on stainless steel, wood, rubber, and high-density foam was also evaluated under multiple factors. Across all treatments, decimal reduction times (D-values) for bacteria ranged from approximately 42 to 182 hours. High-density foam consistently supported the greatest survival of *Listeria monocytogenes*, *Salmonella*, and Shiga toxin–producing *Escherichia coli* (STEC), identifying it as the highest-risk material evaluated. Dry inoculation often increased persistence compared to wet conditions, and higher temperature (27°C) did not consistently accelerate inactivation. Pre-growth medium had minimal influence. Overall, surface material was the dominant factor affecting

bacterial pathogen survival. Meanwhile, virus persistence on FCS was significantly influenced by virus type and temperature. Aichivirus A (AiV) survived longer than Tulane virus (TuV), with higher overall D-values. Both viruses persisted longest at 15°C, with survival decreasing at 27°C. Surface type did not significantly affect virus persistence. Seven C&S treatments have been evaluated for control of bacteria on three FCS (foam, rubber, wood): Clean, Sanitize (SaniDate, Alpet, Oxine), and Clean+Sanitize. Combined C&S consistently achieved ≥94.98% reduction overall. Among sanitize-only treatments, SaniDate showed similar reductions (≥96.07%) on all FCS regardless of cleaning. Alpet was most effective on wood (96.89%), followed by rubber (90.03%), and foam (71.23%; $P < 0.001$). Oxine was least effective on all FCS (≤79.66%) but improved (≥94.98%) when combined with cleaning ($P < 0.001$). Overall, cleaning prior to sanitizing greatly enhances efficacy, and FCS type affects sanitizer efficacy when used alone.

Background

Fresh produce, organic matter, and field debris can be sources of microbial contamination of surfaces in produce packinghouses. Previous research has suggested low pathogen prevalence on both produce (Van Pelt et al., 2018) and produce packinghouse surfaces (Estrada et al., 2020; Sullivan and Wiedmann, 2020). Pathogen transfer to fresh produce via contaminated surfaces is an established route of pathogen entry into the food supply (Possas and Pérez-Rodríguez, 2023), and adequate cleaning and sanitizing (C&S) procedures are essential to limit microbial risks in in produce packing areas. While appropriate C&S procedures for pathogen control on nonporous surfaces within packinghouses have been more extensively researched (Brackett et al., 1999; Olaimat and Holley, 2012; Holvoet et al., 2014; Zhou et al., 2014), the effectiveness of environmental cleaning protocols applied to porous surfaces in packing areas are less understood (Williamson et al., 2019; Vice and Taylor, 2020). Environmental cleaning includes both 1) a pre-cleaning step to physically remove organic soil and microorganisms and 2) application of an EPA-registered chemical agent at the appropriate concentration and exposure time.

Based on an On-farm Readiness Review (OFRR) training held in Michigan in 2022, numerous questions were posed regarding the types of surface materials often observed in packing areas of small to very small operations and the ability to properly clean and sanitize these surfaces as required by the Produce Rule or when adhering to Good Agricultural Practices (GAPs) (A. Philyaw Perez, personal communication, October 31, 2022). Specifically, participants in the OFRR training discussed the types of “unconventional” materials frequently seen in packing areas, and these materials are primarily selected to maintain product quality with less consideration regarding surface cleanability. Moreover, the Southern Regional Integrated Produce Safety Conference in 2020 highlighted food safety aspects of wooden food-contact surfaces (FCS) used during produce production, processing, and distribution (Vice and Taylor, 2020). Vice and Taylor (2020) indicated that little data is available on the efficacy of industrial sanitizers for pathogen reduction when applied to wood. The overall message was that wood [and other porous surfaces] should be cleaned and sanitized, but more evidence is needed. Importantly, unfinished wood is viewed as an FCS material to avoid in packing areas; however, as reviewed by Aviat et al. (2016), the porous nature of wooden surfaces—when compared to smooth surfaces (e.g., stainless steel)— “is not responsible for the limited hygiene of the material used in the food industry”, and these characteristics may even be advantageous to the microbiological status of wood. The authors continue by stating that these rough or porous surfaces often generate unfavorable conditions for pathogens, specifically related to transfer of pathogens from the surface to a food product. Meanwhile, research by Townsend et al. (2022) aimed to determine the main factors associated with *Listeria*-positive surface swabs from fresh produce distribution centers and reported that surface porosity was a top five predictor variable. However, the most important variable was related to whether the surface sampled was wet/moist versus dry. In short, “easy to clean” may also render a surface more likely to release contaminating

pathogens during contact. However, these statements regarding wood FCS may not be applicable to other porous FCS found in packing areas and thus should be further investigated.

As indicated, there is a lack of available data on the effectiveness of C&S practices utilized by produce packinghouses with porous FCS such as unfinished wood, vinyl fabric, high-density foam, carpet, and artificial grass. Previous studies have investigated microbial transfer between produce and packinghouse surfaces (Sharps et al., 2012; Brar and Danyluk, 2013; Nyarko et al., 2018; Williamson et al., 2018), but limited knowledge is available on the efficacy of C&S for pathogen mitigation on porous FCS materials frequently observed in produce packing areas. Nyarko et al. (2018) observed significantly greater levels of *L. monocytogenes* on soiled packinghouse surfaces compared to clean packinghouse surfaces. The authors also reported the influence of surface type on *L. monocytogenes* persistence over time, with foam pads and brushes prolonging survival compared to conveyor belt materials (Nyarko et al., 2018). Moreover, Dunn et al. (2022) detected *L. monocytogenes* on two FCS within cantaloupe packinghouses, including on a metal screw head used to attach a hard foam bumper, i.e., indicating potential for the foam bumper to serve as a pathogen reservoir. Regarding porous FCS, penetration and absorption capacity of a material has been shown to influence bacterial counts (Munir et al., 2019). Although virus removal and transfer from nonporous FCS have been investigated, studies focusing on C&S of nonconventional or porous surfaces for virus reduction are lacking. Virus transfer among gloves and stainless steel has been investigated (Djebbi-Simmons et al., 2020); however, additional studies are needed to quantify risks associated with a wider variety of porous surfaces, pathogen types, and organic matter. To adequately reduce risks related to cross-contamination, the efficacy of current industry practices for sanitation of nonconventional FCS needs to be established. Studies should be performed based on industry feedback to determine if current practices limit cross contamination (i.e., based on pathogen persistence and reduction) from porous packinghouse surfaces to produce—a clearly identified CPS research priority. The proposed work aims to evaluate C&S of common, nonconventional FCS found in packing areas and provide guidance on best practices.

Research Methods and Results

The methods and results for the first objective of this project “Utilize a mixed methods approach to confirm types of porous food-contact surfaces in produce packing areas” have been published (Hamilton et al., 2025), and the article is available as open access. However, for convenience, methods and results have been added to this report as well; the results have been extracted from the discussion as these sections were combined in the published article.

Objective 1 – Methods

Ethics statement. The University of Arkansas Institutional Review Board (Protocol No: 2404535871 [produce safety educators] and 2402518856 [packinghouse visits]) reviewed the study and granted an exemption. Prior to conducting semi-structured interviews, participants were physically provided or were emailed a consent letter and asked if they had reviewed the consent letter and then a verbal “yes” was given to proceed with the interview.

Rationale and recruitment. Semi-structured interviews were conducted using two approaches: first, during in-person visits to packinghouses, and second, with produce safety educators. For each site visit, an individual holding a key operational or managerial role, such as an owner or packinghouse manager, was interviewed. This approach ensured that the interviewee possessed comprehensive knowledge regarding the facility's practices and associated challenges. Food safety educators were selected as subject matter experts due to their extensive knowledge and firsthand experience in providing food safety guidance and support to growers and packinghouse operators. A semi-structured interview relies

on a set of predetermined open-ended questions to guide the conversation, while also allowing flexibility for the interviewer to explore emergent themes and delve deeper into responses, thus combining the benefits of consistency and detailed qualitative data. This approach was optimal for interviewing participants as it permitted exploration of complex experiences and perspectives while ensuring coverage of key topics relevant to packinghouse cleaning and sanitizing practices.

Participants were recruited using a convenience sampling method. Produce safety educators were identified via the Produce Safety Alliance Trainer Directory (https://resources.producesafetyalliance.cornell.edu/directory/) or Cornell's National Good Agricultural Practices Program database (https://cals.cornell.edu/national-good-agricultural-practices-program/collaborators-links/collaborators-state) with the aim of identifying at least one trainer or lead trainer per state. This search resulted in a list of 58 produce safety educators across 40 states. Forty-three educators were contacted via email to request their participation in the study, and 21 educators agreed to participate, offering a diverse range of insights and experiences.

For the recruitment of packinghouse operation participants, Extension Specialists and Agents were identified in five states—Arkansas, Florida, Michigan, North Carolina, and Washington—representing different regions of the U.S. These contacts served as liaisons between the study researchers and the packinghouse operations, assisting in arranging visits according to the study's objectives. In total, 15 packinghouse visits were conducted across the five states. Throughout this objective, operational sizes are categorized based on the categories defined in the Produce Safety Rule. Specifically, large operations are defined as greater than $500,000 in annual (three-year average) gross produce sales; small operations are defined as greater than $250,000 but less than $500,000 in annual (three-year average) produce sales; and very small businesses are defined as greater than $25,000 but less than $250,000 in annual (three-year average) produce sales.

Semi-structured produce safety educator interviews. Two Ph.D.-trained interviewers conducted interviews via Zoom (Zoom Video Telecommunications, San Jose, CA) or Microsoft Teams (Microsoft Corporation, Redmond, WA), depending on the participant's preference and internet availability. The interviewer began each session with a brief overview of the study's purpose before proceeding with the 11 pre-determined questions outlined in **Table 1-1**. When needed, the interviewer posed additional questions to clarify responses. Zoom and Microsoft Teams recordings were auto-transcribed using audio transcription services available on each software platform.

Semi-structured packinghouse operation visits and interviews. A Ph.D.-trained interviewer conducted interviews in-person with the owner, manager, or other qualified person in the packinghouse. The interviewer began each session with a brief overview of the study's purpose before proceeding with the 11 pre-determined questions outlined in **Table 1-1**. When needed, the interviewer posed additional questions to clarify responses. Notes were taken by the interviewer for subsequent thematic analysis by independent researchers.

Data analysis and interpretation. The transcripts and notes from the semi-structured interviews were analyzed using an emergent thematic approach to identify key themes. Two independent researchers manually coded the transcripts and categorized themes into non-mutually exclusive groups (Lune & Berg, 2017). The researchers then met to discuss and consolidate the identified themes. Disagreements in coding and thematic categorization were resolved through regular, iterative discussions between the two researchers. This process involved a return to the original interview data to re-examine contexts and nuances, ensuring that consensus was achieved through collaborative refinement and a shared understanding of the emergent themes. A constant comparison method was employed to identify broad themes across all interviews. The semi-structured interviews were completed by twenty-one produce safety educators and fifteen packinghouse operation participants.

Objective 1 – Results

Semi-Structured Interviews. The average online interview with a produce safety educator lasted approximately one hour (range: 14.0 to 52.8 min; mean: 31.4±13.2 min; median: 30.1 min). In-person, packinghouse operation visits lasted approximately 1 to 2 h, though exact times were not recorded.

Introduction to Themes. The findings of this study underscore the multifaceted challenges and opportunities in enhancing cleaning and sanitation practices within produce packinghouses. From interviews with both produce safety educators and packinghouse operations, three major themes were isolated from the 11 interview questions:

1. Contextual: environmental factors, economic constraints, legacy equipment, materials, cultural and language barriers, regulatory and consumer expectations, motivations.
2. Barriers to risk management and regulatory compliance: knowledge and training, infrastructure, cleaning and sanitizing practices, worker safety and comfort.
3. Research needs: sanitation methods and hygienic design, behavioral and cultural change.

Tables 1-2, 1-3, and **1-4** present each of the three major themes, respectively, along with illustrative quotations from transcribed interviews with produce safety educators to support these themes. The thematic analysis provides critical insights into these areas, supported by illustrative quotations and comparisons to existing literature.

Contextual. The contextual themes identified in the interviews reveal several factors that can impact the ability of produce packinghouse operators to effectively implement cleaning and sanitation protocols (**Table 1-2**). These factors include environmental conditions, economic constraints, legacy equipment, cultural and language barriers, and regulatory and consumer expectations.

Contextual – Environmental Factors. Environmental conditions play a pivotal role in the safety and quality of produce in packinghouses, a fact acknowledged by many interviewees. Challenges commonly identified included temperature differences, condensation, and pest control (**Table 1-2**).

Contextual – Legacy Equipment & Materials. Outdated and legacy equipment (a term interviewees used to describe older, often inherited or retrofitted, machinery and tools not designed with modern food safety and hygienic principles in mind, characterized by materials or designs that may impede effective cleaning and sanitization), particularly in generational farms, may not be designed for easy cleaning, with wood-based materials and rust exacerbating hygiene challenges.

Contextual – Cultural and Language Barriers. Cultural and language barriers can significantly impact communication and the implementation of food safety practices (**Table 1-2**). Many packinghouses have workers, owners, and farmers for whom English is not the primary language, creating challenges in training and ensuring compliance with safety protocols, a finding supported by Ohman et al. (2023). This language barrier can lead to misunderstandings and miscommunications, which can compromise food safety. Interviewees indicated that resistance to change and adherence to traditional methods can hinder the adoption of improved practices. Some operations are reluctant to change long-standing practices, even when new methods could enhance food safety. This resistance to change in practices is commonly referenced by growers as a result of production and output being the main priority.

Contextual – Motivations. Motivational and practical constraints also impact the implementation of food safety practices. Many packinghouse operators are aware of the importance of food safety but are constrained by the cost and practicality of implementing best practices. The financial burden of maintaining high standards can be prohibitive, particularly for smaller packinghouse operations. Operators must often make trade-offs between cost and effectiveness, opting for the next best option below stainless steel, for example. The availability of resources and equipment also influences the

choices made by operators, with many relying on what is readily available or attractively marketed rather than what is most effective for food safety.

Barriers to Risk Management and Regulatory Compliance. The barriers to risk management and regulatory compliance highlight several challenges that impede the effective implementation of food safety practices in produce packinghouses. These barriers include knowledge and training deficiencies, infrastructure limitations, suboptimal cleaning and sanitizing practices, motivational and practical constraints, and concerns related to worker safety and comfort (**Table 1-3**). Addressing these barriers is crucial for enhancing food safety outcomes and ensuring compliance with regulatory standards.

Barriers – Knowledge and Training. A significant barrier to effective risk management in produce packing is the lack of knowledge and training among packinghouse owner/operators and workers. Interviewees expressed that growers who operate packinghouses as well as their associated workers may have limited scientific understanding and formal training in food safety, which could lead to a reliance on outdated methods and word-of-mouth practices rather than evidence-based approaches. As a result, packinghouse operators may encounter confusion regarding the appropriate use of sanitizers and cleaning agents, as they can find the instructions provided on the label to be difficult to interpret, leading to inconsistent and suboptimal practices, a finding supported by Ohman et al. (2023).

Barriers – Infrastructure. Infrastructure limitations were also said to present notable challenges to maintaining food safety standards in packinghouses. Many facilities, particularly very small packinghouse operations with limited resources, face physical constraints such as inadequate space for proper cleaning and sanitation. The design of some facilities and equipment further complicates cleaning efforts, with areas that are difficult to access and clean thoroughly. Additionally, the physical layout of some packinghouses does not allow for efficient product flow and traffic (**Table 1-3**). Inadequate flow can create congestion and impede smooth operations, making it more difficult to maintain proper safety and sanitation practices (Yaptenco & Esguerra, 2012). This inefficient design not only impacts productivity but can also increase the likelihood of cross-contamination and complicate efforts to ensure food safety.

Barriers – Cleaning and Sanitizing Practices. Difficulties in eliminating contamination risks persist even in facilities that have invested in food safety. Suboptimal cleaning and sanitizing practices present another barrier to effective risk management. Many packinghouse operations were observed not cleaning surfaces before sanitizing them, which reduces sanitizer effectiveness. Similar issues were reported by Ohman et al. (2023), based on a survey of 162 growers. In the present study, interviewees described common cleaning methods as hosing down surfaces, scrubbing with dish soap, and/or using isopropyl alcohol wipes, though these methods are insufficient for thorough sanitation. Among operations using sanitizers, interviewees indicated a shift towards peracetic acid (PAA)-based chemistries and products like SaniDate® and Tsunami 100™, particularly among organic growers.

The reliance on visual inspection to determine cleanliness, as opposed to implementing environmental monitoring programs (EMPs), was reported in the present study and may further complicate the effectiveness of cleaning and sanitizing practices. This contrasts with previous studies where a higher proportion of packinghouse operations, typically medium to very large companies (>$250,000 to >$5 million revenue) selling through channels requiring EMPs, reported having an EMP (Critzer et al., 2024; Ohman et al., 2023). Additionally, the lack of understanding of and/or adherence to required sanitizer contact times and the inconsistent use of SOPs for cleaning and sanitizing may contribute to suboptimal practices. These observations are consistent with previous research.

Barriers – Worker Safety and Comfort. Worker safety and comfort are additional considerations that impact food safety practices according to interviewees. Ensuring a comfortable environment for workers is essential for maintaining their focus on safety protocols. However, issues such as hand hygiene remain

problematic, with frequent handwashing leading to cracked hands. Cleaning areas with electrical equipment also poses challenges, as it requires careful handling to avoid accidents, and workers may avoid cleaning those areas due to safety concerns as reported by interviewees in the present study.

Research Needs. The analysis of the packinghouse visits and interviews for produce safety educators highlights several areas where further investigation and development are needed to enhance food safety practices in produce packinghouses. These needs include improved sanitization methods and food-contact surfaces selection in addition to behavioral and cultural change (**Table 1-4**). Addressing these research needs is crucial for advancing food safety outcomes and supporting the long-term sustainability of produce packing operations.

Research Needs – Sanitation Methods and Hygienic Design. A key research priority is the development and evaluation of improved sanitization methods and food-contact surfaces. While many operators described shifting away from chlorine to PAA-based sanitizers, due to perceptions of ease of application, robustness, and efficiency, there remains a need for further research on the effectiveness of various sanitizers and their impact on different surface types. This includes exploring alternative sanitization methods and chemistries especially as some operators have stopped washing their produce altogether (i.e., depending on the commodity), opting instead to clean it with brushes to avoid moisture retention and internalization, which can harbor microorganisms. Another area of research need is the investigation into the environmental impact of different sanitization methods and materials. Some growers have expressed concerns about the environmental effects of certain sanitizers, such as chlorine, which can be caustic to materials and harmful to the environment.

Research Needs - Behavioral and Cultural Change. Behavioral and cultural change is another critical area of research. Many produce safety educators emphasized the need for a shift in mentality from securing markets to protecting consumers and selling wholesome foods (**Table 1-4**). While most growers are committed to the idea of food safety, they may perceive their products as less susceptible to contamination compared to others, or they may face significant challenges in applying general food safety recommendations to their specific operations. Research into effective communication strategies and educational interventions can help foster a stronger culture of food safety among growers and their employees. For example, some growers have been hesitant to engage in food safety discussions, but there has been a gradual shift toward greater openness and concern, particularly as many express stress over the possibility of being involved in a food safety incident.

Objective 2 – Methods

I. Pre-growth Conditions and Bacterial Growth Response – Methods

Strains selection. Pathogen strains were selected from the CPS Strain Collection (Cornell University, Ithaca, NY) based on their association with human clinical cases or produce-related outbreaks (Harrand et al., 2019). The pathogens included *L. monocytogenes* (five serotypes), *Salmonella enterica* (*S. enterica*; five serotypes), and Shiga toxin–producing *Escherichia coli* (STEC; four strains). Additional strain information is provided in **Table 2-1**.

Bacteria culture preparation. Bacterial isolates were streaked from glycerol stocks onto tryptic soy agar (TSA; BD Diagnostic Systems, Sparks, MD) plates and incubated for 24 h at 37°C for *S. enterica* and STEC, or at 35°C for *L. monocytogenes*. A single colony-forming unit (CFU) from each plate was inoculated into 10 mL of tryptic soy broth (TSB; BD Diagnostic Systems) and incubated overnight (16–18 h) with shaking at the corresponding temperatures. Three growth media were evaluated: (i) reduced water activity media (a_w = 0.96 for *S. enterica* and STEC; a_w = 0.95 for *L. monocytogenes*), (ii) minimal media: M9 CA medium (M9; Quality Biological Inc., Gaithersburg, MD) for *S. enterica* and STEC, and Welshimer's Broth,

Modified (Welshimer; HiMedia Laboratories LLC, Kennett Square, PA) for *L. monocytogenes*, and (iii) optimal growth medium (TSB). The reduced water activity media were adjusted by using glycerol (VWR Chemicals BDH, Radnor, PA) at 15.6% (v/v) and 13% (v/v) to achieve an a_w of 0.95 (*L. monocytogenes*) and 0.96 (*S. enterica* and STEC) according to Harrand et al. (2019). Cultures were incubated under four temperature stress conditions (32°C, 27°C, 21°C, 15°C), resulting in a total of 12 growth conditions (3 media × 4 temperatures).

Optical density measurements. Following overnight incubation, cultures were serially diluted (three-fold series) by transferring 0.1 mL of overnight culture into 0.9 mL of the corresponding growth medium in 1.5 mL microcentrifuge tubes and incubated at the designated temperatures. The third dilution (1:1000) was used as the loading sample. For each condition, 200 µL of culture was dispensed into 96-well microplates (Corning Inc., Endicott, NY) with lids in duplicate. Standard curves were generated for each strain under each growth condition prior to sample analysis. Serial dilutions were prepared by adding 0.1 mL of overnight culture to 0.9 mL of 1× phosphate-buffered saline (PBS) in 1.5 mL microcentrifuge tubes. Bacterial enumeration for standard curve development was performed using TSA plate counts for quantification. For optical density (OD) measurement, 200 µL of each standard dilution was loaded into 96-well microplates with lids in duplicate. Growth curves were generated for each strain under each condition to determine OD values across growth phases. OD measurements were obtained at 600 nm using a BioTek Cytation 1 Imaging Reader (Agilent Technologies, Winooski, VT) for cultures incubated at 32°C and 27°C, and a Tecan Infinite M200 Plate Reader (Tecan, Männedorf, Switzerland) for cultures incubated at 21°C and 15°C, which were read manually.

Data analysis and mathematical modeling. The growth kinetics of *L. monocytogenes*, *S. enterica* and STEC in various growth conditions were described using a no lag-phase model. The no lag-phase model is expressed as Eq. (1) (Fang et al., 2013).

$$Y_t = Y_i + Y_{max} - \ln[e^{Y_i} + (e^{Y_{max}} - e^{Y_i})e^{-\mu_{max}t}] \quad (1)$$

where Y_t represent the natural logarithm of the bacterial population at time *t*; Y_i and Y_{max} are the natural logarithms of the initial and maximum bacterial population, respectively; μ_{max} denotes the specific growth rate (h^{-1}).

The United States Department of Agriculture Integrated Pathogen Modelling Program 2013 (Huang, 2014) was employed to develop the no-lag phase model and determine the growth kinetic parameters, including the specific growth rate and maximum population, for each pathogen across different temperatures and media. The performance of the developed no lag-phase models was evaluated by two statistical criteria: root mean squared error (RMSE) and Akaike Information Criterion (AIC). RMSE measures the difference between the actual observed values and the predicted values. A lower RMSE value indicates better model accuracy. The equation for calculating RMSE values was expressed in Eq. (2) (Jia et al., 2020). AIC assesses the model's goodness-of-fit. A smaller AIC value indicates a better model fit. The AIC value was computed using Eq. (3) (Huang et al., 2011).

$$RMSE = \sqrt{\frac{\sum_1^n (y_{obs\_i} - y_{pred\_i})^2}{n-p}} \quad (2)$$

$$AIC = n \times \ln\left(\frac{\sum_1^n (y_{obs\_i} - y_{pred\_i})^2}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \quad (3)$$

where *n* is the number of data points in each growth curve; *p* is the number of parameters in the model; y_{obs_i} is the observed bacterial population (log CFU/mL); y_{pred_i} is the predicted bacterial population (log CFU/mL); *i* is the *i*th data point.

II. Bacterial Pathogen Persistence on FCS – Methods

Strains selection. Pathogen strains were selected from the CPS Strain Collection (Cornell University, Ithaca, NY) based on their association with human clinical cases or produce-related outbreaks (Harrand et al., 2019). The pathogens included *L. monocytogenes* (five serotypes), *Salmonella enterica* (*S. enterica*; five serotypes), and Shiga toxin–producing *Escherichia coli* (STEC; four strains) (**Table 2-1**).

Bacteria culture preparation. For experimental inoculum preparation, each strain was streaked onto tryptic soy agar (TSA; Becton Dickinson, Sparks, MD; Fisher Scientific, Fair Lawn, NJ) and incubated at 35°C for *L. monocytogenes* or 37°C for *Salmonella* and STEC for 24 h. A single colony from each plate was transferred into 10 mL TSB in 50 mL centrifuge tubes and incubated statically overnight (16–20 h) at 35°C for *L. monocytogenes* or 37°C for *Salmonella* and STEC to obtain stationary-phase cultures. The 0.1 mL of each overnight culture was subcultured into 9.9 mL of TSB or minimal medium (M9 for *Salmonella* and STEC, Welshimer's for *L. monocytogenes*) and incubated at 21°C or 27°C for organism and medium-specific durations determined from preliminary growth curves (approximately 24–48 h at 21°C and 8–28 h at 27°C). Following incubation, cultures were centrifuged at 3,500 rpm for 5 min, pellets were washed three times with 1× PBS (pH 7.2), and washed cell suspensions were diluted in PBS and plated on TSA to verify culture concentrations. The overall experimental parameters are presented in **Table 2-2**.

Food-contact surface materials and preparation. Four FCS materials commonly used in produce packinghouses were evaluated: stainless steel (SS), rubber, wood, and high-density foam. Stainless steel, rubber, and foam were cut into 5 cm × 5 cm coupons, and wood coupons were cut to 6 cm × 5 cm to provide an equivalent contact area. All coupons were subjected to dry-cycle autoclaving as an initial decontamination step and allowed to cool before handling. Before inoculation, each coupon was aseptically transferred into a sterile plastic petri dish within a Class II biological safety cabinet (BSC) and exposed to ultraviolet (UV) light for at least 30 min on each side to further reduce background microflora (Baker et al., 2022). Uninoculated coupons for each material were prepared and handled identically to serve as negative controls.

Wet inoculation of FCS. Wet inoculum for each pathogen cocktail was adjusted in PBS, and each coupon was spot inoculated with 20 µL of the appropriate pathogen cocktail, corresponding to an initial target load of approximately 4 log CFU per coupon distributed in 8–10 small droplets across the entire surface area. Inoculated coupons were held in the BSC and allowed air-dry for 30 min to facilitate attachment. Separate sets of coupons were prepared for each pathogen cocktail, surface type, temperature, and sampling time point.

Dry inoculum preparation and dry inoculation of FCS. A dry inoculum was prepared by applying pathogen cocktails to sterile sand as previously described for dry surface exposure studies, with modifications (Girbal et al., 2021; Strawn et al., 2018). Briefly, 17.5 mL of the wet cocktail (approximately 5 log CFU/mL) was added to 100 g of sterile sand in a sterile stomacher bag. The mixture was massaged by hand for 2 min to distribute microorganisms evenly, then spread onto 150 × 15 mm plastic petri dishes using a sterile cell spreader. Sand was dried at 40°C for 24 h to achieve a uniform dry inoculum. After drying, the sand was transferred to a new sterile stomacher bag and mixed by hand for 2 min to break clumps and homogenize the inoculum, then stored at 4°C and used within 2 weeks. For dry inoculation of FCS, individual coupons were placed into a bag containing the inoculated sand and shaken for 2 min to ensure complete surface exposure. Coupons were then removed aseptically, gently tapped to remove loosely adhering sand, and placed into sterile petri dishes. This approach simulated transient contact of FCS with contaminated particulates or residues in dry environments. Wet and dry inoculations were performed separately for each pathogen cocktail, surface type, and temperature.

Storage conditions and sampling schedule. After wet or dry inoculation, FCS coupons were transferred from the BSC to an environmental chamber (Caron model 7000-10-1; Caron Products, Marietta, OH) set

at either 21°C (70°F) or 27°C (80°F) and 50% relative humidity (RH), conditions representative of ambient and warm produce packinghouse environments. Coupons remained uncovered in sterile petri dishes to allow for air exposure without direct physical contact. For each pathogen, survival was monitored over 168 h (7 days). Sampling time points included 0, 2, 4, 12, 24, 48, and 168 h post-inoculation. At each point, a new set of coupons was removed for enumeration to avoid repeated handling.

Recovery of microorganisms from coupons. Recovery procedures were optimized by surface type to maximize detachment of attached cells. For SS, rubber, and wood coupons, 2 mL of sterile PBS was added directly onto the coupon, and the surface was rinsed by repeated pipetting over the entire area 10 times using a calibrated micropipette to elute the persistence cells. The resulting suspension was collected into sterile microcentrifuge tubes. For foam coupons, each coupon was aseptically transferred to a sterile stomacher bag containing 20 mL PBS and processed in a stomacher at 230 rpm for 60 s. Eluates from each coupon were mixed thoroughly, and aliquots were taken immediately for serial dilution and plating.

Microbiological enumeration. For enumeration, serial dilutions were prepared by transferring 0.1 mL of the coupon eluate into 0.9 mL PBS, followed by vortexing. From the appropriate dilutions, 0.1 mL was spread-plated on TSA in duplicate per sample. Plates inoculated with *L. monocytogenes* were incubated at 35°C for 48 h, whereas plates containing *Salmonella* or STEC were incubated at 37°C for 24 h. After incubation, colonies were enumerated, and counts were converted to log CFU per coupon, accounting for dilution factors and plated volumes. Uninoculated control coupons processed in parallel at each time point were used to confirm the absence of background contamination.

Calculation of inactivation parameters. Survival data (log CFU per coupon versus time) were used to estimate first-order inactivation rates and decimal reduction times (D-values), defined as the time required to achieve a 1 log (90%) reduction in viable counts. For each pathogen–surface–inoculation–temperature combination, linear regression was applied to the descending portion of the survival curve, and D-values were calculated as the negative reciprocal of the slope, provided that the coefficient of determination (R^2) met prespecified goodness-of-fit criteria. When non-linear behavior was evident, alternative models (e.g., biphasic) were considered for descriptive comparison, but linear models were retained to facilitate cross-comparison of treatments and to be consistent with previous food-contact surface persistence studies.

III. Virus Persistence on FCS – Methods

Inoculum and surface preparation. An inoculum cocktail containing Tulane virus (TuV) and Aichivirus A (AiV) was prepared using AiV maintenance medium for inoculation. Four surfaces—of two porous (wood, foam) and two nonporous (rubber, stainless steel)—were used in this experiment. Surfaces were cut to a size of 5 cm × 5 cm (wood: 6 cm × 5 cm) coupons and sterilized before use. Prepared surfaces were wrapped in aluminum foil and autoclaved at 121°C for 30 min, then transferred aseptically into sterile petri dishes within a biological safety cabinet. To ensure sterility, the surfaces were exposed to UV light for 30 min prior to surface inoculation.

Surface inoculation. Sterilized surfaces were spot inoculated with 50 µL of TuV-AiV cocktail in 8-10 droplets per coupon to achieve a final concentration of 5 log plaque forming units (PFU) of both viruses on the surfaces. The inoculated surfaces were allowed to air-dry for approximately 30 min (wood, foam) or between 45 to 60 min (rubber, stainless steel). Following drying, the surfaces were then transferred without lids into an environmental control chamber maintained at 50% relative humidity and sampled at different time points (0, 2, 4, 6, 12, 24, 48, 72, 168 h). Dirty surfaces were sampled at 0, 12, and 168 h durations. The inoculum cocktail was diluted 10-fold and plated in technical duplicates to verify the final concentration deposited on each surface.

Virus recovery. At each sampling time point, 2 mL of AiV maintenance medium was used to recover remaining viruses from rubber, stainless steel, and wood by repeated pipetting (7-10 times). For foam samples, each coupon was transferred into a sterile stomacher bag, and 20 mL of 0.1% tris-glyceride beef extract was added. The foam was manually squeezed by hand three times to allow eluent absorption, followed by homogenization, using a stomacher at 230 rpm for 1 min. The foam was then squeezed again to extract the eluate containing the recovered virus, which was diluted as needed for further analysis. Samples recovered immediately after drying were designated as time 0 h.

Data analysis. Experimental data was generated by performing two technical and two biological duplicates to obtain four data points per sample. Each sample was plated in duplicates and averaged to obtain one data point. Experiments were performed in random order to account for potential lurking variables that were not investigated in the study. All PFU values were $\log_{10}$ transformed and used for analyses. D values, time (h) taken to achieve one $\log_{10}$ PFU reduction in virus titer, were estimated using a linear model. The $\log_{10}$ PFU of virus concentration recovered at each time point was used as the response variable which was modelled against duration. The slope of the linear model was then extracted, and the negative reciprocal was computed as the D value. Uncertainty in D values was estimated using 95% confidence intervals obtained via non-parametric bootstrapping (1000 resamples). Bootstrapping was selected because it does not rely on normality assumptions and provides robust inference under conditions of low R^2 and inherent experimental variability.

IV. Cleaning and Sanitizing Treatment for Bacterial Pathogen Removal from FCS – Methods

Inoculum preparation. A single colony from the streaking plates was transferred into 10 mL of TSB and incubated overnight (16–18 h) at 35°C for *L. monocytogenes* or 37°C for *S. enterica,* and STEC. A 0.1 mL aliquot of the overnight culture was subculture into 9.9 mL of TSB or minimal medium (M9 [*S. enterica*, STEC] or Welshimer [*L. monocytogenes*]) in a 50 mL centrifuge tube and incubated at 21°C or 27°C for varying durations based on pre-growth results. Cultures were centrifuged at 3,500 rpm for 5 min, the supernatant was discarded, and the pellet was washed with 5 mL of 1× PBS. The washing step was repeated three times. Serial dilutions were prepared by transferring 0.1 mL of washed culture into 0.9 mL of 1× PBS. Dilutions were plated on TSA and incubated at 35°C for *L. monocytogenes* or 37°C for *S. enterica* and STEC for 24 h to verify culture concentration. The final inoculum was adjusted to approximately 9 log CFU/mL by using 1× PBS and prepared as a cocktail for each bacterium used in the experiments.

Preparation of dirty FCS and inoculation. Clean FCS (wood, rubber, and foam) were cut into 5 × 5 cm pieces and autoclaved prior to use. In a biosafety cabinet, the sterilized surfaces were placed in autoclaved glass petri dishes and exposed to UV light for 15 min before being exposed to autoclaved soil and shaken for 10 s to simulate “dirty” conditions. Prepared FCS were stored in the biosafety cabinet prior to inoculation. Bacteria cocktails (0.1 mL) were inoculated on each surface in 8-10 small drops and left in the biosafety cabinet until dry. Drying time was approximately 30 min for wood surfaces, and 1 h for rubber and foam surfaces. After drying, FCS were randomly designated for 12 h and 1 week holding times and transferred to an environmental chamber maintained at 50% relative humidity at 21 or 27°C.

Sanitizer application on FCS. Seven treatments were included in this study: Clean; Sanitize (SaniDate, Alpet, or Oxine); and Clean + Sanitize (Clean + SaniDate, Clean + Alpet, or Clean + Oxine). For Clean and Clean + Sanitize treatments, FCS were washed in soapy water [10 mL of commercial detergent (Dawn soap) in 2 L of deionized (DI) water] with scrubbing for 30 s. The surfaces were then rinsed for 30 s with DI water dispensed from a carboy prior to sanitizer treatment, if applicable. For sanitizing, each treatment was applied by spraying ~3 mL of 1× PBS or sanitizer solution onto the FCS and allowed to sit for 1 min. Then, 20 mL of neutralizer (D/E broth) was added to each surface. The treated surfaces with

liquids were transferred into sterile stomacher bags and homogenized at 230 rpm for 30 s. The homogenized liquid was transferred into sterile 50 mL centrifuge tubes for serial dilution and enumeration. Percentage reduction was calculated relative to sham-treated controls (1× PBS) processed in parallel. The concentrations of Sanidate and Oxine were tested by using Peracetic Acid EndPoint ID® Test Kits and Chlorine Dioxide Test Kit, respectively, for each experiment.

Bacterial enumeration. Serial dilutions were prepared by transferring 0.1 mL of sample into 0.9 mL of 1× PBS. Dilutions were plated on TSA and incubated as follows: *L. monocytogenes*: 35°C for 48 h; *S. enterica* and STEC: 37°C for 24 h. Colonies were then enumerated to determine bacterial counts.

Statistical analysis The statistical model used in the present project was previously described by Topalcengiz et al. (2025). In short, 72 experimental trials were conducted to evaluate differences in percentage reductions of *S. enterica* and STEC observed among various C&S methods for all combination of factors (treatment, time, surface type, pre-growth medium) for a total of 1,008 samples in this analysis. Percentage reductions were calculated by the equation below:

$$Percentage\ ruduction\ (\%) = \frac{Control\ CFU\ -\ Recovered\ CFU}{Control\ CFU} \times 100$$

Percentage reductions with a negative value assigned a 0 to indicate no log reduction or no product efficacy. Statistical analysis was performed to determine whether treatment, time, surface type, and inoculation pre-growth medium were significant predictors of percentage reduction for *S. enterica* and STEC. Initial analysis of the data using a linear model indicated that the assumptions of normality and homoscedasticity were not met. Therefore, a generalized linear model (GLM) with binomial errors was used. However, the residual deviance was greater than the residual degrees of freedom; therefore, a GLM with quasibinomial errors was used to account for overdispersion. The log link function was applied to model the relationship between recovery percentage and predictor variables. Estimated marginal means were used to calculate treatment means and their 95% confidence intervals. Multiple pairwise comparisons with sidak adjustments were performed to identify statistical differences at $P \leq 0.05$. Due to a lack of significant effect on higher order interactions (four-way and three-way), the model was simplified to only include the two-way interactions. A statistically significant difference was determined when $P \leq 0.05$. All data were analyzed in R (Team, 2021) using packages including car (Fox et al., 2007), broom (Robinson, 2014), tidyverse (Wickham and Wickham, 2017), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2018), emmeans (Lenth et al., 2021), and multcomp (Hothorn et al., 2008).

Objective 2 – Results

I. Pre-growth Conditions and Bacterial Growth Response – Results

All serotypes in all three tested bacteria (*L. monocytogenes*, *S.* enterica, and STEC) reached ~9 log CFU/mL under all tested conditions, except STEC210 (STEC O26:H11) grown at 15°C in a_w medium (~7 log CFU/mL); however, growth rates and time to reach stationary phase differed among media and temperatures. Growth temperature was generally more restrictive than nutrient limitation or water activity.

Listeria monocytogenes. In TSB, the average growth rate increased from 0.196 h^{-1} at 15°C to 0.888 h^{-1} at 32°C. In Welshimer medium, the growth rate was 0.07 h^{-1} at 15°C and increased to 0.24 h^{-1} at 32°C. In a_w medium, the growth rate was 0.08 h^{-1} at 15°C and increased to 0.38 h^{-1} at 32°C. At 15°C, growth in Welshimer and a_w media was similar. However, at 21, 27, and 32°C, growth in a_w medium exceeded that in Welshimer medium, suggesting that nutrient limitation became more restrictive than reduced water activity at temperatures above 15°C. Growth in Welshimer medium remained consistently slower than

in the other two media. Despite these differences, growth rates were comparable among serotypes across temperatures and media conditions (**Table 2-3**). Time to reach stationary phase varied by serotype across all temperatures and media; however, cultures grown in TSB consistently reached stationary phase faster than those in the other two media. At 15°C, L119 (*L. monocytogenes* R9-5506 [4b]; 127.4 h vs. 102.5 h) and L123 (*L. monocytogenes* S10-2161 [1/2a]; 86.9 h vs. 75 h) required more time to reach stationary phase in Welshimer medium than in a_w medium. At 21°C, L119, L121 (*L. monocytogenes* R9-0506 [1/2a]), L122 (*L. monocytogenes* J1-0031 [4a]), and L123 exhibited longer times to stationary phase in Welshimer medium compared with a_w medium. At temperatures above 21°C (27 and 32°C), similar times to stationary phase were observed in a_w and Welshimer media for all serotypes (**Figure 2-1**). Growth curves of *L. monocytogenes* in TSB, a_w 0.95, and Welshimer media at various temperatures (15, 21, 27, and 32°C) are shown in **Figure 2-2**.

Salmonella enterica. In TSB, the average growth rate increased from 0.156 h^{-1} at 15°C to 1.404 h^{-1} at 32°C. In M9 medium, the growth rate was 0.152 h^{-1} at 15°C and increased to 1.076 h^{-1} at 32°C. In a_w medium, the growth rate was 0.068 h^{-1} at 15°C and increased to 0.558 h^{-1} at 32°C. At 15 and 21°C, TSB and M9 showed similar growth; however, at 27 and 32°C, growth in TSB surpassed M9, indicating that nutrient limitation became more restrictive than higher temperature (>21°C). Growth in a_w medium remained consistently slower than in the other two media. Among serotypes, S010 (*S.* Poona R9-5502) showed slower growth at 15 and 21°C in TSB but was comparable to other serotypes at higher temperatures, suggesting greater sensitivity to lower temperatures (**Table 2-4**). Time to reach stationary phase was longest in a_w medium, followed by M9 and TSB, across all temperatures and serotypes. At 15°C, S010 required more time to reach stationary phase in TSB than in M9 (52.5 h vs. 45.8 h), whereas the opposite trend was found in other serotypes under the same conditions. This finding suggests that S010 may be better adapted to nutrient-limited conditions at low temperatures compared to the other serotypes. In addition, S014 (*S.* Newport R9-5252) consistently required more time to stationary phase in a_w medium than the other serotypes at all temperatures, indicating greater sensitivity to water activity (**Figure 2-3**). Growth curves of *S. enterica* in TSB, a_w 0.96, and M9 media at various temperatures (15, 21, 27, and 32°C) are shown in **Figure 2-4**.

***Shiga toxin-producing Escherichia coli*.** A similar pattern was observed in STEC as *S. enterica*. In TSB, the average growth rate increased from 0.16 h^{-1} at 15°C to 1.44 h^{-1} at 32°C. In M9 medium, the growth rate was 0.153 h^{-1} at 15°C and increased to 1.12 h^{-1} at 32°C. In a_w medium, the growth rate was 0.063 h^{-1} at 15°C and increased to 0.518 h^{-1} at 32°C. At 15 and 21°C, TSB and M9 showed similar growth; however, at 27 and 32°C, growth in TSB surpassed M9, indicating that nutrient limitation became more restrictive than higher temperature (>21°C). Growth in a_w medium remained consistently slower than in the other two media across all temperatures. Among strains, STEC210 (STEC O26:H11) exhibited a higher growth rate at 15°C in a_w medium (0.11 h^{-1}) compared to other strains, but showed comparable growth rate under all other conditions, suggesting possible adaptation to low-temperature, reduced water activity conditions (**Table 2-5**). Time to reach stationary phase was longest in a_w medium, followed by M9 and TSB, across all temperatures and strains. At 15°C in a_w medium, time to stationary phase varied substantially among strains, ranging from 20.4 h for STEC210 to 166 h for STEC211 (STEC O157:H7). At 21°C, STEC209 (STEC O104:H4) grown in M9 exhibited a longer time to stationary phase (37.4 h) compared to other strains, but differences were not observed under other conditions (**Figure 2-5**). Growth curves of STEC in TSB, a_w 0.96, and M9 media at 15, 21, 27, and 32°C are shown in **Figure 2-6**.

II. Bacterial Pathogen Persistence on FCS – Results

Overall effects of surface, temperature, inoculum method, and medium. Across all conditions, pathogen persistence on FCS was jointly influenced by surface material, incubation temperature, inoculation method, and pre-growth medium, with D-values spanning approximately 42–182 h under

wet inoculation **Table 2-6** and 44–144 h under dry inoculation at 21°C **Table 2-7**, and approximately 45–112 h (wet) **Table 2-8** and 51–135 h (dry) at 27°C **Table 2-9**. High-density foam consistently supported the greatest persistence for all three pathogen cocktails, whereas wood generally yielded the lowest D-values, particularly at 21°C. Inoculation method had a notable, but surface- and condition-dependent, impact: dry inoculation tended to increase D-values relative to wet inoculation on nonporous and semi-porous surfaces (foam, rubber, stainless steel), whereas differences were minimal on wood under some conditions. Pre-growth in minimal media (Welshimer and M9) produced D-values that were generally similar in magnitude and patterns to those obtained from optimal medium (TSB), indicating that surface type and temperature exerted stronger effects on persistence than inoculum matrix alone.

Pathogen persistence on FCS at 21°C under wet inoculation. At 21°C and 50% RH, wet-inoculated pathogens persisted longest on foam, with D-values for *L. monocytogenes*, *Salmonella*, and STEC up to 182.4 h (95% CI: 119.85–246.44), 107.7 h (74.94–133.06), and 133.2 h (86.14–169.94), respectively, when pre-grown in TSB (**Table 2-6**) Table 3. On stainless steel and rubber, wet-inoculated D-values in TSB for all pathogens ranged from ~48–90 h, intermediate between foam and wood. Wood yielded the shortest D-values under wet inoculation at 21°C in TSB, with *L. monocytogenes*, *Salmonella*, and STEC exhibiting D-values of 47.2 h (39.80–52.04), 42.8 h (29.25–50.59), and 42.7 h (30.10–50.45), respectively. For bacteria grown in minimal media, D-values on foam, rubber, stainless steel, and wood at 21°C generally clustered around 48–54 h, with overlapping confidence intervals, suggesting that medium composition had comparatively modest effects on inactivation kinetics at this temperature.

Pathogen persistence on FCS at 21°C under dry inoculation. Under dry inoculation at 21°C, foam again supported substantial persistence, with D-values in TSB for *L. monocytogenes*, Salmonella, and STEC of 112.7 h (84.09–133.52), 144.4 h (118.02–241.39), and 131.6 h (102.89–153.25), respectively (**Table 2-7**). On rubber and stainless steel with TSB, D-values for the three pathogens ranged from ~79–119 h, indicating enhanced persistence compared to wet inoculation on these surfaces at the same temperature. In contrast, wood showed relatively short D-values under dry inoculation at 21°C in TSB (approximately 44–46.5 h for all pathogens), similar in magnitude to wet-inoculated wood, suggesting that the impact of inoculation method on persistence was attenuated on this porous surface. For *L. monocytogenes* in Welshimer and *Salmonella*/STEC in M9, dry-inoculated D-values on foam, rubber, stainless steel, and wood at 21°C were typically between ~43–122 h, with wood often yielding the lowest values and foam the highest, reinforcing the primary role of surface properties.

Pathogen persistence on FCS at 27°C under wet inoculation. At 27°C, wet-inoculated pathogens remained highly persistent across surfaces, with foam again yielding large D-values in TSB: 89.2 h (58.13–115.20) for *L. monocytogenes,* 111.5 h (82.80–136.10) for *Salmonella*, and 91.6 h (60.15–120.70) for STEC (**Table 2-8**). On rubber and stainless steel in TSB, D-values for all three pathogens were generally between ~78–92 h, comparable to foam and indicating that nonporous and semi-porous surfaces can support extended survival at 27°C. Wood exhibited more variable behavior: for *L. monocytogenes*, wet-inoculated D-values at 27°C in TSB reached 92.5 h (74.02–110.02), comparable to or exceeding foam and stainless steel, whereas *Salmonella* and STEC displayed shorter D-values of 64.3 h (44.51–78.98) and 70.0 h (46.42–87.06), respectively. When *L. monocytogenes* was pre-grown in Welshimer, D-values at 27°C remained high on foam, rubber, and stainless steel (approximately 81–96 h) but were substantially lower on wood (45.5 h, 39.93–48.59), indicating a medium-by-surface interaction in *L. monocytogenes* persistence at elevated temperature. *Salmonella* and STEC grown in M9 at 27°C showed D-values between ~51–89 h across surfaces, with foam and wood often supporting the highest values for *Salmonella* and intermediate values for STEC.

Pathogen persistence on FCS at 27°C under dry inoculation. Dry-inoculated pathogens at 27°C demonstrated long persistence across all surfaces, with foam in TSB again exhibiting the largest D-values for *Salmonella* (134.8 h, 107.01–167.27) and STEC (123.3 h, 88.79–157.63) and high D-values for *L.*

monocytogenes (100.4 h, 84.56–119.01) (**Table 2-9**). Rubber and stainless steel in TSB also supported extended survival, with D-values generally between 111.2 h (81.91-143.45) and 122.6 h (88.91-152.56) for *Salmonella* respectively, and ~79–102 h for *L. monocytogenes* and STEC. Wood, in contrast to 21°C, showed notably high D-values under dry inoculation at 27°C in TSB, particularly for *L. monocytogenes* (109.5 h, 95.39–128.03) and *Salmonella* (117.8 h, 91.97–148.56), with STEC exhibiting a more variable estimate (129.2 h, 87.24–293.21) and lower R^2. In minimal media at 27°C, *L. monocytogenes* in Welshimer showed the highest dry D-values on foam (~87.6 h, 73.91–96.15) and lower values on rubber, stainless steel, and wood (~59–66 h), whereas *Salmonella* and STEC grown in M9 displayed a narrower range of D-values across surfaces (~51–104 h), with wood generally exhibiting the lowest or intermediate D-values.

Impact of incubation temperature on persistence. Comparisons between 21°C and 27°C showed that the effect of temperature on D-values was pathogen-, surface-, inoculation- and medium-dependent rather than uniformly increasing or decreasing persistence. For wet inoculation in TSB, *Salmonella* and STEC on foam displayed similar or slightly reduced D-values at 27°C relative to 21°C, whereas *L. monocytogenes* showed a marked decrease from ~182 h at 21°C to ~89 h at 27°C, indicating temperature-enhanced inactivation for LM under the most supportive foam condition. On wood, however, *L. monocytogenes* D-values in TSB increased from ~47 h at 21°C to ~92 h at 27°C under wet inoculation, while *Salmonella* and STEC exhibited more modest increases, suggesting that elevated temperature can enhance persistence on certain porous materials, possibly via reduced water activity gradients or microenvironmental effects. Under dry inoculation, increases in temperature from 21°C to 27°C typically maintained or increased D-values on foam, rubber, stainless steel, and wood, particularly for *Salmonella* and STEC in TSB, indicating that warm, low-moisture conditions may favor prolonged survival on these surfaces.

Impact of inoculum medium (TSB, Welshimer, M9) on persistence. Pre-growth medium had subtler effects on D-values than surface type and inoculation method, but some patterns emerged. At 21°C, *L. monocytogenes* grown in Welshimer showed relatively uniform D-values across foam, rubber, stainless steel, and wood (~43–98 h) under both wet and dry inoculation, contrasting with the much larger range of D-values observed for TSB-grown *L. monocytogenes*, particularly on foam rubber, stainless steel, and wood (~44–182 h) under both wet and dry inoculation. For *Salmonella* and STEC, pre-growth in M9 at 21°C generally yielded D-values around 44–122 h across surfaces and inoculation methods, again narrower than the range 43-144 h observed in TSB, suggesting that minimal medium pre-growth may reduce the apparent advantage conferred by highly supportive surfaces under some conditions. At 27°C, *L. monocytogenes* in Welshimer exhibited a strong surface effect under wet inoculation, with high D-values on foam, rubber, and stainless steel (~81–97 h) but substantially lower values on wood (~45 h), while dry-inoculated *L. monocytogenes* in Welshimer displayed D-values between ~59 and 88 h across surfaces. *Salmonella* and STEC in M9 at 27°C showed moderate to high D-values (~51–104 h) across surfaces and inoculation methods, but the medium did not consistently reduce persistence relative to TSB for these organisms, indicating that minimal medium pre-growth does not universally attenuate survival potential on FCS.

Impact of surface type on pathogen persistence. Surface material was a dominant determinant of persistence across pathogens, temperatures, and media. Foam consistently supported the longest survival of all three pathogen cocktails under most combinations of temperature, medium, and inoculation method, with many of the largest D-values, particularly for *Salmonella* and STEC. Stainless steel and rubber generally exhibited intermediate persistence, with D-values often similar to each other but lower than foam and higher than wood at 21°C, especially for wet inoculation in TSB. Wood tended to yield the lowest D-values, particularly at 21°C, under both wet and dry inoculation, indicating more rapid inactivation on this porous surface; however, at 27°C and dry inoculation in TSB, wood supported

D-values comparable to or exceeding those on other surfaces for some pathogen–medium combinations, highlighting that temperature and desiccation history can modulate the protective or detrimental role of surface porosity. Overall, these results demonstrate that high-density foam and, under certain warm, dry conditions, wood can function as long-term reservoirs for *L. monocytogenes*, *Salmonella*, and STEC, whereas stainless steel and rubber generally support intermediate persistence.

III. Virus Persistence on FCS – Results

D-value estimations revealed that, overall, AiV exhibited a higher D-value than TuV (**Table 2-10**). A 1 $\log_{10}$ PFU reduction of AiV on the surfaces required a duration of 68.54 (CI: 61.08, 77.66) whereas TuV required 52.71 (48.22, 57.26) to achieve a similar reduction. Moreover, AiV survived the longest on all surfaces compared to TuV under the various conditions explored in the study. Surface cleanliness influenced viral persistence. When coupons were treated with soil to simulate dirty conditions, the D-value observed was 62.54 (56.06, 70.68), compared with 55.15 (50.11, 60.35) under clean conditions. AiV persisted the longest on dirty surfaces with a D-value of 74.92 (61.31, 92.95) compared to clean surfaces (63.57 [54.62, 73.96]). Similarly, TuV had a higher D-value (53.20 [47.24, 60.41]) on dirty surfaces than on clean surfaces (51.65 [45.43, 57.39]).

The survival of viruses on the surfaces was generally enhanced by lower temperatures. Both AiV and TuV survived longer at 15°C and 21°C than at 27°C. For instance, under clean conditions, AiV remained detectable for up to 168 h on survived up to 168 h on stainless steel and rubber both stainless steel and rubber at 15°C and 21°C. TuV persisted for up to 48 h at 15°C on both surfaces but only 24 h and 12 h on stainless steel and rubber, respectively, at 21°C. At 27°C, survivability of AiV decreased to 72 h on both surfaces, whereas TuV lasted for only 12 h on stainless steel and 6 h on rubber. Although viruses generally persisted longer at 15°C, the highest overall D-value was observed at 21°C (63.07 [55.04, 71.69]), followed by 27°C and 15°C which achieved 58.64 (50.92,67.34) and 56.23 (50.27, 63.65) D values, respectively. The observed D-values, however, were dependent on surface cleanliness and virus type. For example, under clean conditions, while TuV exhibited its largest D-value at 21°C (53.98 [43.44, 64.62]), followed by 27°C (68.29 [50.01, 89.95]) and 15°C (42.55 [33.97, 49.23]). In contrast, AiV demonstrated largest D-value at 15°C (92.17 [65.51, 140.93]), followed by 21°C and 27°C with recorded D-values of 70.26 (53.31, 91.44) and 49.12 (36.97, 59.26), respectively (**Figure 2-7**). Overall, lower temperatures prolonged the time needed to achieve a 1 $\log_{10}$ PFU of AiV compared to TuV.

Among surfaces, wood exhibited the largest D-value (105.39 [88.79, 124.93]); however, it is important to note that viral recovery from wood was highly inconsistent, which may have influenced slope estimates and inflated D-values. Additionally, initial viral recovery (0 h) on wood surfaces were low with an average of 1.91 (min: 0; max: 3.78) $\log_{10}$, compared to stainless steel (4.32 [min: 3.41; max: 5.06] $\log_{10}$ PFU), rubber 4.32 [min: 3.4; max: 5.21] $\log_{10}$ PFU)and foam 4.42 [min: 3.48; max: 4.92] $\log_{10}$ PFU). Rubber (53.09 [46.02, 60.79]) showed a slightly higher persistence compared to stainless steel (51.84 [45.80, 58.51]), while the lowest D-value of 47.60 (41.02, 54.21) was recorded on foam.

IV. Cleaning and Sanitizing Treatment for Bacterial Pathogen Removal from FCS – Results

C&S efficacy against *Salmonella*. A factorial ANOVA revealed significant main effects of Treatment, Surface, and Medium on the percentage reduction of *S. enterica* grown at 21°C (Treatment: $P < 0.001$; Surface: $P = 0.004$; Medium: $P < 0.001$), as shown in **Table 2-11**. The main effect of time was not significant ($P = 0.257$), indicating no overall change across time points when averaged across other factors. Several two-way interactions were significant, demonstrating that treatment efficacy varied by FCS type, pre-growth medium, and time duration. For the Treatment × Time interaction ($P < 0.001$; **Figure 2-8**), the Oxine treatment consistently produced the lowest percentage reduction across time points. At 0 h, Oxine alone achieved the lowest reduction (62.5%), whereas the Clean + Oxine

combination produced the highest reduction (99.9%). These results indicate that cleaning substantially enhances Oxine efficacy on dirty FCS, particularly over short time intervals. For the Treatment × Surface interaction ($P < 0.001$; **Figure 2-9**), SaniDate showed similar reductions (≥ 96.5%) on all FCS regardless of cleaning. Alpet was most effective on wood (97.3%), followed by rubber (90.9%), and foam (71.5%). Oxine was least effective on all FCS (≤ 86.5%) but improved to ≥ 92.2% when combined with cleaning. For the Treatment × Medium interaction ($P = 0.05$; **Figure 2-10**), Oxine again showed significantly lower reductions than all other treatments, regardless of pre-growth medium. Post-hoc comparisons did not yield significant pairwise differences within the Surface × Time interaction (**Figure 2-11**), though at the 1-week time point, foam showed a numerically lower reduction (82.9%) compared to wood (92.1%) and rubber (94.0%), a pattern that may reflect the within-group variability observed across conditions. At the 0 h and 12 h time points, reduction percentages ranged from 87.4 to 95.4%. For the Medium × Time interaction ($P < 0.001$; **Figure 2-12**), no significant differences in percentage reduction were observed between the two media at 0 h and 1 week. However, at 12 h, cultures pre-grown in TSB exhibited lower reductions than those grown in M9 (85.8% vs. 95.8%) when subjected to the various cleaning and sanitizing treatments.

C&S efficacy against STEC. A factorial ANOVA revealed significant main effects of Treatment, Surface, and Time on percentage reduction at 21°C (Treatment: $P < 0.001$; Surface: $P = 0.003$; Time: $P < 0.001$), as also shown in **Table 2-11** The main effect of Medium was not significant ($P = 0.346$), indicating no overall difference between the two media when averaged across other factors. Two significant two-way interactions were identified: Treatment × Time ($P < 0.001$) and Surface × Time ($P = 0.05$). No significant differences among C&S treatments were observed for foam or wood surfaces. However, on rubber surfaces, the Oxine treatment produced significantly lower percentage reduction compared with the Clean + Alpet treatment (63.5% vs. 99.3%; **Figure 2-13**), showing Oxine was less effective on percentage reduction of STEC on rubber surface, compared to foam and wood. Across time points (0 h, 12 h, and 1 week), there were no overall differences in percentage reduction among surfaces. However, STEC reduction on wood at 1 week (79.5%) was significantly lower than reductions observed on foam at 0 h (97.7%), and on both foam (96.5%) and rubber (93.6%) at 12 h, with all other treatments showing intermediate reductions (**Figure 2-14**). Notably, this lower reduction on wood may be attributable to the absence of STEC recovery from the control samples at 1 week in some of the wood surface experiments.

Outcomes and Accomplishments

Objective 1

- Financial constraints, knowledge gaps, and repurposed equipment are concerns for packinghouse operations.
- Common surfaces in packing areas include wood, plastic, and stainless steel.
- Cost, compliance, and practicality are drivers of practice and material selection.
- Produce safety educators emphasize the need for hands-on training.

Objective 2 – Bacteria persistence

- Environmental monitoring and sanitation validation programs should prioritize porous polymeric materials, particularly high-density foam, which consistently supported prolonged survival.
- Dry inoculation produced substantially greater D-values under multiple conditions. Sanitation validation and predictive models should incorporate dry contamination data to avoid underestimating environmental persistence.
- A uniform sanitation approach may not be sufficient. Foam and wood surfaces may require enhanced cleaning frequency, alternative materials, or redesign to reduce microbial harborage.
- When feasible, replacing porous materials with cleanable, nonporous alternatives may reduce long-term contamination risk.

Objective 2 – Virus persistence

- Persistence depended on virus type with AiV (a Hepatitis A virus surrogate) persisting significantly longer than TuV.
- Viruses are more likely to persist for an extended period when food-contact surfaces are dirty as compared to a clean surface.
- Temperature influences the persistence of viruses on surfaces with lower temperatures enhancing AiV persistence more than TuV.

Objective 2 – Cleaning and Sanitizing (bacteria only, virus work is still underway)

- Cleaning alone is insufficient to eliminate pathogens on FCS, especially on wood surfaces.
- SaniDate effectively reduced the pathogens on FCS; however, cleaning further enhanced its inactivation efficacy.
- Cleaning is essential for Oxine to achieve optimal pathogen reduction in all surface types and conditions.
- Alpet demonstrated reduced effectiveness on foam and rubber surfaces (for STEC) and required prior cleaning to achieve optimal pathogen reduction on these materials.

APPENDICES

Publications and Presentations

Publication

1. Hamilton, A. N., S. Chandran, K. E. Gibson, and J. Moreira. 2025. Thematic Analysis of Produce Packinghouse Sanitation: Challenges and Recommendations Based on Operator and Educator Insights. *Journal of Food Protection* 88:100587. https://doi.org/10.1016/j.jfp.2025.100587

Manuscripts in preparation

1. H. Shi, F. Torko, M. Ashrafudoulla, B. J. Gonzalez, Z. Jia, and K. E. Gibson. 2026. Growth kinetics of *Listeria monocytogenes, Salmonella enterica*, and Shiga toxin-producing *Escherichia coli* under different pre-growth conditions across varying media and temperatures.
2. M. Ashrafudoulla, H. Shi, F. Torko, and K. E. Gibson. 2026. Persistence of *Listeria monocytogenes*, *Salmonella*, and Shiga toxin-producing *Escherichia coli* on produce packinghouse surfaces.
3. F. Torko, H. Shi, M. Ashrafudoulla, and K. E. Gibson. 2026. Persistence of viruses on food contact surfaces relevant to produce packinghouses.

*Presentations (***3 abstracts have also been accepted for presentation at IAFP 2026 Annual Meeting in New Orleans, LA)*

1. H. Shi, F. Torko, M. Ashrafudoulla, B. J. Gonzalez, Z. Jia, and K. E. Gibson. Growth kinetics of *Salmonella enterica* under different pre-growth conditions across varying media and temperatures. AAFP. Fayetteville, AR. 2025.
2. M. Ashrafudoulla, H. Shi, F. Torko, and K. E. Gibson. Persistence of *Listeria monocytogenes*, *Salmonella*, and Shiga toxin-producing *Escherichia coli* on produce packinghouse surfaces. AAFP. Fayetteville, AR. 2025.
3. F. Torko, H. Shi, M. Ashrafudoulla, and K. E. Gibson. Persistence of viruses on food contact surfaces relevant to produce packinghouses. AAFP. Fayetteville, AR. 2025.

Budget Summary

This project was awarded $206,108 in research funds. All funds have been spent.

Tables and Figures

Objective 1 Tables

Table 1-1. Semi-structured interview discussion guide questions.

Question No.	Variant	Question Content
1	Educator	Can you identify three main challenges you have observed regarding produce packing areas?
	Packinghouse	Can you identify three main challenges you believe you have regarding your produce packing area?
2	Educator	What types of surface materials are used commonly within produce packing areas you have observed?
	Packinghouse	What types of surface materials are used within your produce packing area?
3	Educator	Are there particular surfaces that you are more concerned about, with respect to cross-contamination?
	Packinghouse	Are there particular surfaces that you are more concerned about, with respect to cross-contamination?
4	Educator	What would you say may be the main motivation of growers for choosing their operation's current food contact surface materials?
	Packinghouse	What would you say was your main motivation for choosing your operation's current food contact surface materials?
5	Educator	What types of methods to clean and sanitize surfaces that contact the product have you observed in these operations?
	Packinghouse	What types of methods do you use to clean and sanitize surfaces that contact your product?
6	Educator	What sanitizers are commonly used? Have you observed changes in sanitizers that were more common in the past? If so, why do you believe growers change their sanitizer choices?
	Packinghouse	What sanitizers do you commonly use, and have you used different sanitizers in the past? If so, why did you change your current choices?
7	Educator	What would you say is the main motivation of growers for choosing their operation's current cleaning and sanitation practices?
	Packinghouse	What would you say was your main motivation for choosing your operation's current cleaning and sanitation practices?
8	Educator	How have you observed that growers commonly determine if a food contact surface has been appropriately cleaned or sanitized?
	Packinghouse	How do you determine if a food contact surface has been appropriately cleaned or sanitized?
9	Educator	In terms of environmental conditions (i.e. temperature, humidity, protection against weather events), what do you feel has the most impact on the safety or quality of produce?
	Packinghouse	In terms of environmental conditions (i.e. temperature, humidity, protection against weather events), what do you feel has the most impact on the safety or quality of your products?

10	Educator	What would you describe as the most important educational resources growers consult when developing their cleaning and sanitation practices?
	Packinghouse	What would you describe as the most important educational resources you consulted when developing your cleaning and sanitation practices?
11	Educator	Is there anything you feel I missed and that you would like to share about your experience?
	Packinghouse	Is there anything you feel I missed and that you would like to share about your operation?

Table 1-2. Contextual themes

Themes	Illustrative Quotes
Environmental Factors	• "You're not gonna avoid [condensation] because of the temperature differences, but I know…folks have concerns about the water dripping from the roof to the boxes." • "Open sheds can be more challenging to control pests and animals coming in." • "They just have to rely on the weather to act appropriately for them to continue the work."
Economic Constraints	• "We have very small and medium sized growers…they don't have many resources." • "The main challenge is like a lack of resources…and lack of those tools and supplies." • "The first thing they always say is the cost to get GAP certified."
Legacy Equipment	• "Some just aren't designed to be cleaned very well. So a lot of 90 degree angles, and even the materials they are made of are hard to clean." • "Wood-based materials, rust on equipment." • "Legacy equipment in generational farms."
Materials	• "Wood can trap in microorganisms. Okay, and then it's difficult to clean and it can actually cross-contaminate the other produce that was in the same container." • "There's no way that you can clean a brush and sanitize a brush. And those are food-contact surfaces." • "You also see the stainless that's all pitted and impossible to clean." • "Screen doors" [used for drying] • "Those plastic containers that have been used very vigorously, and the surfaces are very raw, can actually hold those microorganisms."
Cultural and Language Barriers	• "We cannot forget that a lot of farmers English is not their primary language. And they're not just sometimes in the general national narrative. They think of them as farm workers, which I find I really struggle with that because there's a lot of farm owners and operators that are not [speaking] English" • "Most of the packinghouses using Hispanic workers for the majority." • "Industry norms battling. Some of those industry perceptions are just like this is the way we've always done it." • "Younger people care more [about safety] and are starting to take over businesses."
Regulatory and Consumer Expectations	• "Beating an audit is easily the biggest [motivation when choosing equipment and sanitizers], maybe equally matched with the buyers." • "They're now GAP [good agricultural practices] certified, and they need to do it certain way" • "Many of the consumers don't have a positive feeling about chlorine. Okay, the thing is that chlorine is a chemical compound. Okay, so that's the main thing that they try to avoid chlorine and then use peroxyacetic acid." • "They are aware and conscientious that they don't wanna get anyone sick, but there has been a cost to all of this."
Motivations	• "They are aware and conscientious that they don't wanna get anyone sick, but there [has] been a cost to all of this."

- "I think they're looking for the trade-off of what's the next best option below stainless steel."
- "They wanna change, many of them. But because of the lack of resources, sometimes they have to use the same items."

Table 1-3. Barriers to Risk Management and Regulatory Compliance

Themes	Illustrative Quotes
Knowledge and Training	• "Lack of scientific knowledge and basic knowledge of cleaning and sanitizing." • "They don't use appropriate techniques…like clean and sanitize their produce before they pack." • "Many different degrees of stainless steel or grades. I think [that] one of the biggest problems is that they don't know the difference between 316, 380, and 304."
Infrastructure	• "Physical lack of space/time for proper cleaning and sanitation." • "Most places just don't have enough abundant space to adequately move things around."
Cleaning and Sanitizing Practices	• "Not usually cleaning before sanitizing." • "They're either just hosing out to clean, if it's not if it's just large particles, if there's like schmutz and slime, then a scrub with dish soap and then air dry after rinsing or additional spray with a hand spray bottle of sanitizer." • "Yeah, there are some people who still do use a pressure washer." • "They're already using some of those isopropyl alcohol wipes." • "There's definitely been a shift to SaniDate® and [Tsunami 100™], so more of the PAA's [peroxyacetic acid]. • "Some non-organic growers, I think, who are using like quaternary ammonium products and bleach is still used. But PAA, it seems PAA has taken over for both organic and non-organic growers." • "A visual observation [is used to determine if something is clean/sanitized.] Yeah, I mean, I don't have anyone who's doing any sort of, you know, rapid tests in in New Hampshire. I've definitely seen in larger places…using ATP testing." • "Currently, it's really sort of a visual clean and a check off on the process."
Worker Safety and Comfort	• "Comfortable environment for the workers so they can focus on safety." • "Hand hygiene is still problematic, because if you wash enough your hands crack." • "Cleaning areas with electrical equipment."

Table 1-4. Research Needs

Themes	Illustrative Quotes
Sanitation Methods and Hygienic Design	• "Some growers are shifting from chlorine to PAA, because it is easier to apply…no pH control, durability, efficiency is higher." • "Some of them are not washing their produce anymore. They clean it with brushes, but they don't wash it. Because they are afraid of internalization and leaving moisture on the surface for microorganisms." • "More research on food contact surfaces" • "Picture based labels for sanitizers"
Behavioral and Cultural Change	• "The mentality needs to be changed from securing markets to protecting people or selling wholesome foods." • "I do feel in the past 6 years that I've been doing this job, there tends to be a lot less resistance now to food safety conversations than there was even when I started 6 years ago." • "So, you know, if there's some ways like as a community that we can come up with common messaging, so that everybody's gonna say the same thing every time, and so that there's no contradicting that goes on."

Objective 2 Tables

Table 2-1. Pathogens included in this study.

ID in this study	Pathogen	Strain (Serotype)	Outbreak
L119	*L. monocytogenes*	R9-5506 (4b)	Packaged salad, 2016
L121	*L. monocytogenes*	R9-0506 (1/2a)	Cantaloupe, 2011
L122	*L. monocytogenes*	J1-0031 (4a)	Human
L123	*L. monocytogenes*	S10-2161 (1/2a)	Soil spinach field
L124	*L. monocytogenes*	R2-0503 (1/2b)	Human, 1994
S007	S. Javiana	R9-5273	Tomatoes, 2002
S010	*S.* Poona	R9-5502	Cucumber 2015
S012	*S.* Typhimurium	R9-5409	Peanut butter, 2008–2009
S014	*S.* Newport	R9-5252	Tomatoes, 2002 and 2005
S019	*S.* Litchfield	R9-5344	Cantaloupe, 2008
STEC209	STEC O104:H4	R9-5257	Human, 2009
STEC210	STEC O26:H11	R9-5512	Raw clover sprouts, 2012
STEC211	STEC O157:H7	R9-5271	Baby spinach, 2006
STEC212	STEC O111:H8	R9-5345	Apple cider

Table 2-2: The overall experimental parameters

Variables	**Levels**
Bacteria	*Listeria monocytogenes* (5 serotypes)*, Salmonella* (5 serotypes), and *Shiga toxin-producing Escherichia coli (STEC)* (4 strains)
Inoculum Matrix	Optimal medium (TSB) and minimal medium (M9 and Welshimer)
Inoculation method	Wet and Dry
Contact Surface	Stainless steel (SS), Rubber, Foam, Wood
Temperature (°C)	21, and 27
Relative humidity	50%
Time (h)	0, 2, 4, 12, 24, 48, 72, 168
Reps	2 technical replicates per 2 experimental trials reps for each treatment

Table 2-3. Growth rates of *L. monocytogenes* from various temperature and media conditions.

Serotype	Medium/Temp.	Growth Rate (μ_{max}, h^{-1})			
		15°C	21°C	27°C	32°C
Average	TSB	0.196	0.47	0.738	0.888
	a_w 0.95	0.08	0.148	0.326	0.38
	Welshimer	0.07	0.094	0.252	0.24
L119	TSB	0.19	0.55	0.76	0.94
	a_w 0.95	0.07	0.15	0.31	0.34
	Welshimer	0.04	0.08	0.22	0.24
L121	TSB	0.19	0.39	0.72	0.88
	a_w 0.95	0.07	0.14	0.25	0.31
	Welshimer	0.07	0.11	0.26	0.23
L122	TSB	0.2	0.46	0.72	0.88
	a_w 0.95	0.08	0.14	0.35	0.41
	Welshimer	0.08	0.09	0.27	0.25
L123	TSB	0.21	0.47	0.75	0.87
	a_w 0.95	0.11	0.19	0.38	0.46
	Welshimer	0.09	0.07	0.23	0.27
L124	TSB	0.19	0.48	0.74	0.87
	a_w 0.95	0.07	0.12	0.34	0.38
	Welshimer	0.07	0.12	0.28	0.21

Table 2-4. Growth rates of *S. enterica* from various temperature and media conditions.

Serotype	Medium/Temp.	Growth Rate (μmax, h^{-1})			
		15°C	21°C	27°C	32°C
Average	TSB	0.156	0.374	0.958	1.404
	a_w 0.96	0.068	0.172	0.442	0.558
	M9	0.152	0.394	0.784	1.076
S007	TSB	0.19	0.41	0.96	1.36
	a_w 0.96	0.06	0.15	0.43	0.53
	M9	0.13	0.27	0.8	1.16
S010	TSB	0.1	0.25	0.97	1.34
	a_w 0.96	0.07	0.18	0.46	0.57
	M9	0.19	0.49	0.86	1.15
S012	TSB	0.16	0.34	0.93	1.38
	a_w 0.96	0.08	0.19	0.48	0.55
	M9	0.13	0.32	0.57	0.84
S014	TSB	0.14	0.44	0.93	1.37
	a_w 0.96	0.06	0.16	0.38	0.53
	M9	0.12	0.42	0.83	1.08
S019	TSB	0.19	0.43	1	1.57
	a_w 0.96	0.07	0.18	0.46	0.61
	M9	0.19	0.47	0.86	1.15

Table 2-5. Growth rates of STEC from various temperature and media conditions.

Serotype	Medium/Temp.	Growth Rate (μmax, h^{-1})			
		15°C	21°C	27°C	32°C
Average	TSB	0.160	0.330	0.970	1.440
	a_w 0.96	0.063	0.145	0.448	0.518
	M9	0.153	0.363	0.800	1.120
STEC209	TSB	0.17	0.3	0.93	1.44
	a_w 0.96	0.05	0.14	0.45	0.52
	M9	0.15	0.26	0.86	1.19
STEC210	TSB	0.16	0.33	0.92	1.37
	a_w 0.96	0.11	0.15	0.49	0.48
	M9	0.15	0.39	0.74	1.15
STEC211	TSB	0.18	0.37	0.94	1.32
	a_w 0.96	0.04	0.17	0.48	0.57
	M9	0.18	0.43	0.77	1.17
STEC212	TSB	0.13	0.32	1.09	1.63
	a_w 0.96	0.05	0.12	0.37	0.5
	M9	0.13	0.37	0.83	0.97

Table 2-6: Inactivation kinetics of bacteria at 21°C based on pre-growth media (TSB, minimal media [MM]) and surface type following WET inoculation.

Surface	Medium	Bacteria	D-value (log CFU h^{-1}) $^{-1}$ (95% CI)	R^2
Foam	TSB	*L. monocytogenes*	182.40 (119.85-246.44)	0.47
		Salmonella	107.72 (74.94-133.06)	0.67
		STEC	133.16 (86.14-169.94)	0.63
Rubber	TSB	*L. monocytogenes*	49.88 (43.50-53.95)	0.92
		Salmonella	52.89 (45.52-58.46)	0.88
		STEC	48.21 (38.91-53.99)	0.86
SS	TSB	*L. monocytogenes*	70.23 (60.07-76.73)	0.92
		Salmonella	90.51 (67.92-108.36)	0.76
		STEC	68.76 (53.39-78.11)	0.84
Wood	TSB	*L. monocytogenes*	47.24 (39.80-52.04)	0.92
		Salmonella	42.84 (29.25-50.59)	0.76
		STEC	42.68 (30.10-50.45)	0.76
Foam	MM	*L. monocytogenes*	47.84 (45.75-52.03)	0.97
		Salmonella	47.96 (45.93-50.58)	0.97
		STEC	47.78 (46.20-50.69)	0.98
Rubber	MM	*L. monocytogenes*	53.72 (43.74-61.02)	0.84
		Salmonella	53.57 (43.17-60.57)	0.85
		STEC	52.67 (43.80-58.94)	0.87
SS	MM	*L. monocytogenes*	51.95 (43.02-58.43)	0.85
		Salmonella	51.88 (43.12-58.84)	0.84
		STEC	54.25 (43.88-61.97)	0.81
Wood	MM	*L. monocytogenes*	48.76 (34.21-58.59)	0.73
		Salmonella	48.24 (33.46-58.01)	0.74
		STEC	48.73 (33.30 -59.09)	0.72

Shiga toxin–producing *Escherichia coli,* STEC; SS, Stainless steel

Table 2-7: Inactivation kinetics of bacteria at 21°C based on based on pre-growth media (TSB, minimal media [MM]) and surface type following DRY inoculation.

Surface	Medium	Bacteria	D-value (log CFU h^{-1}) $^{-1}$ (95% Cl)	R^2
Foam	TSB	*L. monocytogenes*	112.67 (84.09-133.52)	0.80
		Salmonella	144.36 (118.02-241.39)	0.62
		STEC	131.56 (102.89-153.25)	0.82
Rubber	TSB	*L. monocytogenes*	84.71 (73.78-104.54)	0.88
		Salmonella	79.13 (63.37-87.50)	0.91
		STEC	82.30 (67.76-90.64)	0.91
SS	TSB	*L. monocytogenes*	100.15 (76.76-122.10)	0.87
		Salmonella	94.90 (73.45-108.95)	0.86
		STEC	118.86 (88.87-140.30)	0.83
Wood	TSB	*L. monocytogenes*	44.22 (41.30-52.45)	0.96
		Salmonella	45.78 (42.92-52.31)	0.96
		STEC	46.51 (44.35-52.16)	0.98
Foam	MM	*L. monocytogenes*	98.86 (87.37-106.34)	0.95
		Salmonella	102.15 (91.84-108.71)	0.95
		STEC	122.48 (92.20-150.88)	0.72
Rubber	MM	*L. monocytogenes*	43.15 (41.59 -46.22)	0.97
		Salmonella	44.56 (42.75-47.61)	0.97
		STEC	47.75 (46.15-50.24)	0.98
SS	MM	*L. monocytogenes*	65.08 (58.08-69.77)	0.97
		Salmonella	44.70 (42.56-49.23)	0.97
		STEC	74.11 (64.38-81.47)	0.92
Wood	MM	*L. monocytogenes*	45.47 (41.86-47.47)	0.97
		Salmonella	44.21 (39.35-46.83)	0.96
		STEC	56.02 (50.99-59.54)	0.95

Shiga toxin–producing *Escherichia coli,* STEC; SS, Stainless steel

Table 2-8: Inactivation kinetics of bacteria at 27°C based on pre-growth media (TSB, minimal media [MM]) and surface type following WET inoculation.

Surface	Medium	Bacteria	D value (log CFU h^{-1}) $^{-1}$ (95% Cl)	R^2
Foam	TSB	*L. monocytogenes*	89.18 (58.13-115.20)	0.62
		Salmonella	111.52 (82.80-136.10)	0.67
		STEC	91.60 (60.15-120.70)	0.56
Rubber	TSB	*L. monocytogenes*	92.09 (61.39-116.38)	0.61
		Salmonella	78.32 (56.63-94.92)	0.68
		STEC	78.89 (49.17-102.04)	0.60
SS	TSB	*L. monocytogenes*	82.85 (59.85-104.49)	0.62
		Salmonella	90.27 (56.70-122.75)	0.59
		STEC	82.72 (56.03-108.21)	0.56
Wood	TSB	*L. monocytogenes*	92.48 (74.02-110.02)	0.75
		Salmonella	64.32 (44.51-78.98)	0.71
		STEC	69.96 (46.42-87.06)	0.69
Foam	MM	*L. monocytogenes*	96.74 (79.52-113.28)	0.77
		Salmonella	72.01 (54.61-84.89)	0.80
		STEC	78.74 (58.05-94.02)	0.75
Rubber	MM	*L. monocytogenes*	80.65 (55.48-102.65)	0.63
		Salmonella	89.32 (58.43-118.19)	0.52
		STEC	73.58 (52.38-88.59)	0.73
SS	MM	*L. monocytogenes*	93.25 (67.30-112.89)	0.68
		Salmonella	73.43 (61.07-81.45)	0.90
		STEC	51.31 (44.51-55.82)	0.92
Wood	MM	*L. monocytogenes*	45.46 (39.93-48.59)	0.94
		Salmonella	80.07 (55.10-97.76)	0.72
		STEC	58.90 (50.28-65.27)	0.87

Shiga toxin–producing *Escherichia coli,* STEC; SS, Stainless steel

Table 2-9: Inactivation kinetics of bacteria at 27°C based on based on pre-growth media (TSB, minimal media [MM]) and surface type following DRY inoculation.

Surface	Medium	Bacteria	D value (log CFU h^{-1}) $^{-1}$ (95% Cl)	R^2
Foam	TSB	*L. monocytogenes*	100.41 (84.56-119.01)	0.78
		Salmonella	134.82 (107.01-167.27)	0.60
		STEC	123.28 (88.79-157.63)	0.63
Rubber	TSB	*L. monocytogenes*	101.64 (81.75-126.48)	0.77
		Salmonella	111.25 (81.91-143.45)	0.67
		STEC	94.84 (62.53-122.39)	0.48
SS	TSB	*L. monocytogenes*	94.71 (77.39-107.28)	0.82
		Salmonella	122.61 (88.91-152.56)	0.62
		STEC	79.16 (69.15-86.61)	0.90
Wood	TSB	*L. monocytogenes*	109.50 (95.39-128.03)	0.71
		Salmonella	117.81 (91.97-148.56)	0.54
		STEC	129.21 (87.24-293.21)	0.48
Foam	MM	*L. monocytogenes*	87.62 (73.91-96.15)	0.92
		Salmonella	94.78 (72.37-110.92)	0.81
		STEC	103.89 (83.45-118.62)	0.86
Rubber	MM	*L. monocytogenes*	59.05 (52.17-62.46)	0.97
		Salmonella	61.23 (54.32-65.97)	0.96
		STEC	59.34 (55.83-62.10)	0.97
SS	MM	*L. monocytogenes*	66.03 (61.91-70.21)	0.97
		Salmonella	62.57 (58.18-66.25)	0.97
		STEC	59.29 (57.52-61.74)	0.99
Wood	MM	*L. monocytogenes*	66.34 (49.47-76.95)	0.83
		Salmonella	51.38 (47.09-53.65)	0.97
		STEC	51.25 (45.61-54.36)	0.97

Shiga toxin–producing *Escherichia coli,* STEC; SS, Stainless steel

Table 2-10: D values of Aichivirus A and Tulane virus based on surface cleanliness and temperature.

Virus	Cleanliness	Temperature (°C)	D-Value (95% CI)	R^2
AiV	Dirty	15	78.27 (55.07, 112.92)	0.24
		21	104.58 (65.84, 190.04)	0.29
		27	73.90 (54.43, 108.87)	0.43
	Clean	15	92.17 (65.52, 140.94)	0.16
		21	70.26 (53.31, 91.44)	0.23
		27	49.12 (36.97, 59.26)	0.38
TuV	Dirty	15	48.79 (41.22, 57.31)	0.42
		21	64.43 (49.77, 91.09)	0.49
		27	53.14 (3.29, 88.54)	0.33
	Clean	15	42.55 (33.97, 49.23)	0.49
		21	53.98 (43.44, 64.62)	0.34
		27	68.29 (50.01, 89.95)	0.28

AiV: Aichivirus A; TuV: Tulane virus; CI: confidence interval

Table 2-11. Degrees of freedom (Df), *F*-values, and *P*-values for the main effect and two-way interactions of independent variables (treatment, surface, medium, and time point) on the percentage reduction of *S. enterica* and STEC grown at 21°C.

Variable	Df	*S. enterica*		STEC	
		F-value	*P*-value	*F*-value	*P*-value
Treatment	6	51.95	< 0.001	13.95	< 0.001
Surface	2	5.54	0.004	5.86	0.003
Medium	1	21.29	< 0.001	0.89	0.346
Time	2	1.36	0.257	11.52	< 0.001
Treatment : Surface	12	10.02	**< 0.001**	4.31	**< 0.001**
Treatment : Medium	6	2.12	**0.05**	0.92	0.480
Treatment : Time	12	3.65	**< 0.001**	1.59	0.069
Surface : Medium	2	0.53	0.591	2.74	0.066
Surface : Time	4	6.83	**< 0.001**	2.39	**0.050**
Medium : Time	2	8.55	**< 0.001**	1.84	0.160

Objective 2 Figures

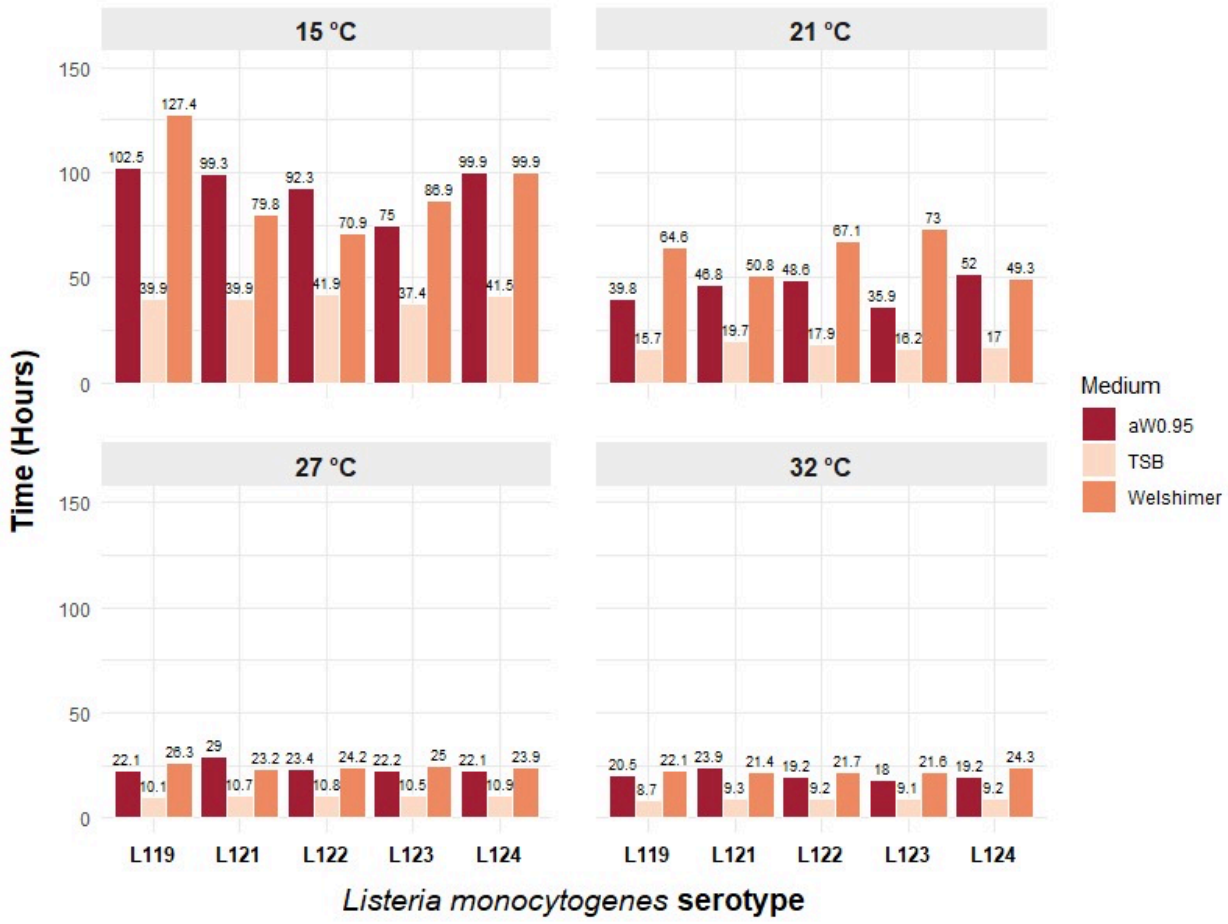
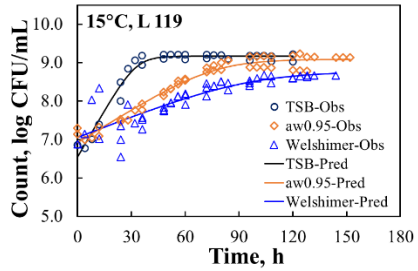
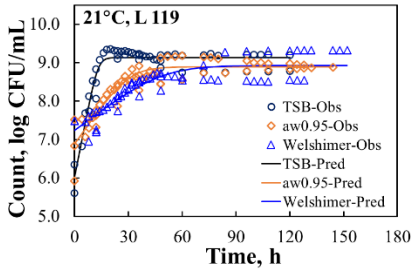
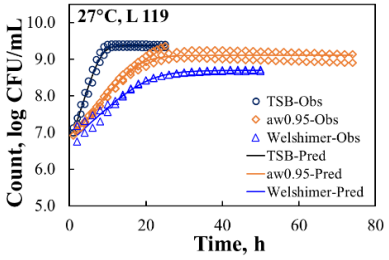


Figure 2-1. Time to reach stationary phase of *L. monocytogenes* (five serotypes) in TSB, a_w 0.95, and Welshimer media at various temperatures (15, 21, 27, and 32°C).


15°C, L 119
Count, log CFU/mL
Time, h
TSB-Obs
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred


21°C, L 119
Count, log CFU/mL
Time, h
TSB-Obs
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred


27°C, L 119
Count, log CFU/mL
Time, h
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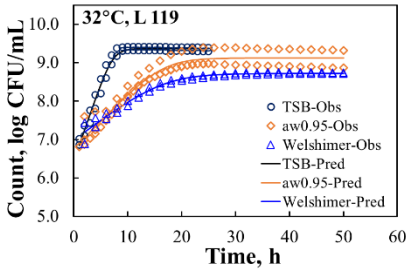
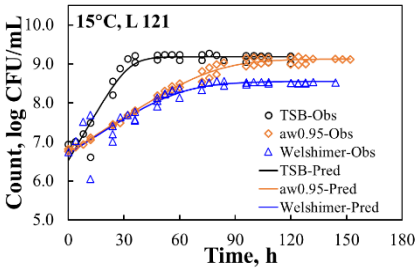
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred


32°C, L 119
Count, log CFU/mL
Time, h
TSB-Obs
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred


15°C, L 121
Count, log CFU/mL
Time, h
TSB-Obs
aw0.95-Obs
Welshimer-Obs
TSB-Pred
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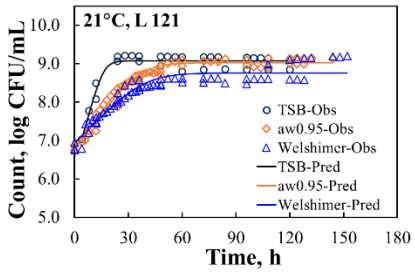
Welshimer-Pred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Count, log CFU/mL
Time, h
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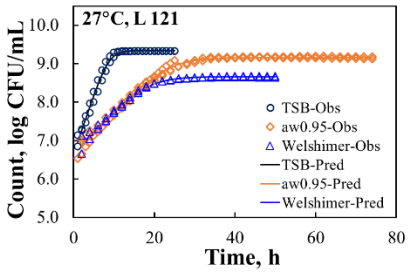
Welshimer-Pred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Count, log CFU/mL
Time, h
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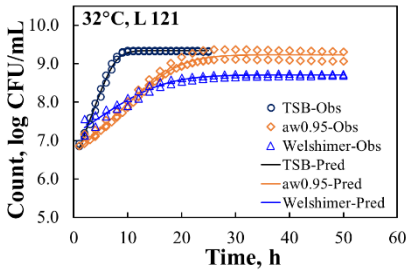
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred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Count, log CFU/mL
Time, h
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aw0.95-Obs
Welshimer-Obs
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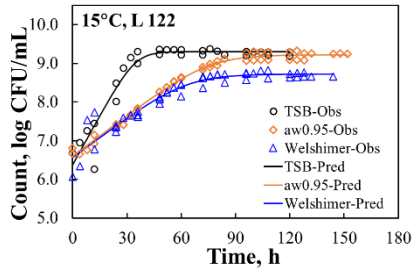
Welshimer-Pred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Count, log CFU/mL
Time, h
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Welshimer-Obs
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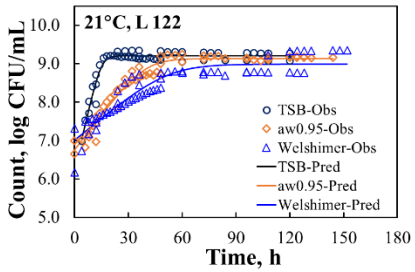
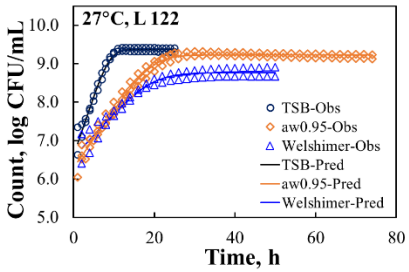
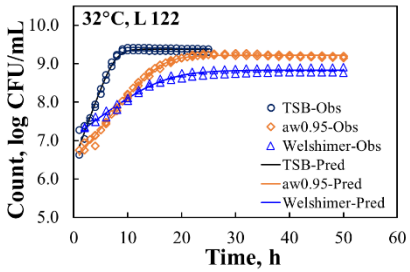
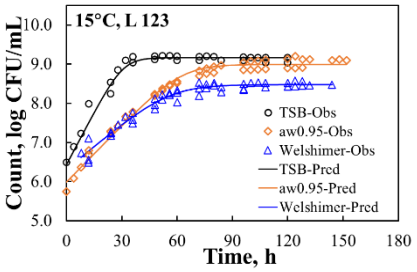
Welshimer-Pred


21°C, L 122
Count, log CFU/mL
Time, h
TSB-Obs
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred


27°C, L 122
Count, log CFU/mL
Time, h
TSB-Obs
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred


32°C, L 122
Count, log CFU/mL
Time, h
TSB-Obs
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred


15°C, L 123
Count, log CFU/mL
Time, h
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Welshimer-Obs
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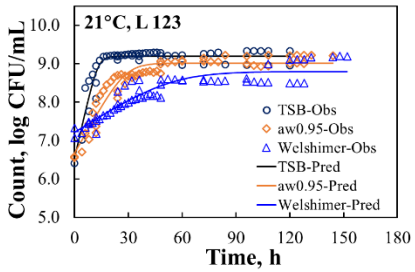
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Count, log CFU/mL
Time, h
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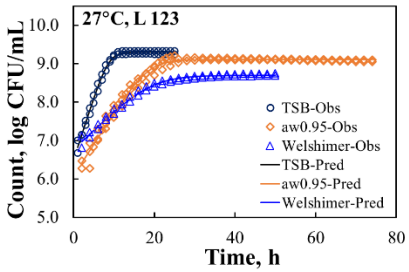
27°C, L 123
Count, log CFU/mL
Time, h
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aw0.95-Obs
Welshimer-Obs
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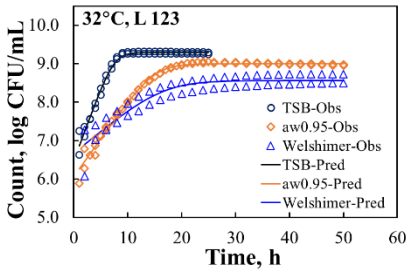
Welshimer-Pred


32°C, L 123
Count, log CFU/mL
Time, h
TSB-Obs
aw0.95-Obs
Welshimer-Obs
TSB-Pred
aw0.95-Pred
Welshimer-Pred

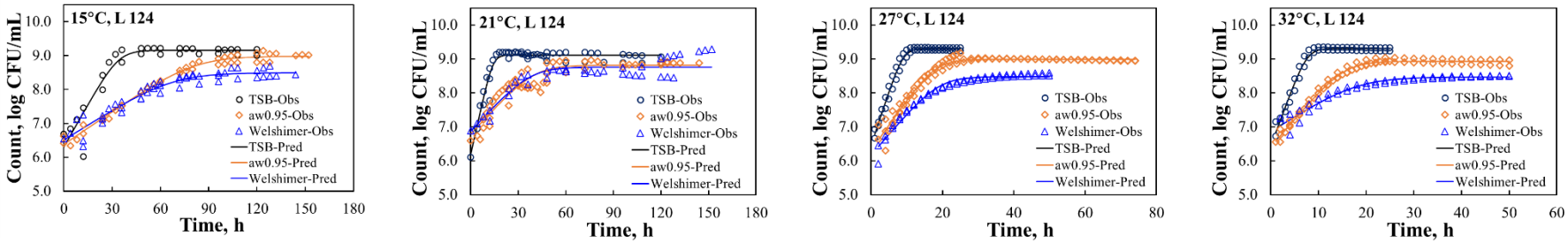


Figure 2-2. Growth curves of *L. monocytogenes* in TSB, a_w 0.95, and Welshimer media at various temperatures (15, 21, 27, and 32°C). Black circles, orange diamonds, and blue triangles indicate the observed *L. monocytogenes* populations (log CFU/mL) in TSB, a_w 0.95, and Welshimer media, respectively. Corresponding black, orange, and blue solid lines represent the predicted populations based on the no-lag phase model.

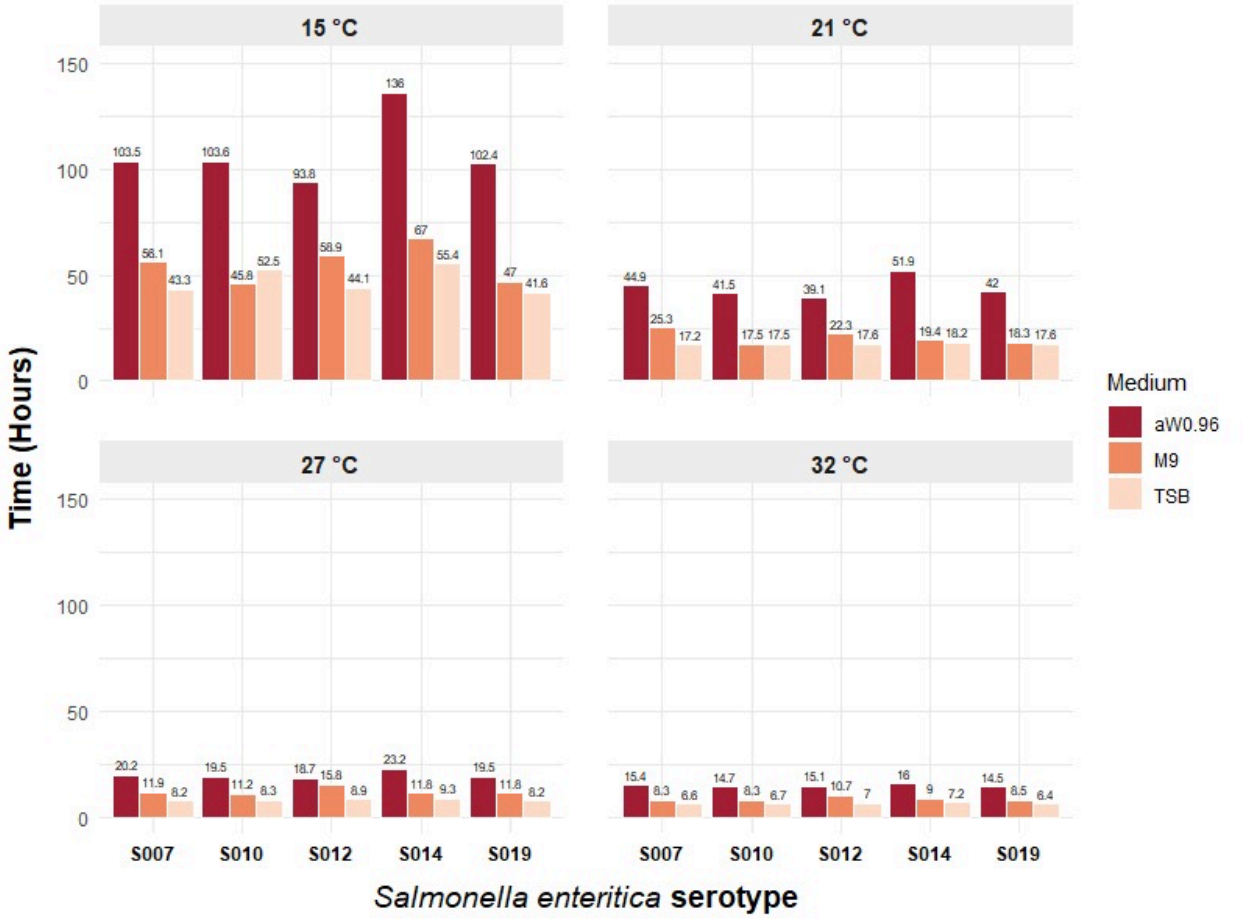
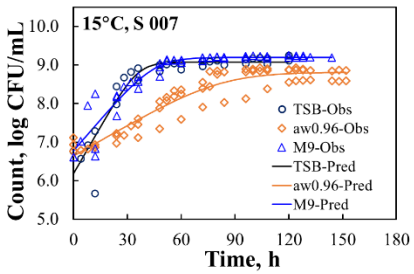


Figure 2-3. Time to reach stationary phase of *S. enterica* (five serotypes) in TSB, a_w 0.96, and M9 media at various temperatures (15, 21, 27, and 32°C)


15°C, S 007
Count, log CFU/mL
Time, h
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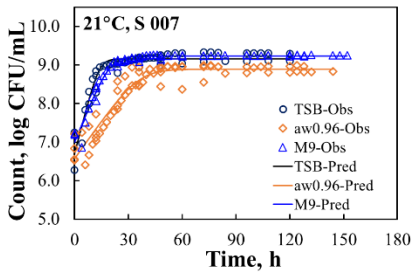
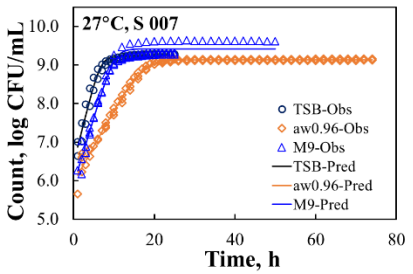
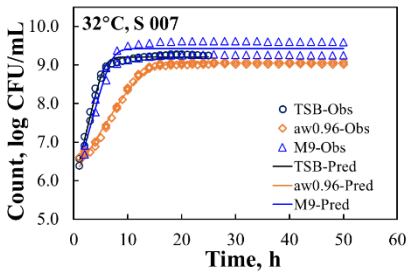
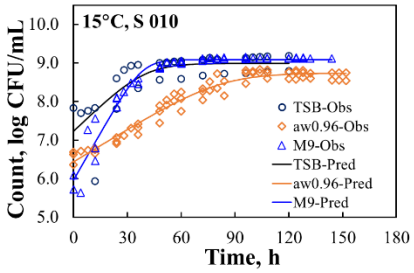
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


21°C, S 007
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


27°C, S 007
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


32°C, S 007
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


15°C, S 010
Count, log CFU/mL
Time, h
TSB-Obs
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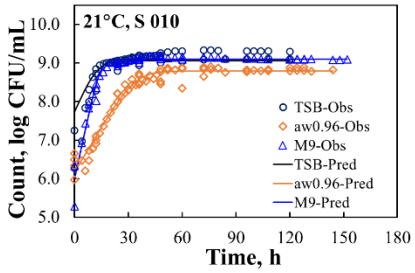
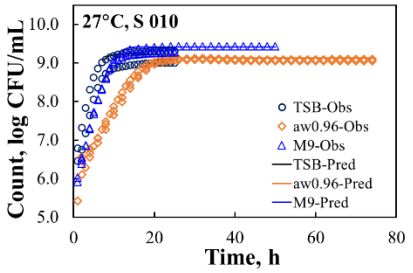
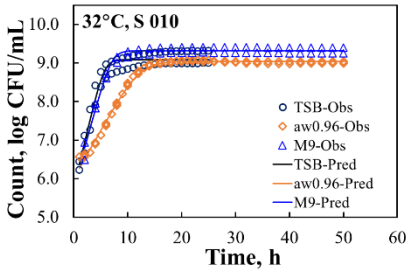
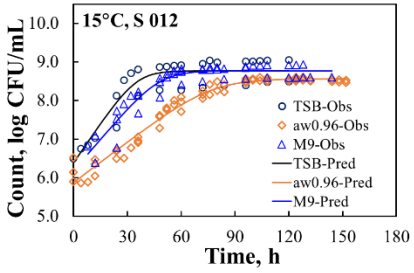
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


21°C, S 010
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


27°C, S 010
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


32°C, S 010
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


15°C, S 012
Count, log CFU/mL
Time, h
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M9-Obs
TSB-Pred
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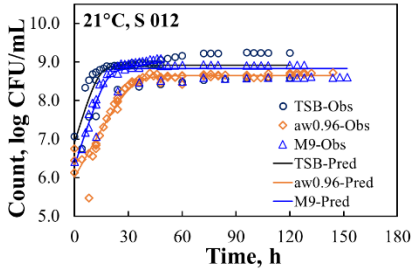
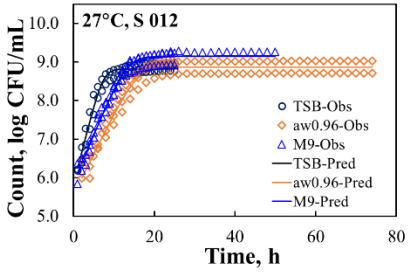
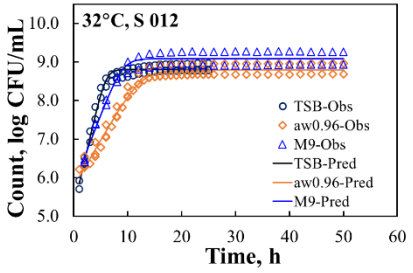
M9-Pred


21°C, S 012
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


27°C, S 012
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


32°C, S 012
Count, log CFU/mL
Time, h
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aw0.96-Obs
M9-Obs
TSB-Pred
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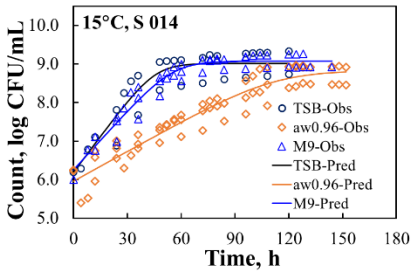
M9-Pred


15°C, S 014
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M9-Obs
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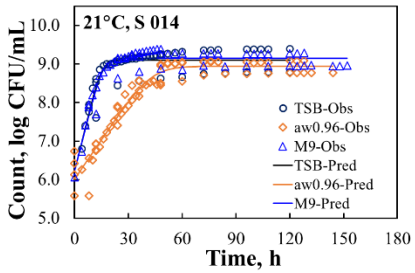
21°C, S 014
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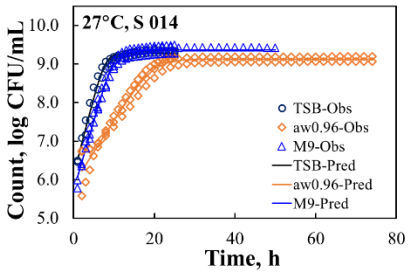
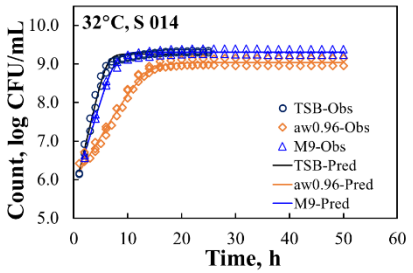
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


27°C, S 014
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred


32°C, S 014
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred

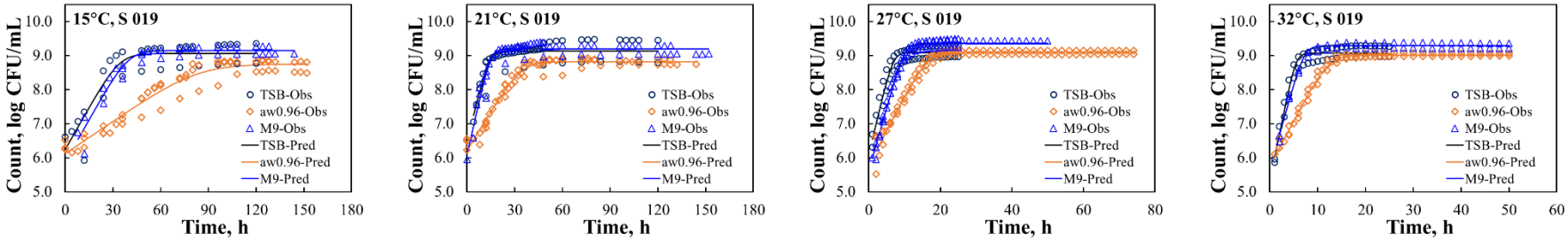


Figure 2-4. Growth curves of *S. enterica* in TSB, a_w 0.96, and M9 media at various temperatures (15, 21, 27, and 32°C). Black circles, orange diamonds, and blue triangles indicate the observed *S. enterica* populations (log CFU/mL) in TSB, a_w 0.96, and M9 media, respectively. Corresponding black, orange, and blue solid lines represent the predicted populations based on the no-lag phase model.

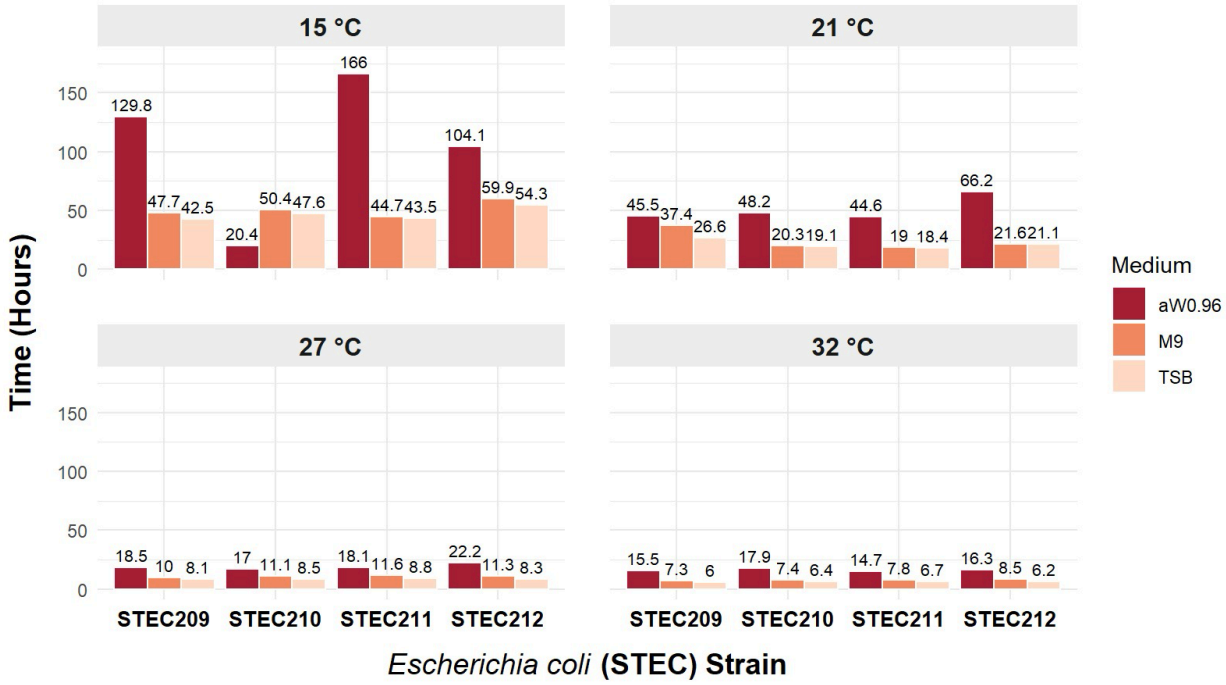


Figure 2-5. Time to reach stationary phase of STEC (four strains) in TSB, a_w 0.96, and M9 media at various temperatures (15, 21, 27, and 32°C).

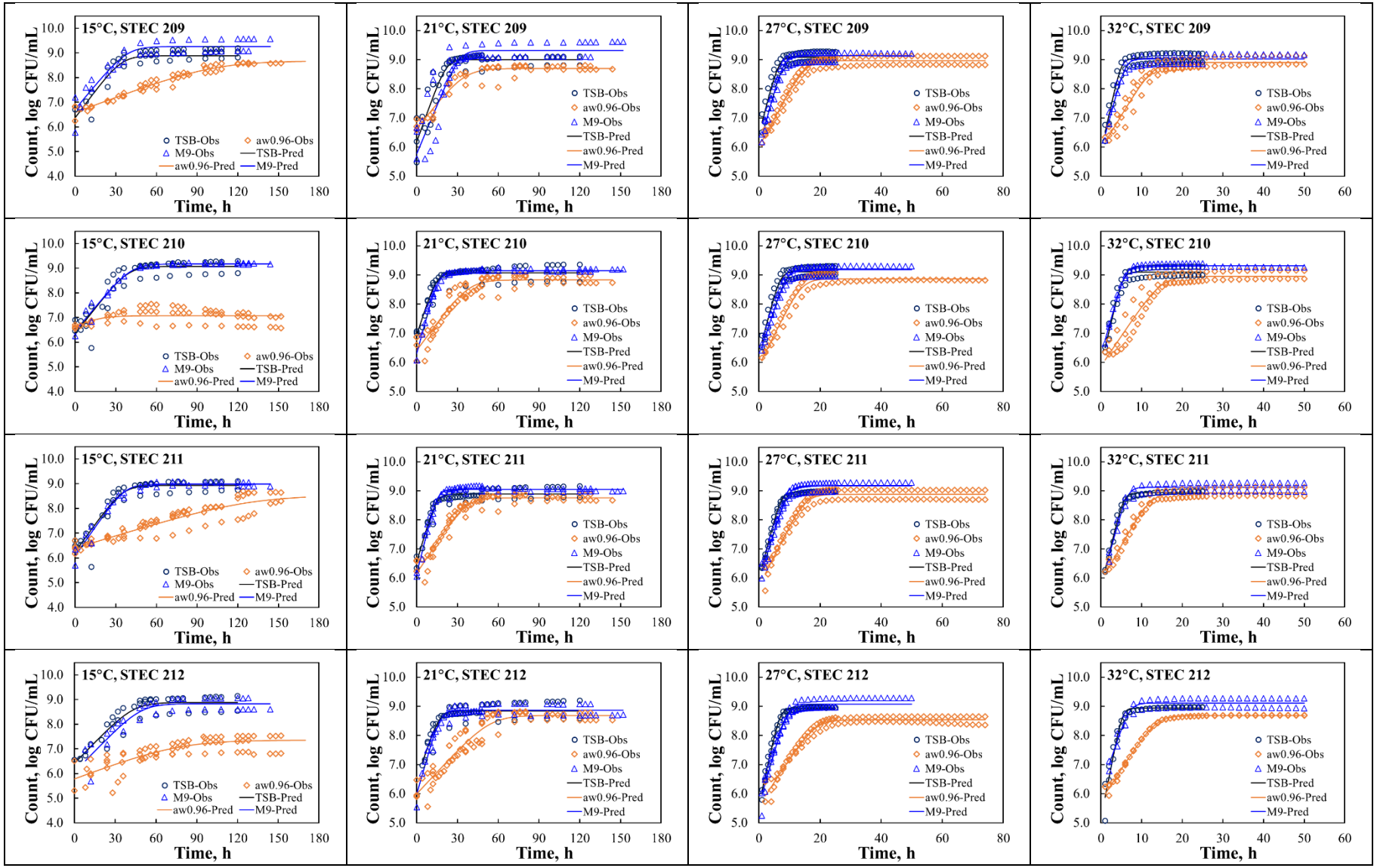

15°C, STEC 209
21°C, STEC 209
27°C, STEC 209
32°C, STEC 209
15°C, STEC 210
21°C, STEC 210
27°C, STEC 210
32°C, STEC 210
15°C, STEC 211
21°C, STEC 211
27°C, STEC 211
32°C, STEC 211
15°C, STEC 212
21°C, STEC 212
27°C, STEC 212
32°C, STEC 212
Count, log CFU/mL
Time, h
TSB-Obs
aw0.96-Obs
M9-Obs
TSB-Pred
aw0.96-Pred
M9-Pred

Figure 2-6. Growth curves of STEC in TSB, a_w 0.96, and M9 media at various temperatures (15, 21, 27, and 32°C). Black circles, orange diamonds, and blue triangles indicate the observed STEC populations (log CFU/mL) in TSB, a_w 0.96, and M9 media, respectively. Corresponding black, orange, and blue solid lines represent the predicted populations based on the no-lag phase model.

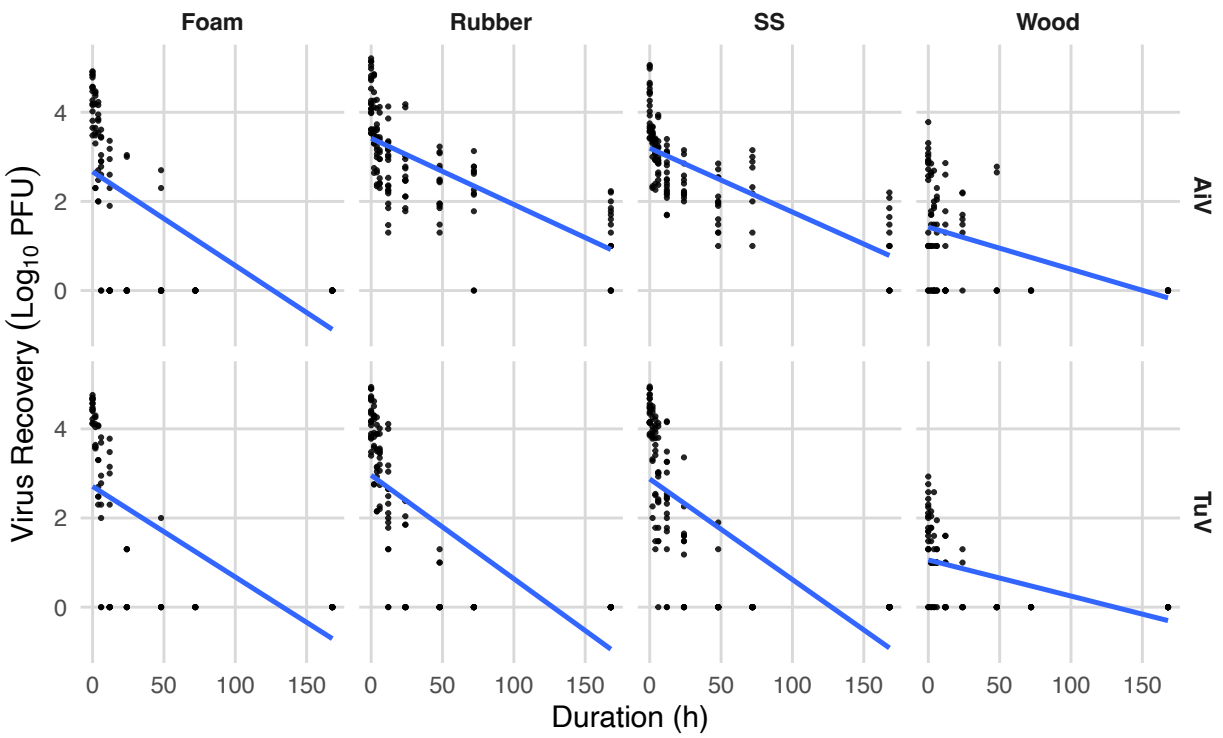


Figure 2-7. Persistence of Aichivirus A (AiV) and Tulane virus (TuV) on foam, rubber, stainless steel (SS), and wood surfaces. Viral recovery (log_{10} PFU) was quantified over specific durations (h), and linear regression models (blue lines) were fitted to determine inactivation rates and corresponding D-values.

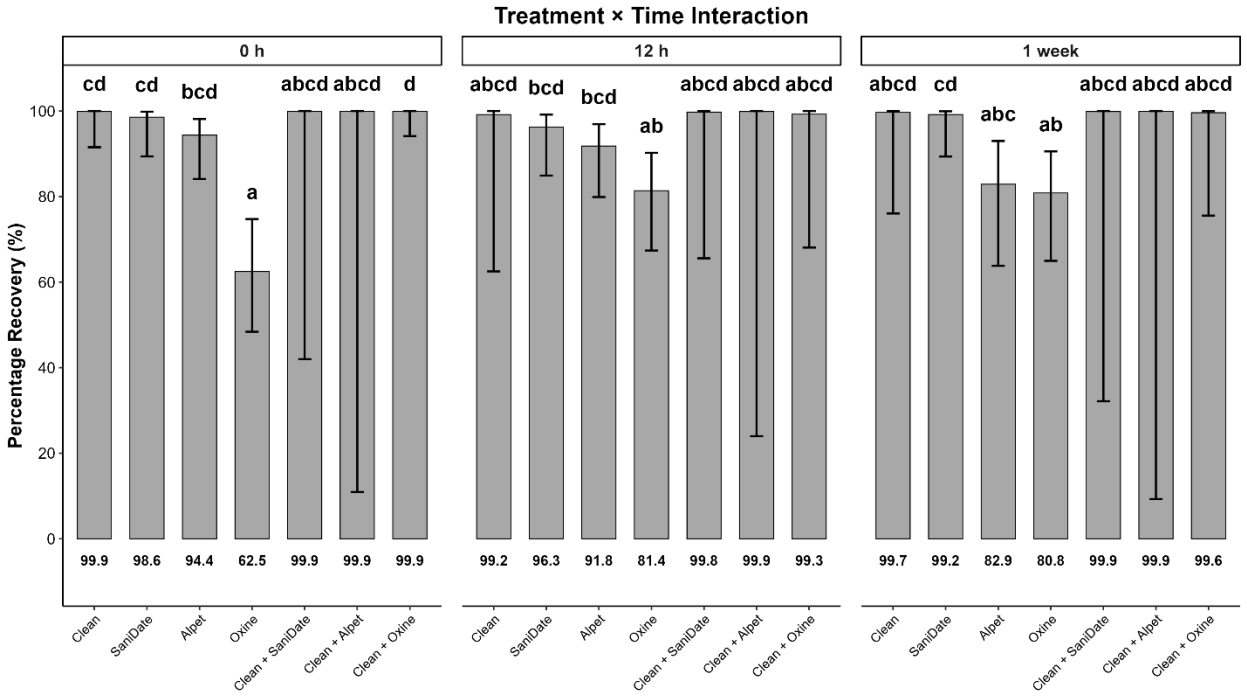


Figure 2-8. Estimated percentage reduction (%) derived from a generalized linear model with quasibinomial errors for cleaning and sanitizing *S. enterica* grown at 21°C across different C&S treatments, FCS, pre-growth media, and time points (n = 504). Data are shown for seven treatments evaluated at three time points. Statistical differences among treatments are indicated by different compact letters above the error bars ($P \leq 0.05$).

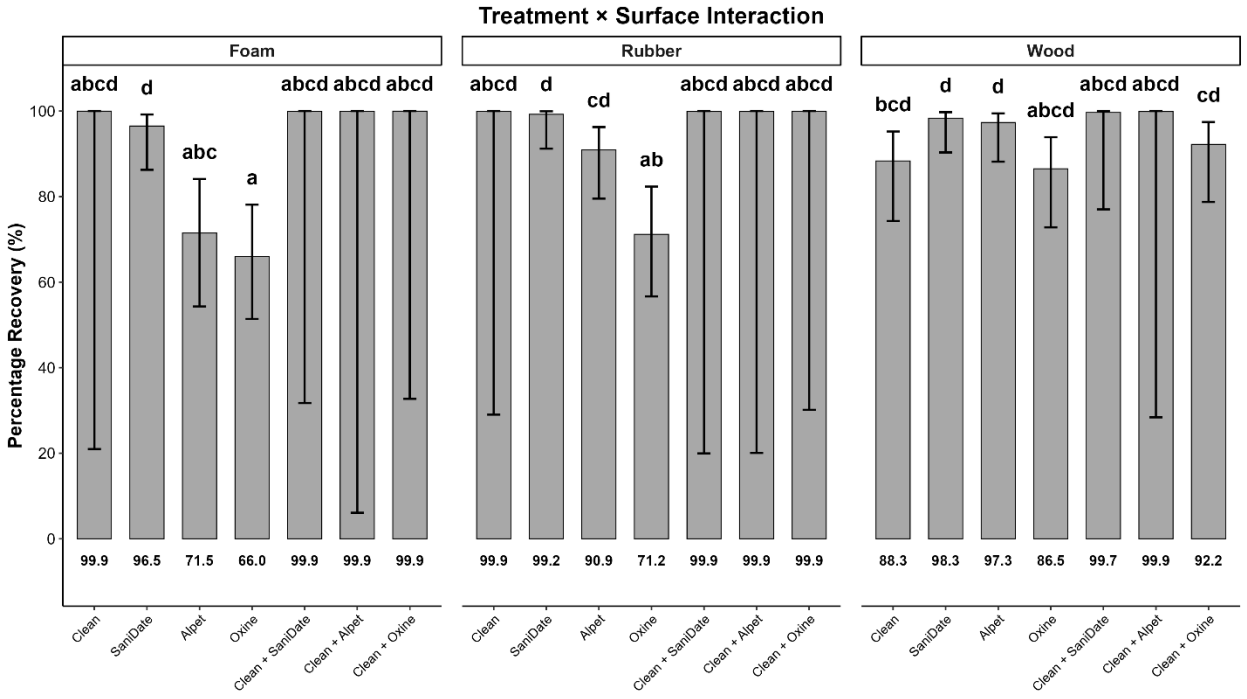


Figure 2-9. Estimated percentage reduction (%) derived from a generalized linear model with quasibinomial errors for cleaning and sanitizing *S. enterica* grown at 21°C across different C&S treatments, FCS, pre-growth media, and time points (n = 504). Data are shown for seven treatments evaluated on three FCS. Statistical differences among treatments are indicated by different compact letters above the error bars ($P \leq 0.05$).

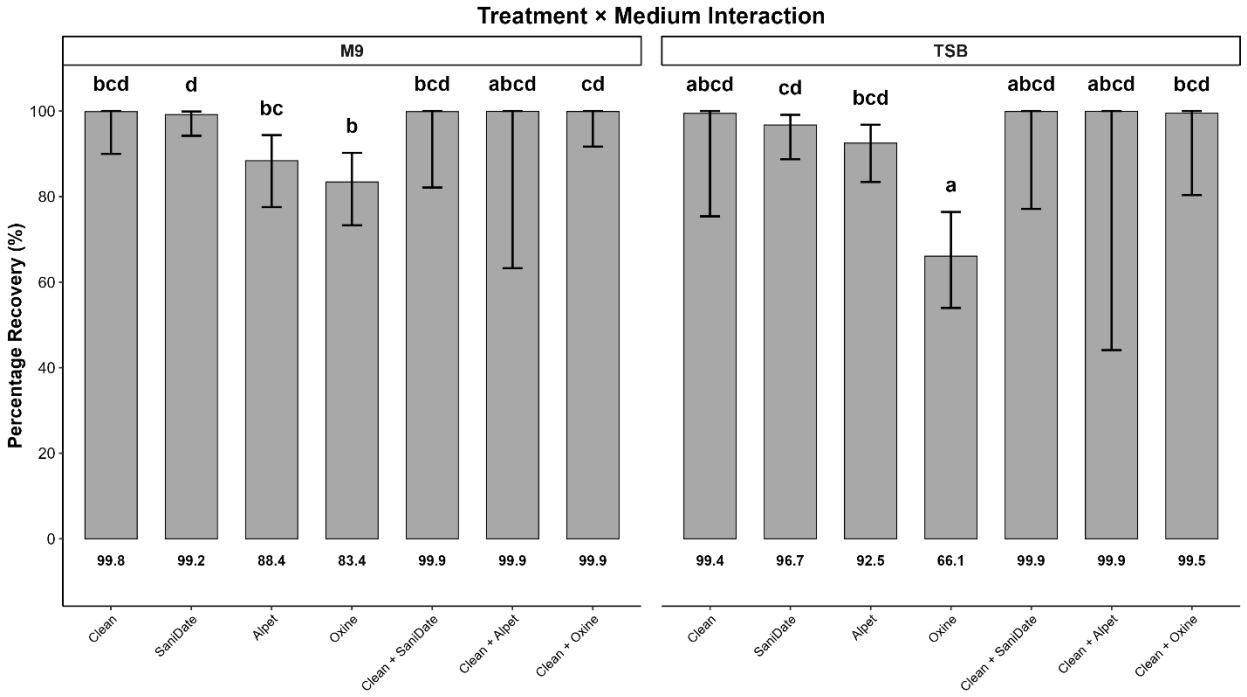


Figure 2-10. Estimated percentage reduction (%) derived from a generalized linear model with quasibinomial errors for cleaning and sanitizing *S. enterica* grown at 21°C across different C&S treatments, FCS, pre-growth media, and time points (n = 504). Data are shown for seven treatments evaluated at two pre-growth media. Statistical differences among treatments are indicated by different compact letters above the error bars ($P \leq 0.05$).

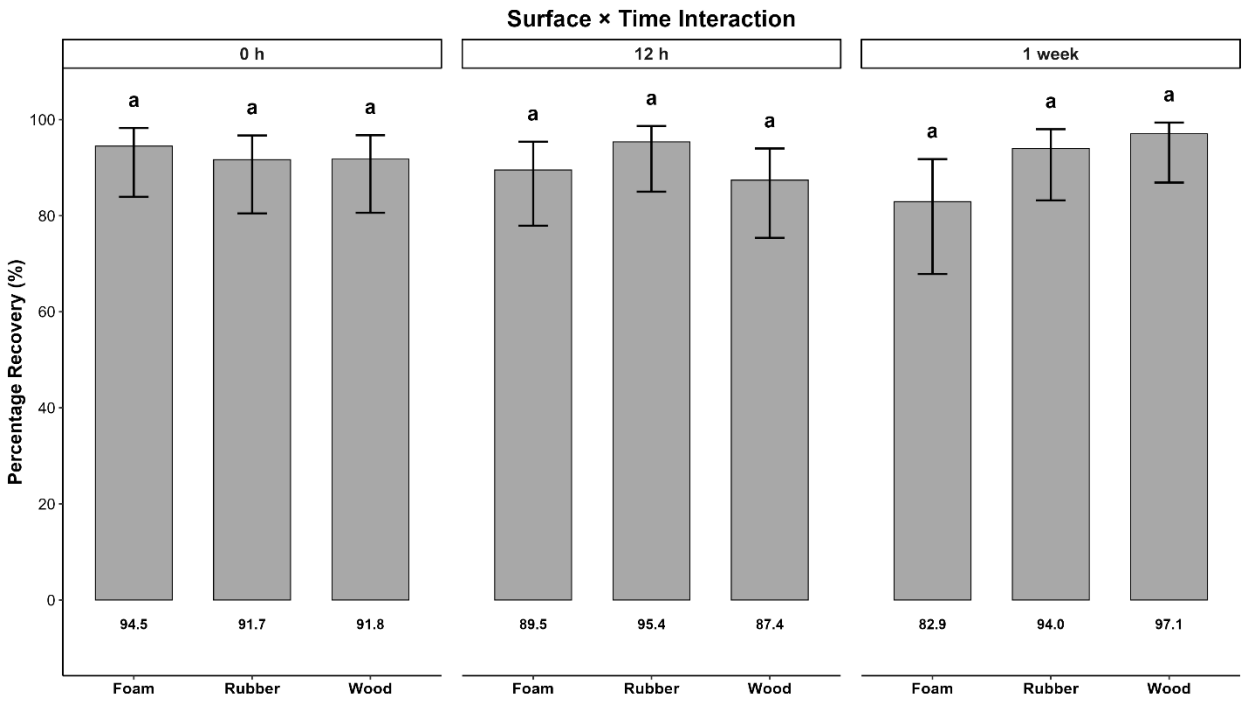


Figure 2-11. Estimated percentage reduction (%) derived from a generalized linear model with quasibinomial errors for cleaning and sanitizing *S. enterica* grown at 21°C across different C&S treatments, FCS, pre-growth media, and time points (n = 504). Data are shown for three FCS evaluated at three time points. Statistical differences among FCS are indicated by different compact letters above the error bars ($P \leq 0.05$).

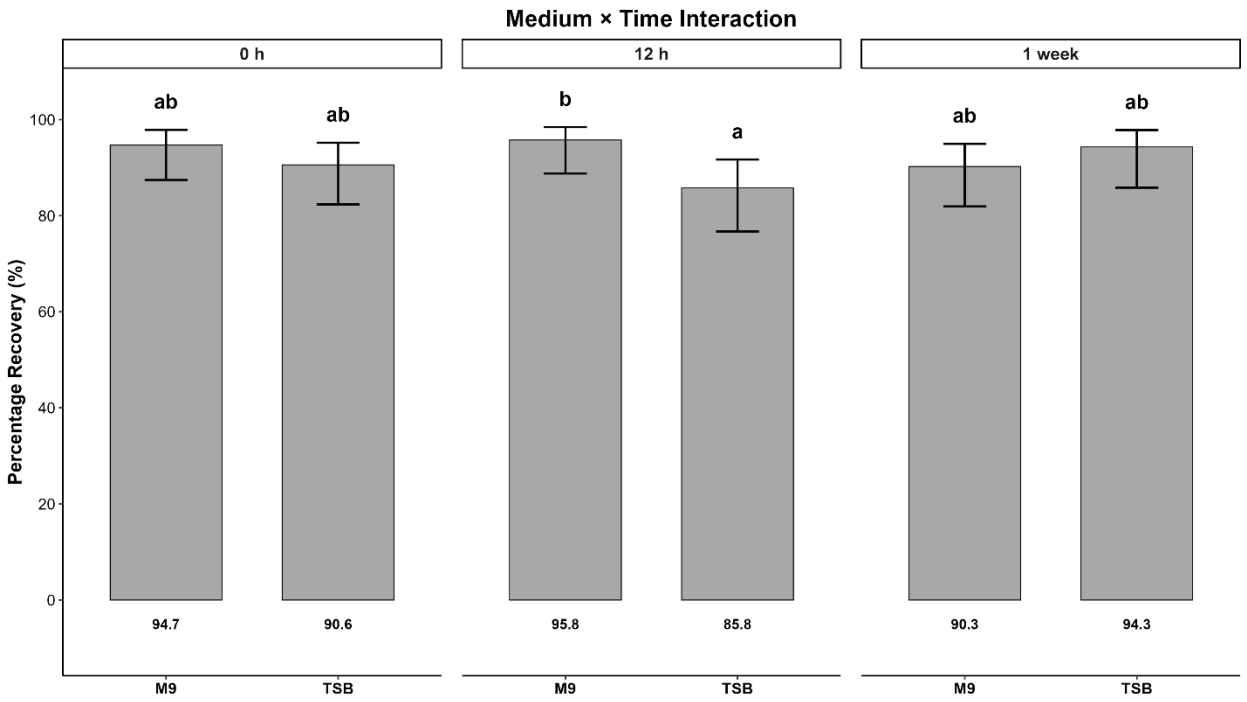


Figure 2-12. Estimated percentage reduction (%) derived from a generalized linear model with quasibinomial errors for cleaning and sanitizing *S. enterica* grown at 21°C across different C&S treatments, FCS, pre-growth media, and time points (n = 504). Data are shown for two pre-growth media evaluated at three time points. Statistical differences between media are indicated by different compact letters above the error bars ($P \leq 0.05$).

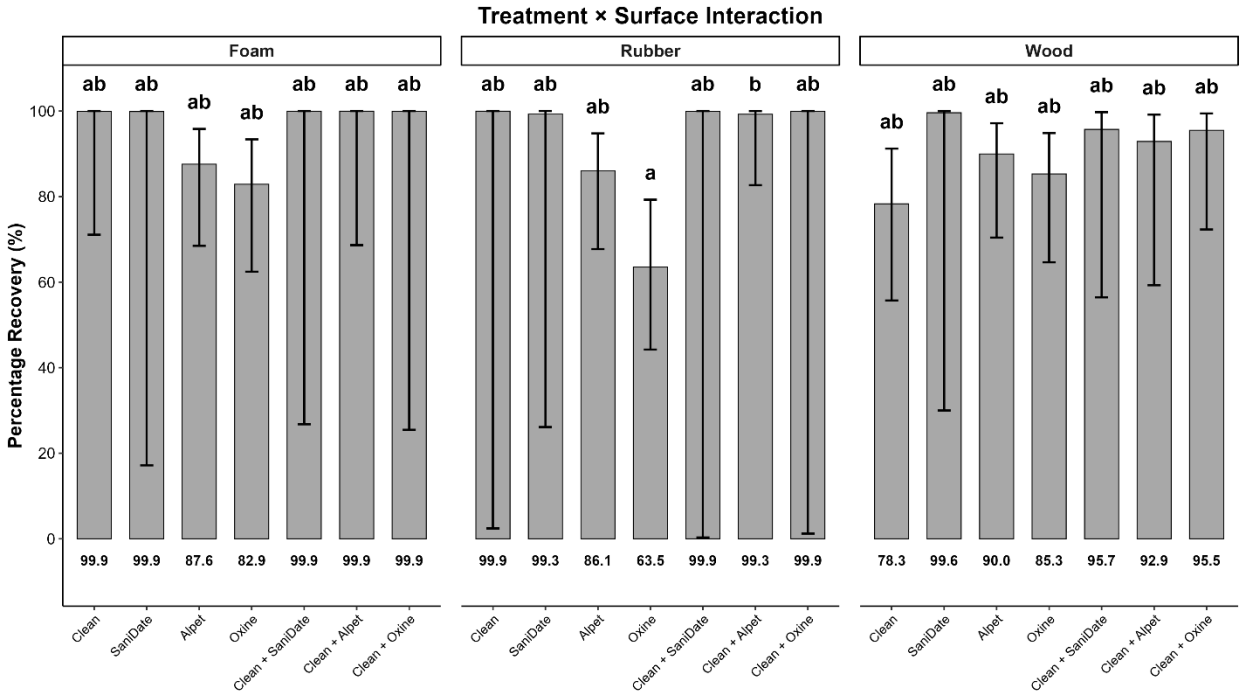


Figure 2-13. Estimated percentage reduction (%) derived from a generalized linear model with quasibinomial errors for cleaning and sanitizing STEC grown at 21°C across different C&S treatments, FCS, pre-growth media, and time points (n = 504). Data are shown for seven treatments evaluated on three FCS. Statistical differences among treatments are indicated by different compact letters above the error bars ($P \leq 0.05$).

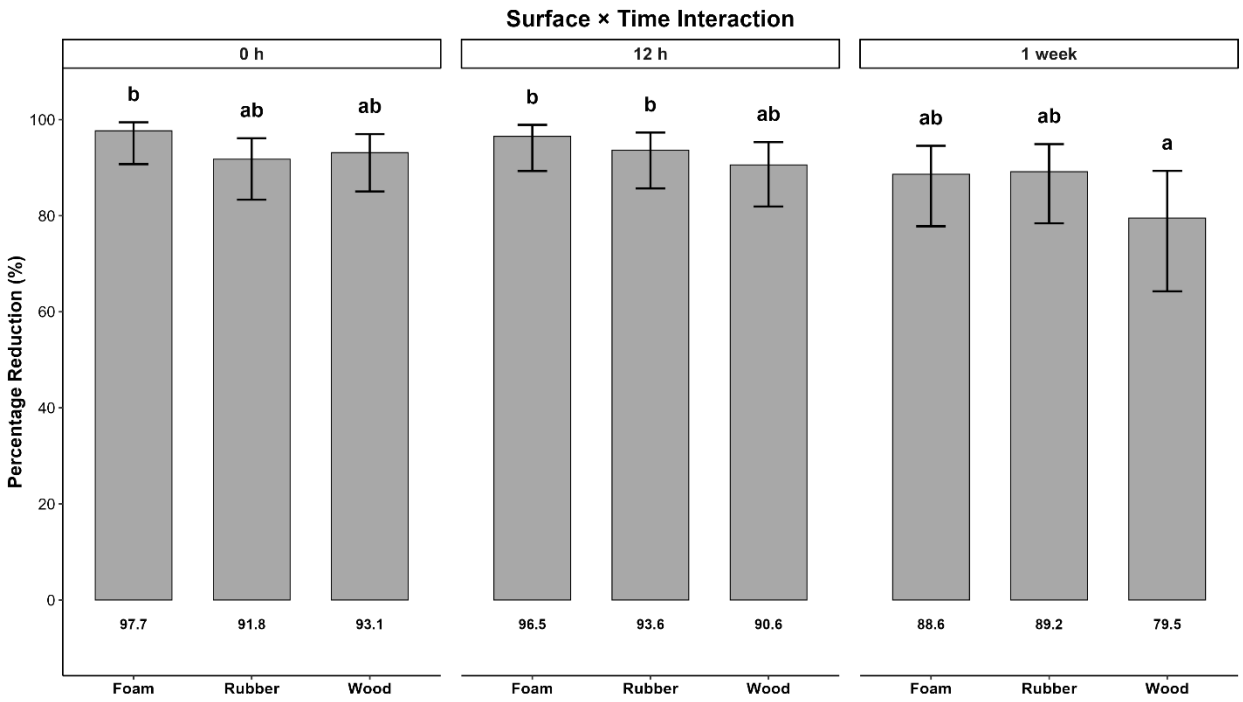


Figure 2-14. Estimated percentage reduction (%) derived from a generalized linear model with quasibinomial errors for cleaning and sanitizing STEC grown at 21°C across different C&S treatments, FCS, pre-growth media, and time points (n = 504). Data are shown for three FCS evaluated at three time points. Statistical differences among FCS are indicated by different compact letters above the error bars ($P \leq 0.05$).

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