CENTER for PRODUCE SAFETY

CPS 2018 RFP
FINAL PROJECT REPORT

Project Title
Modeling tools for design of science-based *Listeria* environmental monitoring programs and corrective action strategies

Project Period
January 1, 2019 – December 31, 2019 (extended to February 28, 2020)

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Objectives

1. *Develop a series of computer models, representing different produce processing facilities, to be validated with industry data collected through an on-going complementary USDA Specialty Crop Research Initiative (SCRI)-funded project at Cornell, as well as historical industry data where applicable.*
2. *Evaluate differential corrective actions in response to Listeria spp. and environmental monitoring plans in the modeled fresh produce processing facilities.*

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FINAL REPORT

Abstract

While contamination of fresh produce with *Listeria monocytogenes* (Lm) may occur throughout the field-to-consumer chain, contamination events are often traced back to sources in the environment and equipment of processing facilities. Hence environmental monitoring (EM) programs with appropriate corrective actions have become a key tool to control Lm and reduce the risk of finished product contamination. Along with implementing science-based *Listeria* EM programs, development of improved approaches to appropriate follow-up actions in response to *Listeria* detection is essential for the produce industry. In particular, industry needs appropriate tools to optimize the response to *Listeria* presence/absence testing across various product handling areas, as these responses are specifically taken to control Lm contamination of processing plant environments and prevent finished product contamination. However, practically speaking, science-based *Listeria* control programs cannot be designed solely based on testing data. We therefore used a modeling and computational approach where we leveraged our existing agent-based model (ABM) prototype for food processing facilities to develop four new models, each tailored to one of four produce operations: two fresh-cut facilities and two packinghouses. These models allow us to simulate the *Listeria* dynamics in these produce operation environments. Inputs such as location of employees, water and traffic levels, equipment cleanability, and product flow are all incorporated into these models. This data allows us to evaluate whether site-specific attributes may justify differential EM strategies for *Listeria* spp. and responses taken within produce processing facilities. Each individual ABM is validated for the specific facility using longitudinal sampling data, collected through the current study and our ongoing complementary project funded by the USDA Specialty Crop Research Initiative (SCRI) (PI, Dr. Martin Wiedmann). We have used these four models to generate data to support site-specific and risk-differentiated practices for the produce industry. For each of the participating facilities, these models provide a customized tool for evaluating risk of contamination and to aid decision-making about food safety in the facility, while the simulation results provide the broader produce industry with generalizable learnings regarding their *Listeria* control programs. The results of this study fundamentally reverse the “one-size-fits-all” mentality towards *Listeria* control and contamination prevention for a fresh produce industry that is characterized by varied and changing practices, capabilities and requirements.

Background

This project addresses the fresh produce industry’s need for scientific support of best practices in response to *Listeria* detection within complex environments of fresh produce processing facilities. While contamination of ready-to-eat food products with *Listeria* monocytogenes (Lm) may occur throughout the field-to-consumer chain, contamination events are often traced back to sources in processing facilities. For the fresh produce industry, recent outbreaks and recalls of Lm have been traced back to contamination in the environment and equipment of processing facilities (Ferreira et al., 2014; CDC, 2017). The link between contaminated environments and finished product contamination highlights the importance of finding and eliminating *Listeria* sources in fresh produce processing facilities, as Lm may be transferred to products during operations. Hence environmental monitoring (EM) programs with appropriate corrective actions have become a key tool to control Lm and reduce the risk of finished product contamination.

This is emphasized in the more specific government guidance documents, such as FDA's recent FSMA Preventive Controls for Human Food Rule and Draft *Listeria* Guidance, as well as in the more prescriptive supply chain and buyer EM requirements that are increasingly informing industry practices.

While Lm is the only human pathogen in the genus *Listeria*, testing for the genus *Listeria* is most often utilized in routine EM programs rather than specifically detecting Lm. As conditions that support *Listeria* spp. presence are indicative of conditions that may also sustain Lm, this practice is thought to provide a preventive approach to finding and eliminating contamination sources. Numerous reviews and guidance documents present details for implementation of *Listeria* EM programs (Suslow and Harris, 2000; Tompkin, 2002; United Fresh, 2013) and "seek and destroy" methodology (Malley, Butts and Wiedmann, 2015) in different sectors of the food industry, but it is recognized that even within each industry sector, facilities will have unique *Listeria* risks. Development of improved approaches to implement science-based *Listeria* EM programs, especially appropriate follow-up actions in response to *Listeria* detection, is essential for the produce industry. More precisely, industry needs a foundation for taking the optimal action in response to *Listeria* presence/absence testing across various product handling areas, as these responses are specifically taken to control Lm contamination of processing plant environments and prevent finished product contamination. This research is particularly timely as a systematic (scoping) review, conducted by the project team, determined that in the 197 identified research studies and guidance documents on *Listeria* EM and control, fresh produce was the focus in only 12, compared with 66 on meat, 52 on fish and seafood, and 51 on dairy (Zoellner et al., 2018), confirming the need for more data upon which industry may establish appropriate response and monitoring strategies.

Science-based *Listeria* control programs cannot be designed solely based on testing data. For example, it is not feasible to test different corrective action and sampling approaches in each individual facility (or even the number of facilities required to have representation of the industry's diversity) to determine which sampling approaches and corrective actions are most effective. Simulation and risk assessment models, on the other hand, are very useful for decision-making and have been demonstrated for management of Lm control strategies in food processing environments (Akingbade et al., 2013; Tenenhaus-Aziza et al., 2014), especially where experimental approaches would not be feasible or ethical and where empirical data are difficult to obtain (e.g., due to sensitive nature of compliance). There is increasing support for providing locally relevant and evidence-based solutions to fresh produce growers for in-field decision-making (Monaghan et al., 2017); we posit that a similar approach should also be applied to EM programs and associated corrective actions in fresh produce processing facilities, as these facilities differ considerably in design, processes, infrastructure, etc. We thus developed computer models for four different produce processing facilities in order to evaluate, using simulations, scenarios for (i) corrective actions in response to *Listeria* detection (in response to CPS Research Priority 1.1.4) as well as (ii) routine EM programs (in response to CPS Research Priority 1.1.1).

We simulated *Listeria* spp. contamination routes and detection on surfaces during facility operations by adapting our existing agent-based model (ABM), developed through funding from the American Frozen Food Institute to evaluate and optimize EM in frozen food processing plants (Zoellner et al., 2019). An ABM, also called an individual based model, is comprised of individuals or agents that are unique and autonomous in their characteristics and interactions with each other and their environment (Railsback and Grimm, 2012). This method allows for simulation of behaviors of systems with complex and dynamic conditions that could not be described by analytically tractable compartmental mathematical models, albeit at a cost of high

programing and computational requirements. Nevertheless, the promise of ABMs in utilizing vast data types and sources for predicting pathogen dynamics to inform policy and individual decision-making has been previously articulated in pre-harvest food safety (Bergholz and Wiedmann, 2016) and validated in agriculture and public health settings (Martinez-Lopez et al., 2011; Rubin et al., 2013; Codella et al., 2015). Importantly, ABMs have specifically been used to support design and implementation of infection control and surveillance programs in hospital environments (Rubin et al., 2013; Codella et al., 2015). While previous models have focused on *Listeria* interaction with food product matrices, our existing ABM method involves discretization of the complex production facility and its characteristics to focus on *Listeria* interaction with the equipment and environment. In the current study, the new models were further extended to include a range of cleaning/sanitation and follow-up procedures, and were parameterized and validated for application to fresh produce processing facilities based on extensive sampling data collected through a complementary SCRI-funded project at Cornell (PI Dr. Wiedmann). The validated models provide a tool for rapid simulation-based experimentation of different corrective actions and EM plans, otherwise unfeasible in field studies.

Research Methods and Results

Facility selection and historical data. Four models were created for this study: two representing fresh-cut produce facilities and two representing produce packinghouses. The two fresh-cut operations were year-round while the two packinghouses were seasonal. The operations were selected from participants in a previous study by our group where one year's worth of environmental sampling data was available (Sullivan et al., in preparation). The models for each operation represent either one or two main rooms where product is washed, dried, cut (in the case of the fresh-cut operations), and packed. As only the main production rooms were modeled, only zones 1–3 were represented in the developed ABMs for those facilities.

Historical data for all four operations was collected as part of our complementary SCRI-funded project at Cornell (Sullivan et al., in preparation). Briefly, sites in zones 2 and 3 were sampled using individually packaged sponges hydrated with 10 mL DE neutralizing buffer (3M, St. Paul, MN), with two gloves per sponge. The sponge samples were collected at least 3–4 hours into production. Sponge samples were analyzed to determine presence of *Listeria* spp. using the FDA "BAM method" as detailed in Chapter 10 of the Bacteriological Analytic Manual (FDA, 2017): *Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods*.

Modeling approach and implementation. All four models have multiple components that are specific to the given facility and were developed based on in-person observations and information received from facility personnel. The floorplan of each facility was imported into the respective model from a csv file that contained a grid of squares (3,854 squares for Facility A; 8,256 for Facility B; 29,008 for Facility C; and 47,300 for Facility D) labeled with an integer serving as a code that indicates whether the square represents a wall, door, or floor in the facility blueprint (model interface shown in **Figure 1**). Each grid square represents one scaled square of the floor area in the facility (i.e., patch). The scale of the patch was dependent on the size of the operation. For Facility A, each patch represented a 30x30cm area. For Facility B, each patch represented a 50x50cm area. For Facilities C and D, each patch represented a 25x25cm area. An identical grid of ceiling patches was also included and was located in a parallel plane at the height of the modeled rooms. The same system was used to create and import maps that indicate the traffic and water levels in each patch of the operation throughout

the course of a day, with the map that was being implemented (i.e., high, medium, low, none) dependent on which shift was occurring or if there was a shift change. The equipment and employees (agents) were also imported via a csv file, which contained information about the agent's name, x and y coordinates in the model's two-dimensional plane, zone, height, surface area, and whether or not the agent is considered cleanable during routine sanitation. The four models had between 172–649 agents (**Table 1**). The height of each agent represented the height within the facility itself. Therefore, if an employee or piece of equipment was located on a mezzanine, then the height would include the height of the mezzanine. Mezzanines were represented as additional patches that were placed between the floor and ceiling patches at the height of the mezzanine. Therefore, in the 3D space, there could be (from low to high): a floor patch, an agent, a mezzanine patch, another agent, and a ceiling patch. Connections between agents were imported into the model using two files, with one containing a list of pairs of agents that have a bi-directional relationship (i.e., *Listeria* can transfer in either direction) and the other containing a list of pairs of agents that have a uni-directional relationship (i.e., *Listeria* can only transfer from the first agent to the second, without moving back). Finally, a file containing the list of hourly "events" (i.e., empty, pre-production, production, clean) was imported into the model, which contains the event that occurs each hour of each day in a week in a 24x7 matrix.

The model algorithm (code) was written in the open source program NetLogo 6.0 (Wilensky, 1999). The code was adapted for this study from our previously developed agent-based model of a food facility (Zoellner et al., 2019) by tailoring the code to the specifics of each of four facilities. Briefly, the set-up of the *in silico* facility environment involves the importing of the agents and agent attributes, links, events, and maps. To initialize the model world, the initial environment is set to a 0% *Listeria* prevalence among agents and patches. The start of the 2-week simulation begins on Sunday at 12:01 am. Throughout the week, *Listeria* can be introduced into the environment via three ways: (i) contaminated raw product, (ii) zone 4 cross-contamination, and (iii) random noise. The number of employees that appear in the room are pre-set and based on the time of day (and therefore the shift). *Listeria* can spread throughout the model in four ways: (i) patch-to-patch spread, (ii) agent-to-agent spread through the directed or undirected links, (iii) zone-to-zone spread from neighboring agents, and (iv) zone-to-patch spread. Patch-to-patch spread only occurs if water or traffic levels in the area of patches are greater than 0 at a given hour of the day. Condensation is also included in the model with the possibility that *Listeria* can be transferred from the mezzanine underside or ceiling to the agents or patches directly below. *Listeria* growth and survival is dependent on the water level, as well as if the event is "clean" (i.e., routine cleaning and sanitation), which results in a reduction in the concentration of any *Listeria* present in accordance with the sanitation log reduction parameter.

Input parameters. The input parameters for the models were determined using (i) in-person observations, (ii) published literature, or (iii) an expert elicitation (**Table 2**). In rare instances, select parameters were assumed. The expert elicitation was used for the estimation of six of the input parameters. The survey used for the expert elicitation was adapted from a survey used in our previous study (Zoellner et al., 2019), with updates made to include scenarios specific to fresh-cut produce. The survey was sent to and completed by six people who had expertise on *Listeria* in food facilities: four experts that work in academia and two experts that work in the produce industry.

Validation. Several methods were used to validate the models, including syntax checking, visual testing, print statements, spot tests with agent monitors, code reviews, parameter validity, different seed generators, and predictive validation. The predictive validation of each of the models was done using historical data collected during a previous study (Sullivan et al., in

preparation) and data collected as part of the current project. Code was written into the models that allowed the simulation of sample collection during production and on days that were consistent with the sample collection of the historical data. The *Listeria* prevalence of the historical samples were compared against the average prevalence of the corresponding *in silico* sampling sites from 10,000 iterations of the model's 2-week simulation. As zone 1 sites were not sampled in historical data, they could not be validated using this method. Models for the two fresh-cut facilities have been successfully validated (**Tables 3–6**), while validation is in the process for models for the two packinghouses.

Simulation and sensitivity analysis. To assess the *Listeria* dynamics within the modeled facilities, simulation analyses are performed for the validated models. Multiple simulation iterations (i.e., trials) were completed, generating simulated data that allowed us to characterize the *Listeria* prevalence on each zone during four phases of production: pre-op, beginning of shift, mid-shift, and post-shift. Preliminary results for the simulation analyses of the fresh-cut facility models revealed that, regardless of sample collection day, the *Listeria* prevalence is greater for each successive phase of production during a given week's simulation (**Figure 2**). Simulation analysis of the fresh-cut models also revealed an interesting feature of those facilities. The facilities tend to have more water on the floors and equipment as compared to packinghouses, therein allowing more *Listeria* to spread throughout the environment throughout the course of a day. However, the intensity of the fresh-cut facility's cleaning and sanitation programs, which is captured within the model, seems to mitigate this risk, as is reflected in historical data. With our models, we can observe how *Listeria* levels evolve as we alter the input parameters such as equipment cleanability and cleaning intensity. A sensitivity analysis on validated models was performed using R (from r-project.org) using the prcc() command (Stevenson, 2018; Iooss et al., 2018) to determine the partial rank correlation coefficients to identify which of the 53 key parameters affect select outcomes in the model, such as prevalence per zone. Tornado plots are then generated of the significant parameters after Bonferroni correction ($p < 0.05/53$) to visualize the extent and directionality of the parameter influence on the outcome of interest. Since we modeled multiple operations, the preliminary sensitivity analysis reveals which key parameters are having the greatest impact on *Listeria* levels in each specific operation (**Figure 3**). For example, the fresh-cut operations revealed that the probability of zone 4 introduction (i.e., input parameter *pr*) has the greatest influence on *Listeria* prevalence.

Scenario analysis. The validated models are then used to conduct scenario analyses to evaluate environmental monitoring programs and determine the optimal sampling plans given four scenarios (**Table 7**). The first scenario is the baseline and represents the sampling program that is currently conducted by the facility during routine sampling. The second scenario represents the FDA Listeria Draft Guidance Recommendation (FDA-CFSAN, 2017), which specifies that at least 5 samples should be collected per line on food contact surfaces, and 5 samples per line on non-food contact surfaces. The third scenario represents a "random" sample collection, where during each iteration of the simulation the sponge samples can be collected from any of the three zones. For the fourth scenario, sponges were only collected from zone 3. All scenarios are simulated at three different sample collection times: (i) 1 hour into production, (ii) 4 hours into production (as is recommended by FDA [FDA-CFSAN, 2017]), and (iii) 10 hours into production.

Scenario analysis demonstrated the utility of an *in silico* approach to the evaluation of sampling plans. Specifically, we evaluated the "diagnostic" accuracy of the sampling program as measured by the sampling sensitivity, specificity and positive predictive value. Sampling

sensitivity was defined as the proportion of contaminated agents (i.e., site in the facility) that are selected for sampling. Sampling specificity was defined as the proportion of non-contaminated agents that are not selected for sampling; related to that is a more informative measure: 1-specificity, which is the proportion of non-contaminated agents that are selected for sampling (and therefore these collected samples were wasted on non-contaminated sites). Finally, the positive predictive value was defined as the proportion of contaminated agents among all sampled. The sampling sensitivity and specificity for zones 1–3 were compared across twelve sampling plans (four sampling schemes at three time points). Additionally, positive predictive values were calculated for each scenario and time combination. The results are plotted in **Figure 4** for Facility A and **Figure 5** for Facility B. Visual comparisons of the accuracy of the tested sampling programs were performed. It should be noted that statistical comparisons would not be meaningful in this case, considering that the magnitude of statistically significant difference that can be detected is inversely related to the number of iterations (i.e., a large number of iterations would allow detection of small differences between sampling programs even if not meaningfully different).

For Facility A, 1,000 iterations (i.e., simulations) were conducted. Each sampling scenario consisted of the collection of 30 samples. Scenario 1, which represented the facility's existing sampling plan, had a mid-range sensitivity when compared to the other scenarios. Scenario 2, which represented the FDA recommendation, also had a mid- to low-range sensitivity. Scenario 3, representing samples collected from a "random" distribution of zones, resulted in the lowest sensitivity, particularly when samples were collected mid-production. Finally, scenario 4, representing only zone 3 samples, resulted in the highest sensitivity, particularly at the start of production. Scenario 4 appears to result in a consistently higher sensitivity for all sampling collection times, when compared to the other three scenarios. The positive predictive value remained consistent across all scenarios and times. These data suggest that scenario 4 can result in the highest sampling sensitivity. However, it is important for facility personnel to have the additional information provided by zone 1 and zone 2 sampling (i.e., the presence of *Listeria* spp. in close proximity to food), even when the prevalence is low, as this can be an indicator that *Listeria* controls are robust.

For Facility B, 100 iterations were conducted. Each sampling scenario consisted of 105 samples. Scenarios 1 and 2 resulted in mid-range sampling sensitivity when compared against the alternative scenarios. Additionally, samples collected during the first shift for these two scenarios appear to have a slightly higher sampling sensitivity. Similar to Facility A, scenario 3 appears to have the lowest sampling sensitivity. This suggests that a sampling scheme with sites from a random assignment of zones is not optimal for detecting *Listeria* prevalence. Scenario 4, similar to Facility A, has a consistently high sampling sensitivity for all time collection points. However, similar to the findings of Facility A, this may not be the optimal sampling scheme, as it does not provide information on *Listeria* spp. prevalence in zones 1 and 2.

Our tailored and validated models are also capable of evaluating corrective actions. We are currently finalizing validation of the models for two packinghouses. This will be followed by investigating and finalizing the scenario analysis to determine the efficacy of select corrective actions.

Outcomes and Accomplishments

This project has resulted in four computer models, each representing a produce operation: two fresh-cut facilities and two produce packinghouses. The analyses of the validated models produced detailed simulation-based site-specific data on frequency, timing, level and transmission of contamination with *Listeria* spp. in each of the modeled produce operations. More specifically, the models demonstrate that site-specific characteristics, such as frequency and level of contamination, connectivity to other equipment and environmental surfaces, and poor cleanability, suggest risk-differentiated areas of a facility. The developed and validated models allow for further rapid virtual experimentation and evaluation of a variety of sampling plans, interventions, and corrective actions. For example, we compared the sampling scheme currently used by an operation against three alternative sampling schemes. Additionally, these various sampling schemes were compared during different times during production (i.e., simulating sample collection at 3 different time points). Further contamination scenarios and controls can be studied to determine site-specific characteristics that justify differential sanitation responses while still providing *Listeria* contamination control and meeting market demands.

Since we modeled multiple fresh produce processing facilities, the data reveal key parameters that are having the greatest impact on *Listeria* levels in each type of operation. With our models, we can observe how *Listeria* levels evolve as we alter these and other input parameters such as equipment cleanability and cleaning intensity.

Summary of Findings and Recommendations

It is impractical for facility personnel in the produce industry to try out and compare every sampling scenario or every corrective action. They need a time- and cost-saving approach that is science-based to determine what approach may be most effective. Modeling can help with this. We need to keep exploring modeling-based approaches, as each time we create a computer model to help elucidate a problem, we are incorporating learnings from our previous models. As we continue to develop our modeling-based approaches, we get better at simulating real-world situations, therein allowing us to run scenarios in a few minutes on a computer rather than spending days, months, or years doing a trial and error in-field test. This will ultimately allow industry to make better decisions such as how to detect potential issues quicker and with fewer samples.

Overall, this project has resulted in a series of simulations, across models of multiple fresh produce processing facilities that can be used to evaluate different EM sampling strategies for their ability to detect contamination events typical for produce processing facilities. These simulations also have the potential to be used to evaluate different corrective actions, taken after detection of a positive environmental sample, for their ability to control or eliminate *Listeria*.

This project would not have been possible without the participation of our incredible industry collaborators. Their willingness to participate and communicate was crucial to this project’s success.

Note: Guideline documents and a workshop for the fresh produce industry are in preparation for later in 2020, and will allow transfer of knowledge regarding the utility of agent-based models as decision support tools and the model-based inference concerning sampling and corrective actions. Before presenting the documents or workshop, the validity of the tool we have developed needs to be confirmed by experts in the field.

APPENDICES

Publications (in preparation)

G. Sullivan, C. Zoellner, C. Barnett-Neefs, M. Wiedmann, and R. Ivanek. 2020. Using in silico models for design and optimization of science-based *Listeria* environmental monitoring programs in fresh-cut produce facilities. Manuscript in preparation.

C. Barnett-Neefs, G. Sullivan, C. Zoellner, M. Wiedmann, and R. Ivanek. 2020. Evaluation of corrective action strategies for produce processing facilities using modeling tools. Manuscript in preparation.

Presentations

Ivanek, R. (March 6, 2019). EnABLe: Environmental monitoring with an Agent-Based model of *Listeria*. International Food Information Council (IFIC) & Foundation. Educational webcast.

Ivanek, R. (June 18, 2019). Modeling tools for design of science-based *Listeria* environmental monitoring programs and corrective action strategies. Lightening Report at CPS Research Symposium, Austin, TX.

Ivanek, R. (October 17, 2019). Digital Agriculture: Food for Health. Cornell University Council and Trustee Council Annual Meeting.

Sullivan, G., C. Zoellner, C. Barnett-Neefs, M. Wiedmann, and R. Ivanek. (June 2020). Modeling tools for design of science-based *Listeria* environmental monitoring programs and corrective action strategies. Poster at CPS Research Symposium (webinar).

Budget Summary

The total funds awarded to this project were $203,988. The team had the required funds to carry out the project. As of 31 March 2020 the majority of funds have been expended and were used to support personnel on the project (salary and wages ($109,323) and fringe benefits ($10,676)), travel ($421), supplies ($14,089), and other direct costs, which included students' tuition ($55,742). Indirect costs were $9,601.

Tables and Figures (see below)

Tables 1–7 and Figures 1–5

Table 1. Summary of agent characteristics in models by zone.

	Zone 1 [a]	Zone 2	Zone 3	Employees
Facility A				
Number of agents	130	120	30	34
Distance from floor (m)	1.2 [0.9, 4.6] [b]	0.9 [0.9, 4.6]	0.9 [0.0, 1.8]	2.1 [1.2, 2.7]
Surface area (cm^2)	7432 [156, 66147]	15396 [914, 92903]	3871 [625, 15396]	156 [156, 156]
Number (%) not cleanable	3 (2.3%)	2 (1.7%)	11 (36.7)	0 (0.0%)
Facility B				
Number of agents	321	219	158	155
Distance from floor (m)	1.2 [0.3, 3.0]	1.2 [0.9, 4.0]	0.3 [0.0, 3.4]	1.2 [1.2, 1.5]
Surface area (cm^2)	6000 [156, 22296]	15000 [5000, 33445]	5000 [729, 10000]	156 [156, 156]
Number (%) not cleanable	34 (10.6%)	34 (15.5%)	41 (25.9%)	0 (0.0%)
Facility C				
Number of Agents	134	117	15	13
Distance from floor (m)	0.9 [0.15, 1.8]	0.5 [0, 1.13]	0 [0, 0.8]	0.01 [0.01, 1.3]
Surface area (cm^2)	625 [100, 138294]	2500 [100, 30000]	1250 [40, 43088]	156.25 [156, 156]
Number (%) not cleanable	9 (6.7%)	93 (79.5%)	6 (40.0%)	0 (0.0%)
Facility D				
Number of Agents	60	39	37	36
Distance from floor (m)	0.9 [0, 1.6455]	0 [0, 2]	0 [0, 0.01]	0.88 [0, 0.985]
Surface area (cm^2)	500 [240, 239765]	27500 [1000, 86968.8]	3178.5 [405, 11184]	156 [156, 156]
Number (%) not cleanable	4 (6.7%)	6 (15.4%)	8 (21.6%)	0 (0.0%)

[a] Agents that are representing Employees are considered zone 1 and therefore are also included in the Zone 1 agents summary.

[b] Values given are median [5th–95th percentile], unless otherwise stated.

Table 2. Input parameters for agent-based model developed for facility A (corresponding tables for models for facilities B–D not shown for brevity).

Symbol	Description	Equation/Distribution	Mean	5th–95th percentile	Reference
p_z	Probability that *Listeria* spp. is introduced into the room via objects from Zone 4	$10^{\text{Pert (-2.3,-0.9,-0.2, 4.8)}}$	0.13	[0.02, 0.35]	expert opinion
N_z	Amount of *Listeria* spp. introduced per object from Zone 4 (CFU)	$10^{\text{Pert (0,1.9,3.3,4.2)}}$	156	[5.6, 624]	expert opinion
R_d	Prevalence of *Listeria* spp. in produce on day d, for d = Monday, Tuesday, Wednesday, Thursday, Friday	$10^{\text{Pert (-2.3, -0.6, -0.6, 5.4)}}$	0.16	[0.06, 0.24]	expert opinion
N_R	Concentration of *Listeria* spp. per contaminated produce (CFU/g)	Gamma(0.0019, 0.019)	0.1		Chen et al. 2016
α	Proportion of *Listeria* spp. transferred to an equipment surface upon contact with a contaminated produce	$10^{\text{Normal (−0.28, 0.2)}}$	0.56	[0.24, 1]	Hoelzer et al. 2012
p_r	Probability that a random event introduces *Listeria* spp. from outside the room	$10^{\text{Pert (-4.3, -0.9, -0.6, 4.6)}}$	0.07	[0.004, 0.2]	expert opinion
	Probability of random introduction to ceiling	—	0.05	—	—
	Probability of random introduction to floor	—	0.85	—	—
	Probability of random introduction to equipment	—	0.1	—	—
N_r	Amount of *Listeria* spp. introduced per random event (CFU)	$10^{\text{Pert (0.2, 3.3, 3.7, 3.3)}}$	1251	[41, 3851]	expert opinion
K	Environmental carrying capacity of *Listeria* spp. (CFU/ml)	—	10^5	—	FDA/FSIS. 2013
GT	Generation time (h) of *Listeria* spp. on environment surfaces (10 °C)	Uniform (47, 155)	101	[53, 149]	Ziegler et al. 2018
μ	Maximum specific growth rate (h^{-1}) of *Listeria* spp. on environment surfaces (10 °C)	=In(2)/GT	0.046	[0.03, 0.075]	Giménez et al. 2004
p_t	Probability that contact on floor from foot and equipment traffic is sufficient to spread *Listeria* spp. to adjacent patch	Pert (0.03, 0.25, 0.65, 4)	0.27	[0.10, 0.48]	Chambers et al. 2009

c_i	Contact rate between the contaminated patch and the adjacent patch given the traffic level i = high, low, negligible	chigh = 60/patch/hr, clow = 12/patch/hr, cneg = 0.2/patch/hr	—	—	observed
p_w	Probability that environmental *Listeria* spp. is transported to adjacent patches via (visible) water	Uniform (0.01, 0.05)	0.03	[0.012, 0.048]	assumed
β	Transfer coefficient for *Listeria* spp. transmission among patches via traffic and water	Uniform (0.0, 0.05)	0.025	[0.002, 0.048]	assumed
p_f	Probability that produce falls to the floor during any given hour of production	Uniform (0.05, 0.10)	0.075	[0.05, 0.097]	observed
p_c	Probability of a condensation transfer event given *Listeria* spp. is present	Uniform (0.01, 0.02)	0.015	[0.01, 0.02]	assumed
η_d	Log10 reduction of *Listeria* spp. from washing and sanitation on day d, for d=Monday, Tuesday, Wednesday, Thursday, Friday	Pert (−8, −6, −1.5, 4)	−5.6	[−7.4, −3.5]	FDA/FSIS. 2013
γ	Probability that a cleanable agent was not properly cleaned at the end of the shift	0.01	—	—	assumed

Table 3. Validation of model predictions against historical data for Facility A, arranged by equipment category.

	Model			Historical				
Equipment	**Probability of contamination[1]**	**95th Percentile[2]**	**99th Percentile[2]**	**# tested**	**# positive**	**Probability of contamination**	**5% CI[3]**	**95% CI[3]**
control-panel	0.0002	0.0000	0.0000	9	0	0.00	0.00	0.30
door	0.0073	0.0000	0.3333	10	0	0.00	0.00	0.28
drain	0.0390	0.3333	0.5000	11	0	0.00	0.00	0.26
floors	0.0019	0.0019	0.0769	65	0	0.00	0.00	0.06
frame	0.0120	0.0909	0.1538	39	0	0.00	0.00	0.09
ladder	0.0018	0.0000	0.0000	10	0	0.00	0.00	0.28
misc	0.0009	0.0000	0.0000	15	0	0.00	0.00	0.20
packing	0.0153	0.0000	1.0000	4	0	0.00	0.00	0.49
squeegee	0.0063	0.0000	0.3333	12	0	0.00	0.00	0.24
trash-gray	0.0006	0.0000	0.0000	5	0	0.00	0.00	0.43
trash-white	0.0003	0.0000	0.0000	4	0	0.00	0.00	0.49
trash-yellow	0.0000	0.0000	0.0000	5	0	0.00	0.00	0.43
wall	0.0009	0.0000	0.0000	12	1	0.08	0.01	0.35
weigher	0.0014	0.0000	0.0000	5	0	0.00	0.00	0.43

[1] Probability of contamination predicted by the model represents the average prevalence for all iterations.

[2] 95th and 99th percentiles are shown for model predictions (5th percentile for all equipment categories was 0, consistent with the low probability of contamination in the facility and therefore is not shown).

[3] 5% and 95% Confidence Interval (CI) for historical data are calculated using a Wilson score interval, a binomial proportion confidence interval.

Table 4. Validation of model predictions against historical data for Facility A, arranged by location category.

	Model			Historical				
Location	**Probability of contamination[1]**	**95th Percentile[2]**	**99th Percentile[2]**	**# tested**	**# positive**	**Probability of contamination**	**5% CI[3]**	**95% CI[3]**
a-b	0.0009	0.0000	0.0000	12	1	0.08	0.01	0.35
a-flume	0.0049	0.0000	0.2005	10	0	0.00	0.00	0.28
a-line	0.0148	0.1667	0.2857	23	0	0.00	0.00	0.14
a-under-room	0.0014	0.0000	0.0000	7	0	0.00	0.00	0.35
b-flume	0.0143	0.0000	1.0000	4	0	0.00	0.00	0.49
b-line	0.0178	0.1667	0.2857	17	0	0.00	0.00	0.18
coring	0.0193	0.1667	0.3333	15	0	0.00	0.00	0.20
elevator	0.0013	0.0000	0.0000	5	0	0.00	0.00	0.43
packing	0.0043	0.0000	0.2000	29	0	0.00	0.00	0.12
scoring	0.0104	0.0000	0.5000	5	0	0.00	0.00	0.43
spin-dryer	0.0002	0.0000	0.0000	4	0	0.00	0.00	0.49
weigher-platform	0.0010	0.0000	0.0000	10	0	0.00	0.00	0.28
floors	0.0019	0.0000	0.0769	65	0	0.00	0.00	0.06

[1] Probability of contamination predicted by the model represents the average prevalence for all iterations.
[2] 95th and 99th percentiles are shown for model predictions (5th percentile for all location categories was 0, consistent with the low probability of contamination in the facility and therefore is not shown).
[3] 5% and 95% Confidence Interval (CI) for historical data are calculated using a Wilson score interval, a binomial proportion confidence interval.

Table 5. Validation of model predictions against historical data for Facility B, arranged by equipment category.

	Model			Historical				
Equipment	**Probability of contamination[1]**	**95th Percentile[2]**	**99th Percentile[2]**	**# tested**	**# positive**	**Probability of contamination**	**5% CI[3]**	**95% CI[3]**
chain	0.0001	0.0000	0.0000	1	0	0.00	0.00	0.79
door	0.0983	1.0000	1.0000	13	1	0.08	0.01	0.33
drain	0.1337	1.0000	1.0000	18	2	0.11	0.03	0.33
dryer	0.0049	0.0000	0.2035	17	1	0.06	0.01	0.27
floor	0.0019	0.0000	0.0000	17	0	0.00	0.00	0.18
frame	0.0188	0.2000	0.3333	19	0	0.00	0.00	0.17
mezzanine	0.0003	0.0000	0.0000	2	0	0.00	0.00	0.66
pallet-jack	0.0577	0.5000	1.0000	7	0	0.00	0.00	0.35
table	0.0001	0.0000	0.0000	1	0	0.00	0.00	0.79
trash	0.2620	1.0000	1.0000	1	0	0.00	0.00	0.79

[1]Probability of contamination predicted by the model represents the average prevalence for all iterations.
[2]95th and 99th percentiles are shown for model predictions (5th percentile for all equipment categories was 0, consistent with the low probability of contamination in the facility and therefore is not shown).
[3]5% and 95% Confidence Interval (CI) for historical data are calculated using a Wilson score interval, a binomial proportion confidence interval.

Table 6. Validation of model predictions against historical data for Facility B, arranged by location category.

	Model			Historical				
Location	**Probability of contamination[1]**	**95th Percentile[2]**	**99th Percentile[2]**	**# tested**	**# positive**	**Probability of contamination**	**5% CI[3]**	**95% CI[3]**
Tray Pack	0.019	0.000	0.500	5	0	0.000	0.000	0.434
Flume	0.168	0.500	0.750	22	1	0.045	0.008	0.218
Packing	0.105	1.000	1.000	20	0	0.000	0.000	0.161
Transition	0.015	0.167	0.333	31	3	0.097	0.033	0.249
Floor	0.002	0.000	0.000	18	0	0.000	0.000	0.176

[1] Probability of contamination predicted by the model represents the average prevalence for all iterations.
[2] 95th and 99th percentiles are shown for model predictions (5th percentile for all location categories was 0, consistent with the low probability of contamination in the facility and therefore is not shown).
[3] 5% and 95% Confidence Interval (CI) for historical data are calculated using a Wilson score interval, a binomial proportion confidence interval.

Table 7. Number of samples collected from each zone for each of 4 simulated sampling scenarios evaluated by the agent-based models for Facilities A and B (fresh-cut facilities).

	Sampling Scenarios[1]:			
	1 – Baseline[2]	2 – FDA[3]	3 – Random[4]	4 - Only Zone 3[5]
Model A				
Zone 1	0	10		0
Zone 2	10	10	Random 30	0
Zone 3	20	10		30
Model B				
Zone 1	0	35		0
Zone 2	45	35	Random 105	0
Zone 3	60	35		105

[1] All four scenarios were tested at three time points: 1 hour into production, 4 hours into production, and 10 hours into production.

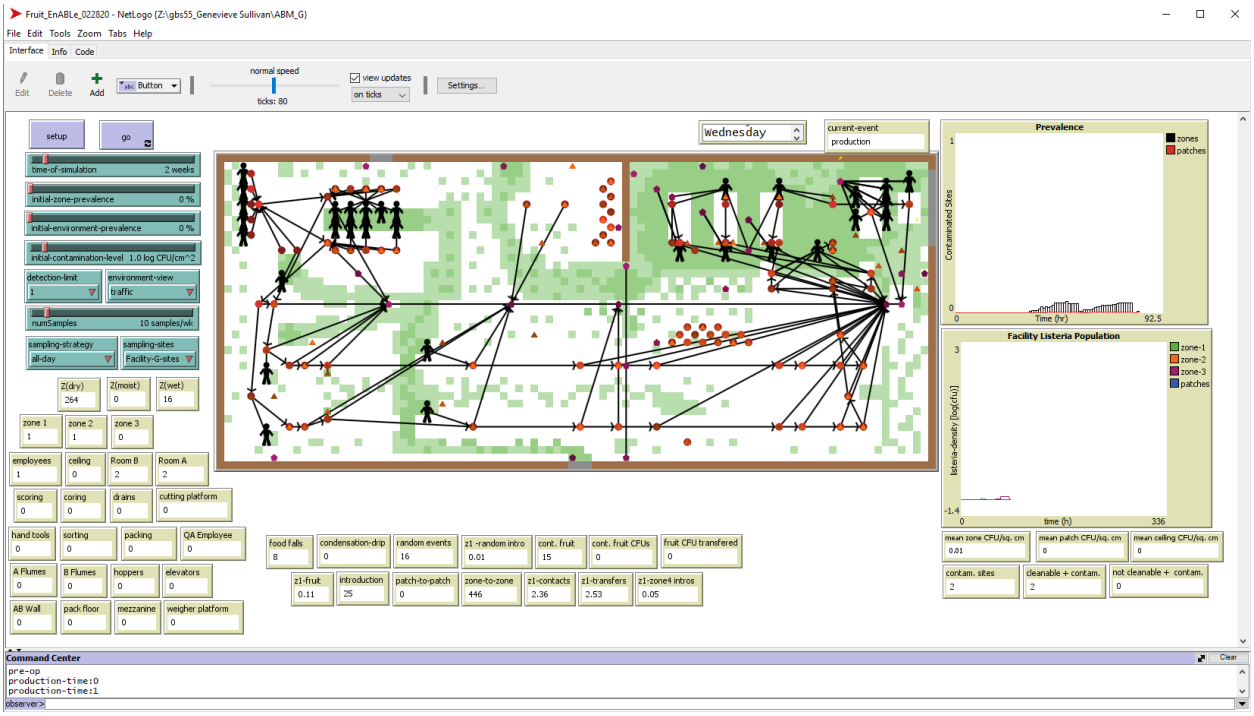
[2] The numbers of samples for the Baseline scenario were adapted from the facility's current sampling plans.

[3] The numbers of samples for the FDA scenario were based on the recommendation provided by the FDA Draft Guidance (FDA-CFSAN, 2017), which states *"We recommend that even the smallest processors collect samples from at least 5 sites of FCS and 5 sites of non-FCS on each production line for RTE foods".* Facility A had two lines. Facility B had seven lines.

[4] The numbers of samples collected for the Random scenario are randomly selected from any of the three zones (with the pre-specified number collected from the floor).

[5] The numbers of samples for the "Only Zone 3" scenario only included samples from zone 3, including the pre-specified number of floor samples.

Figure 1. NetLogo model interface for Facility A (top) and Facility B (bottom).



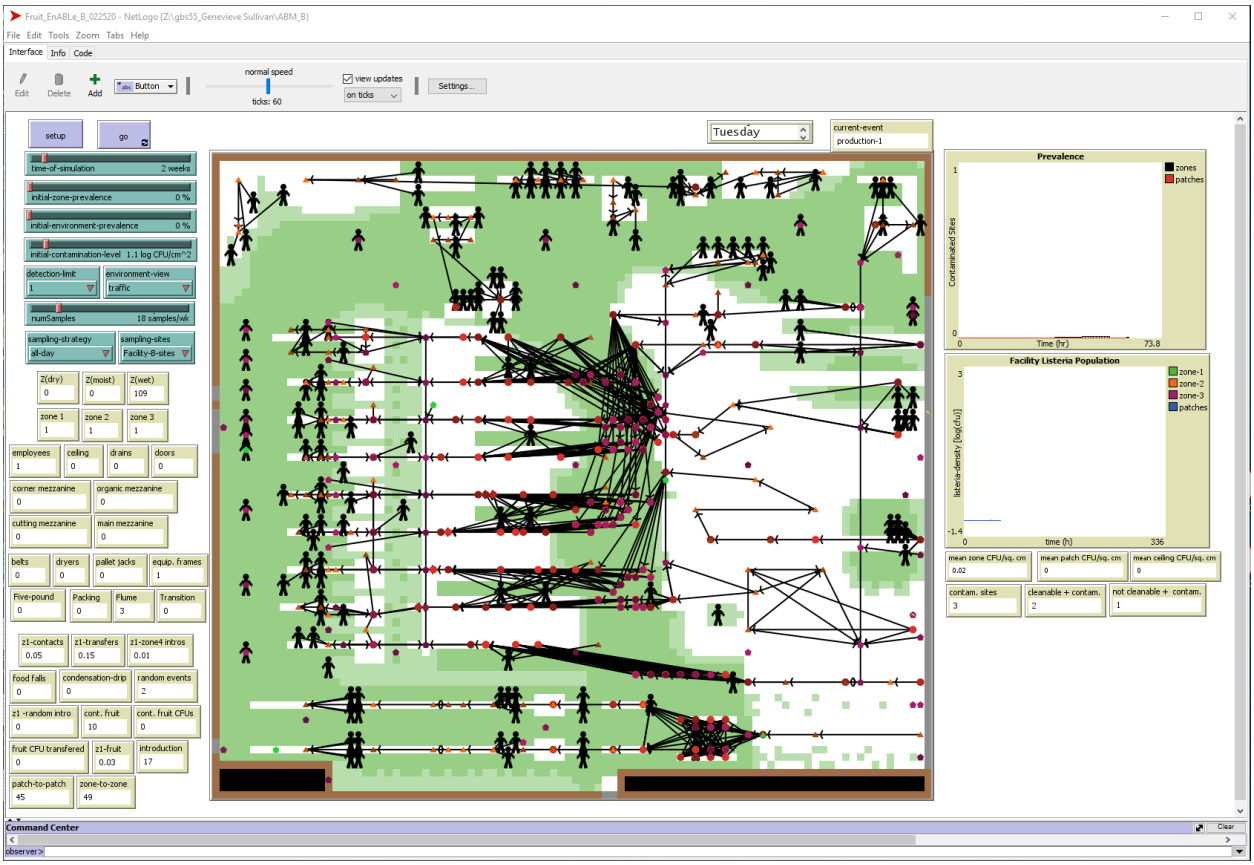


Figure 2. Violin plots showing the predicted prevalence of positive agents in all zones on Wednesday (pre-production, beginning of shift, mid-production, post-production) for Facility A (top) and Facility B (bottom). The central white dot represents the median value, the black bar represents the interquartile range (IQR), the black line represents 95% confidence interval, and the outer shape represents the kernel density plot of all possible values (the thickest section indicates the mode).

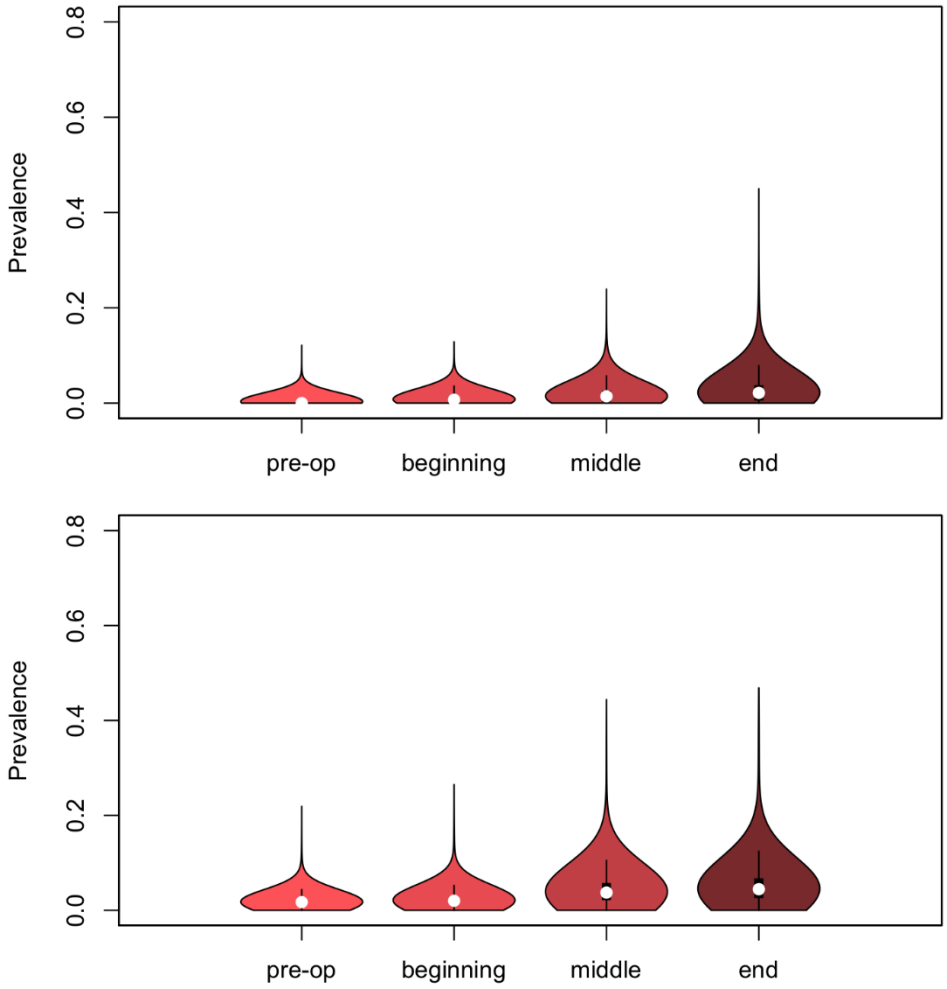


Figure 3. Sensitivity analysis for Facility A (top) and Facility B (bottom) reveals key parameters significantly affecting *Listeria* prevalence detected in zones 2 and 3. (Notations: p.zone4.intro= p_z; fruit.conc= N_R; p.random.noise=p_r; zone4.load=N_z; p13=probability of contact from contaminated zone 1 to zone 3; p31=probability of contact from contaminated zone 3 to zone 1; p32=probability of contact from contaminated zone 3 to zone 2; p33=probability of contact from contaminated zone 3 to zone 3; tc32= probability of *Listeria* spp. transfer from zone 3 to zone 2 given contact; tc33= probability of *Listeria* spp. transfer from zone 3 to zone 3 given contact.)

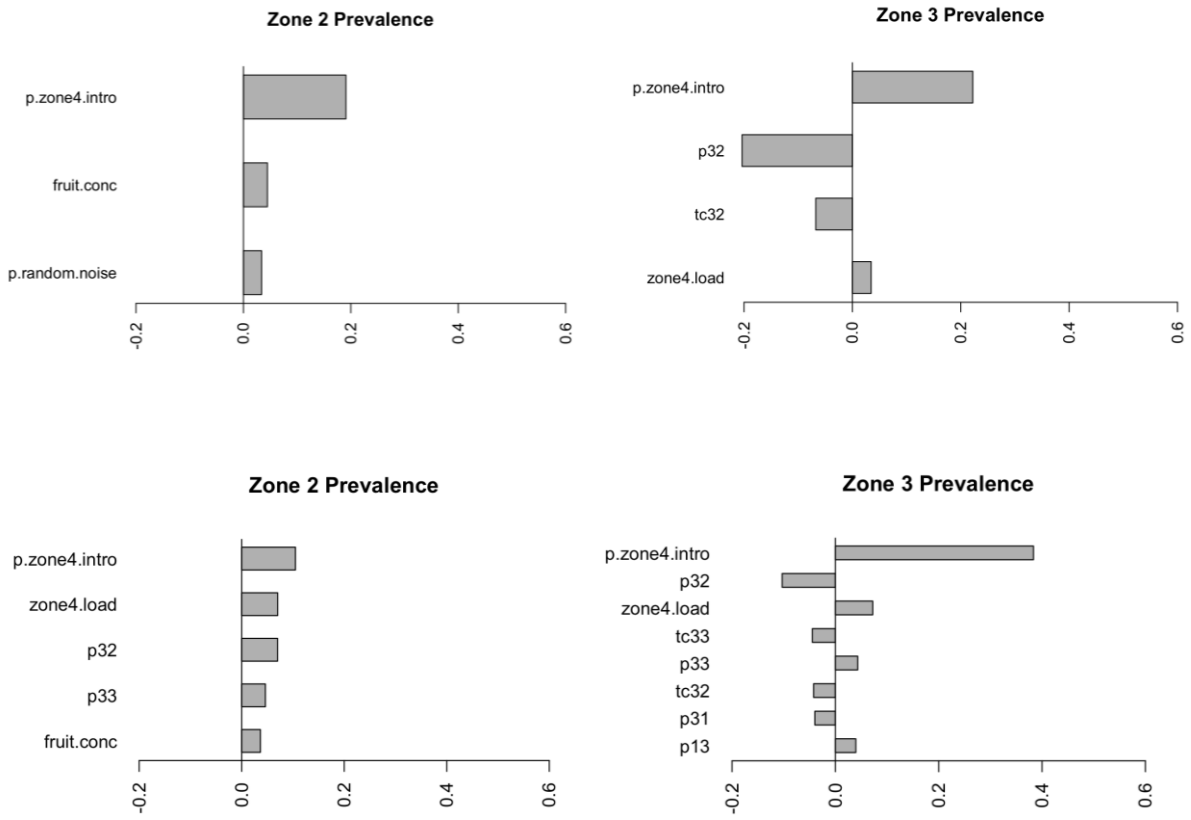


Figure 4. Box plots for scenario analysis for Facility A showing how the Sensitivity (Se) and Positive Predictive Value (PPV) of the sampling scheme compares among 4 tested sampling scenarios, each evaluated for 3 different times during production. Boxplots show model simulation results as the median (black bar), interquartile range (box), 5th–95th percentile (black whiskers), and outliers (black circles outside of whiskers).

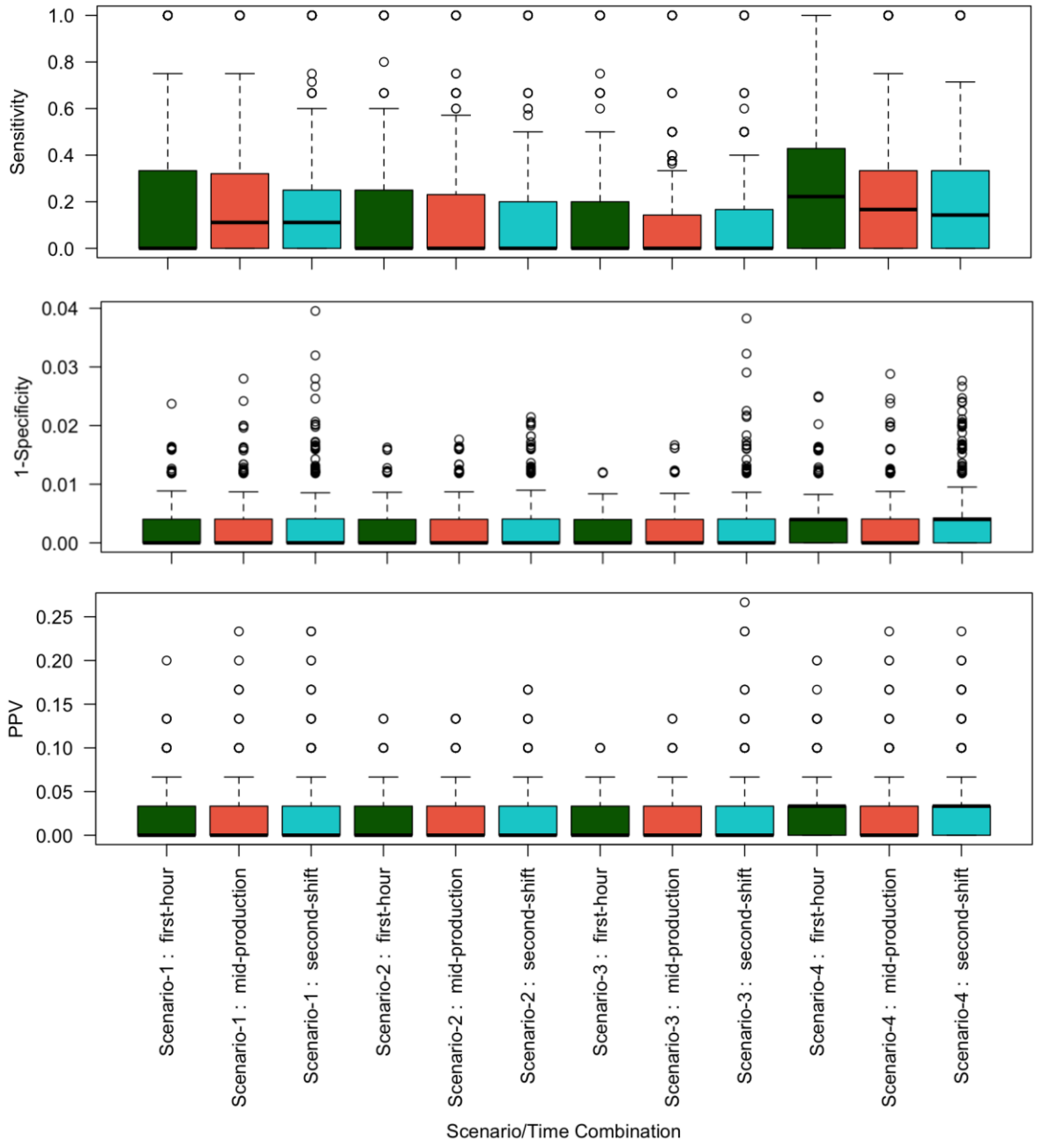
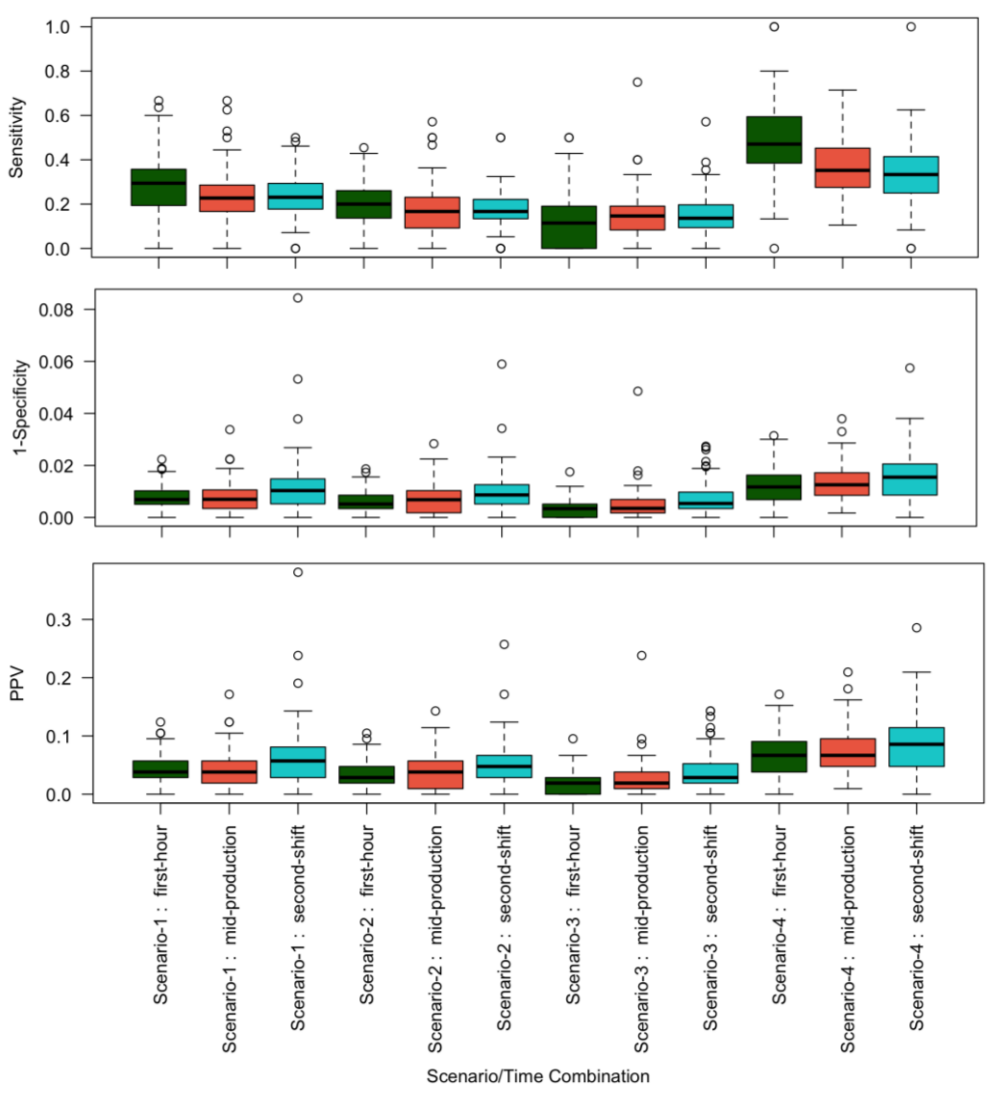


Figure 5. Box plots for scenario analysis for Facility B showing how the Sensitivity (Se) and Positive Predictive Value (PPV) of the sampling scheme compares among 4 tested sampling scenarios, each evaluated for 3 different times during production. Boxplots show model simulation results as the median (black bar), interquartile range (box), 5th–95th percentile (black whiskers), and outliers (black circles outside of whiskers).



References Cited

1. Akingbade, D., N. Bauer, S. Dennis, D. Gallagher, K. Hoelzer, J. Kause, R. Pouillot, M. Silverman, and J. Tang. 2013. Draft Interagency Risk Assessment—*Listeria monocytogenes* in Retail Delicatessens Technical Report. Interagency Retail Listeria monocytogenes Risk Assessment Workgroup. https://www.fda.gov/downloads/Food/FoodScienceResearch/RiskSafetyAssessment/UCM351328.pdf

2. Bergholz, P.W., and M. Wiedmann. 2016. Toward agent-based models for pre-harvest food safety. IBM J Res & Dev. 60(5/6): Paper 8. doi: 10.1147/JRD.2016.2596378.

3. CDC (Centers for Disease Control and Prevention). 2017. "Listeria (Listeriosis)." Updated on 28 June 2017. https://www.cdc.gov/listeria/index.html. Accessed on 24 January 2018.

4. Chambers, M. K., Ford, M. R., White, D. M., Barnes, D. L. & Schiewer, S. 2009. Transport of fecal bacteria by boots and vehicle tires in a rural Alaskan community. J. Environ. Manage. 90, 961–966, https://doi.org/10.1016/j.jenvman.2008.03.008.

5. Chen Y, Burall LS, Luo Y, Timme R, Melka D, Muruvanda T, Payne J, Wang C, Kastanis G, Maounounen-Laasri A, De Jesus AJ, Curry PE, Stones R, K'Aluoch O, Liu E, Salter M, Hammack TS, Evans PS, Parish M, Allard MW, Datta A, Strain EA, Brown EW. 2016. *Listeria monocytogenes* in stone fruits linked to a multistate outbreak: enumeration of cells and whole-genome sequencing. Appl Environ Microbiol 82:7030 –7040. doi:10.1128/AEM.01486-16.

6. Codella, J., N. Safdar, R. Heffernan, and O. Alagoz. 2015. An agent-based simulation model for *Clostridium difficile* infection control. Med Decis Making. 35:211. doi: 10.1177/0272989X14545788.

7. CPS (Center for Produce Safety). 2018 RFP Research Priorities Summary. https://www.centerforproducesafety.org/amass/documents/document/422/2018%20CPS%20Research%20Priorities_FINAL.pdf

8. FDA. 2017. Bacteriological Analytical Manual. Chapter 10. Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods https://www.fda.gov/food/laboratory-methods-food/bam-detection-and-enumeration-listeria-monocytogenes (Accessed on: 28th February, 2020)

9. FDA-CFSAN. Control of Listeria monocytogenes in Ready-to-Eat Foods: Guidance for Industry. Draft Guidance. (Food and Drug Administration, 2017). Available at, http://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/UCM535981.pdf#page=1&zoom=auto,-121,792 (Accessed on: 28th February, 2020).

10. FDA/FSIS. 2013. Interagency Risk Assessment: Listeria monocytogenes in Retail Delicatessens; Technical Report. 1–175 (United States Department of Agriculture-Food Safety and Inspection Service). Available at,

https://www.fsis.usda.gov/wps/wcm/connect/c0c6dfbc-ad83-47c1-bcb8-8db6583f762b/Lm-Retail-Technical-Report.pdf?MOD=AJPERES (Accessed on: 28th February, 2020).

11. Ferreira, V., M. Wiedmann, P. Teixeira, and M. Stasiewicz. 2014. *Listeria monocytogenes* persistence in food associated environments: epidemiology, strain characteristics, and implications for public health. J Food Prot. 77: 150–170.

12. Giménez, B. & Dalgaard, P. 2004. Modelling and predicting the simultaneous growth of Listeria monocytogenes and spoilage microorganisms in cold-smoked salmon. J. Appl. Microbiol. 96, 96–109, https://doi.org/10.1046/j.1365-2672.2003.02137.x.

13. Hoelzer, K., Pouillot, R., Gallagher, D., Silverman, M.B., Kause, J., Dennis, S. 2012. Estimation of *Listeria monocytogenes* transfer coefficients and efficacy of bacterial removal through cleaning and sanitation. Int. J. Food Microbiol. 157, 267–277, https://doi.org/10.1016/j.ijfoodmicro.2012.05.019 .

14. Iooss, B., Janon, A. & Pujol, G. Package 'sensitivity': Global Sensitivity Analysis of Model Outputs. Available at, https://cran.r-project.org/web/packages/sensitivity/sensitivity.pdf (Accessed on: 28th February, 2020)

15. Malley, T.J., J. Butts, and M. Wiedmann. 2015. The Seek and Destroy Process: *Listeria monocytogenes* process controls in the Ready-to-Eat (RTE) meat and poultry industry. J Food Prot. 78: 436-445.

16. Martinez-Lopez, B., B. Ivorra, D. Ngom, A.M. Ramos, and J.M. Sanchez-Vizcaino. 2011. A novel spatial and stochastic model to evaluate the within and between farm transmission of classical swine fever virus: II Validation of the model. Vet Micro. 155:21. doi: 10.1016/j.vetmic.2011.08.008.

17. Monaghan, J.M., J.C. Augustin, J. Bassett, R. Betts, B. Pourkomailian, and M.H. Zwietering. 2017. Risk assessment or assessment of risk? Developing an evidence-based approach for primary producers of leafy vegetables to assess and mange microbial risks. J Food Prot. 80(5):725. doi: 10.4315/0362-028X.JFP-16-237.

18. Railsback, S.F., and V. Grimm. Agent-based and individual-based modeling: A practical introduction. Princeton, NJ: Princeton University Press, 2012.

19. Rubin, M.A., M. Jones, M. Leecaster, K. Khader, W. Ray, A. Huttner, B. Huttner, D. Toth, T. Sablay, R.J. Borotkanics, D.N. Gerding, and M.H. Samore. 2013. A simulation-based assessment of strategies to control *Clostridium difficile* transmission and infection. PLoS ONE. 8(11):e80671. doi:10.1371/journal.pone.0080671.

20. Stevenson, M. Package 'epiR': Tools for the Analysis of Epidemiological Data. Available at, https://cran.r-project.org/web/packages/epiR/epiR.pdf (Accessed on: 28th February, 2020)

21. Sullivan, G., M. Wiedmann. 2020. Detection and prevalence of *Listeria* in produce packinghouses and fresh-cut facilities. Manuscript in preparation.

22. Suslow, T., and L. Harris. 2000. Guidelines for controlling *Listeria monocytogenes* in small- to medium-scale packing and fresh-cut operations. University of California ANR, no. 8015:1–8. http://anrcatalog.ucanr.edu/pdf/8015.pdf

23. Tenenhaus-Aziza, F., J.J. Daudin, A. Maffre, and M. Sanaa. 2014. Risk-based approach for microbiological food safety management in the dairy industry: The case of *Listeria monocytogenes* in soft cheese made from pasteurized milk. Risk Anal. 34(1):56. doi: 10.1111/risa.12074.

24. Tompkin R.B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. J Food Prot. 65 (4):709–25.

25. United Fresh. 2013. Guidance on Environmental Monitoring and Control of *Listeria* for the Fresh Produce Industry. http://www2.unitedfresh.org/forms/store/ProductFormPublic/search?action=1&Product_productNumber=42425

26. Wilensky, U. 1999. NetLogo. Center for Connected Learning and Computer-Based Modeling, Northwestern University, Evanston, IL. http://ccl.northwestern.edu/netlogo/.

27. Ziegler M, Rüegg S, Stephan R, Guldimann C. 2018. Growth potential of *Listeria monocytogenes* in six different RTE fruit products: impact of food matrix, storage temperature and shelf life. Ital J Food Saf. 2018 Oct 8;7(3):7581. doi: 10.4081/ijfs.2018.7581.

28. Zoellner, C., Ceres, K., Ghezzi-Kopel, K., Wiedmann, M., Ivanek, R. 2018. Design Elements of Listeria Environmental Monitoring Programs in Food Processing Facilities: A Scoping Review of Research and Guidance Materials. Compr Rev Food Sci Food Saf. 17:1156–1171. doi: 10.1111/1541-4337.12366

29. Zoellner, C., R. Jennings, M. Wiedmann, and R. Ivanek. 2019. EnABLe: An agent-based model to understand *Listeria* dynamics in food processing facilities. Scientific Reports 9:495, Nature Publishing group, 10.1038/s41598-018-36654-z. https://rdcu.be/bh8oZ