CENTER for PRODUCE SAFETY

CPS 2019 RFP
FINAL PROJECT REPORT

Project Title
Environmental microbial risks associated with vented produce in distribution centers

Project Period
November 1, 2019 – December 31, 2020 (extended to June 30, 2021)

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Objective

1. *Collect current cleaning and sanitizing practices through a survey and on-site observations in parallel with microbial sampling within storage and handling areas in at least 30 distribution centers.*

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FINAL REPORT

Abstract
Little is known regarding the environmental sanitary condition of food distribution centers. As these centers often handle fresh produce that is exposed to the environment (i.e., in unsealed, vented packing), a better understanding of the potential for microbial hazards was needed. Environmental sampling was conducted in 18 distribution centers in the United States between December 2019 and March 2021. At each facility, 40–70 sponge swabs in Dey-Engley neutralizing buffer were used to sample surfaces, including floors, drains, forklifts, trailers, conveyers, shelving, and other surfaces near fresh produce. Adenosine triphosphate (ATP) swabs were also collected with a corresponding subset of sponge swabs. Swabs were analyzed for *Listeria* spp. using the U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA BAM) method within 24 h of collection. *Listeria* spp. were isolated in 12 of the 18 (66.7%) facilities, with *Listeria* prevalence ranging from none detected to present on nearly 1/3 of swabs. Shipping docks and cleaning storage areas were frequently positive for *Listeria* spp., followed by combined shipping and receiving docks and receiving docks. Floors, trailers, and cleaning supplies were the most frequently positive surfaces. ATP was determined to be a poor predictor of *Listeria* spp. positive samples. These data indicate that *Listeria* spp. are prevalent in the distribution center environment and may pose a potential hazard if *Listeria monocytogenes* harborage concomitantly occurs.

Background
The Preventive Controls for Human Food Rule requires facilities handling food that may be exposed to the environment to assess their food safety risks. Most foods passing through distribution centers have a low contamination risk, as they arrive and remain fully sealed while transiting through the center. The exception, however, is fresh produce. While fruits and vegetables are typically packaged in a primary container (e.g., clamshell, plastic wrap) and a secondary container (e.g., cardboard box) for shipment, these materials are generally vented to facilitate gas and vapor exchange due to continued postharvest metabolic activity. The vented, unsealed containment may put product at risk if environmental contamination is present; however, little was known previously about the overall sanitary condition of distribution centers that handle fresh produce.

This project provides the first publicly available data on the prevalence and distribution of *Listeria* spp., an index for the foodborne pathogen *L. monocytogenes*. This data was obtained by collecting environmental samples throughout 18 distribution centers in the United States. Sample locations were documented according to general and primary location in the distribution center, and surface material was also recorded. Samples were processed within 48 h for the presence of *Listeria* spp., and corresponding relative humidity, temperature, and ATP data were collected. Finally, management at each facility were interviewed to determine frequency of cleaning and sanitation regimes, unique facility features, shift change information, and other information that may provide insight into the environmental data.

Research Methods and Results

Sponge Swabs
Sampling regions of interest included all areas of the facility through which fresh produce transited, including dry storage, refrigerated storage coolers and rooms, and loading docks. Particular attention was placed on areas or surfaces in close proximity to produce, including

shelving, pallets, and bins. Items that moved throughout a facility were also examined when available, and included cleaning supplies, forklifts, pallet jacks, and carts. No foods or food contact surfaces were examined. Sampling locations were identified upon arrival, based upon visual examination of the facility. Each sampled location was recorded and photographed using an iPhone, but identifying facility features or personnel were not captured. Management was given the opportunity to review all images prior to the team's departure from the facility, and images were deleted when requested.

Relative humidity and temperature were recorded in each sampled area. 3M Sponge-Sticks with 10 mL Dey-Engley neutralizing buffer (3M, St. Paul, MN) were compressed to remove excess moisture, removed from the sterile bag using gloved hands, and used to sponge an approximately 30 cm x 30 cm (1 ft x 1 ft) surface according to the US FDA "Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples." Surfaces were sponged approximately 10 times vertically with one side of the sponge, and then 10 times horizontally using the opposite side of the sponge, then a final 10 times using the same side used for vertical swabbing. Irregularly shaped or sized surfaces were swabbed in their entirety and the approximate area recorded or described (e.g., sample 1 – drain surface). Sponges were returned to the buffer-containing bag and sealed. Forty to 70 sponge samples were collected at each facility, and immediately shipped on ice to the Strawn laboratory (Virginia Tech) for processing (within 24 h) according to the FDA BAM method for *Listeria* spp. All isolates were confirmed using PCR. Identifying facility location was not disclosed to the laboratory to ensure confidentiality.

Adenosine Triphosphate (ATP) Samples
Fifteen to 20 ATP swabs were collected at each facility and in locations adjacent to microbial swab locations. As per the manufacturer's instructions, a Hygiena Supersnap swab (Hygiena, Camarillo, CA) was used to sample a 10 cm x 10 cm square area, measured using a pre-cut template. Each area was sampled by rotating the swab while sweeping across the surface vertically, horizontally, and finally, vertically. The swab was immediately placed in the Hygiena SystemSURE PLUS ATP Measurement System (Hygiena) for measurement, and results were recorded.

Air Samples
Ten to 15 impaction samples were collected in each facility. For each sample, 1000 L of air was impacted onto standard agar (7%) plates. The agar was transferred (scraped) into Whirl-Pak bags using a sterile L-spreader, covered with 90 mL of buffered Listeria enrichment broth (BLEB), and hand massaged to homogenize the agar and BLEB. Bags were placed on ice for transport to the Strawn lab.

Upon arrival at the Strawn lab, bags were incubated at 30 °C for 4 hours, at which time a 360 µl aliquot of *Listeria* selective enrichment supplement was added to each bag. Bags were returned to 30 °C for 24 and 48 h, then the mixture from each sample bag was streaked onto Listeria CHROMagar and Modified Oxford agar. Plates were incubated at 30 °C for 48 h, and up to two positive samples were PCR confirmed, retained, and preserved at −80 °C.

Survey Data
Observation and survey data were collected at each facility to determine environmental risk management practices. This included determining how product moved through facilities, handling and management practices, observational analyses, standard operation procedure and records review, interviews, and responses to a written survey. These data elucidated common themes, including shared practices, handling procedures, or sanitation regimes. Grouped responses were then coded into categories to yield a meaningful interpretation of the data.

Results

Listeria spp. were detected in 12 of 18 (66.7%) facilities surveyed, with positive sample prevalence ranging from not detected to approximately 1/3 of samples positive for *Listeria* spp. (**Figure 1**). Interestingly, the facility with the highest number of *Listeria* positive samples had recently undergone major renovations.

Listeria spp. were found most frequently at the shipping, receiving, or combined shipping/receiving docks and in areas where cleaning supplies were stored (**Figure 2**). *Listeria* spp. were found in less than 4.0% of samples in cold storage areas, the merge, and 50 °F rooms.

Surfaces frequently positive for *Listeria* spp. included floors, the interior of trailers, and from cleaning supplies (**Figure 3**). Shelving, equipment (e.g., forklifts, pallet jacks) and walls were positive for *Listeria* spp. in less than 3.0% of swabs.

Surfaces comprised of metal were the least frequently positive for *Listeria* spp., while epoxy patches and puddles/liquid samples were positive in greater than 12% of samples (**Figure 4**). Surfaces made from fabric, rubber, concrete, wood/cardboard, or plastic were also positive for *Listeria* spp.

Log(x+1) transformed ATP data across facilities ranged from 0.0 to approximately 9.0 RLU (relative light units) (**Figure 5**). However, logistic regression (not shown) indicated that ATP appears to be a poor indicator for the presence of *Listeria* within the distribution center environment. ATP is still an important component of a cleaning and sanitation regime as a verification tool, but it should not be used in lieu of a robust microbial environmental monitoring program.

The management survey indicated that floors are among the most frequently cleaned surfaces within the majority of distribution centers (**Figure 6**).

Outcomes and Accomplishments

The project team completed 18 onsite visits and collected environmental data and survey data at all locations. Data analysis and manuscript preparation are ongoing. One manuscript, a systematic review, was published in June 2021. The co-authors are currently preparing a second manuscript, *Adenosine Triphosphate (ATP) Bioluminescence as an Indicator of Listeria spp. in Distribution Centers that Handle Fresh Produce*, to submit in August 2021. The combined quantitative and qualitative survey data will be submitted for publication in fall/winter 2021, and the metagenomic analysis will be completed and submitted for publication in spring 2022. Additionally, an industry whitepaper (i.e., guidance document), *Environmental Risk Assessment and Hazard Management for Distribution Centers* (working title) is currently in preparation for fall 2021 submission.

During the project the team partnered with three additional collaborators to further the impact of the study. Dr. Abhinav Mishra (UGA Department of Food Science and Technology) works with microbial risk assessment, and joined the team to add a modeling component to the data analysis. Dr. Trevor Suslow suggested the team add a metagenomics component to the study, so at each facility, an additional set of swabs was collected for DNA extraction, which occurred at both the Strawn and Dunn labs. To assist with this portion of analysis, the team is collaborating with Dr. Henk den Bakker (UGA Center for Food Safety), and CPS permitted the use of project funds to support costs associated with Dr. den Bakker's work. The addition of these scientists and their respective expertise will significantly increase the value and impact beyond the work initially proposed.

Summary of Findings and Recommendations

Although *Listeria* spp. were isolated within most distribution centers, the study did not confirm the presence of *L. monocytogenes* in order to facilitate company recruitment. However, as *Listeria* spp. are considered appropriate index organisms for *L. monocytogenes*, this work suggests the potential for contamination by *L. monocytogenes* within the distribution center environment. Knowing this, targeted, robust cleaning and sanitation regimes may be advisable to mitigate this potential hazard within the distribution center environment. Current cleaning programs may require remediation. For instance, the management survey indicated that floors are among the most frequently cleaned surfaces within the majority of distribution centers (Figure 6), yet they also most frequently yielded positive *Listeria* spp. samples (Figure 3). Therefore, more robust sanitation operation procedures may be advisable. Reliance on or integration of ATP within an environmental monitoring program may be necessary as the current data suggest a poor association between ATP and presence of *Listeria* spp. While ATP is a valuable resource to determine adequacy of cleaning activities, it should not be the primary component of an environmental monitoring program to the exclusion of microbial swabs.

While the study indicates a likelihood for environmental contamination by *L. monocytogenes*, the study made no attempt to estimate the potential risk to fresh produce. First, thorough characterization of the *Listeria* spp. commonly isolated within distribution centers should ascertain if pathogenic species are present. Additionally, further work to determine the likelihood of pathogen ingress through vented packaging under conditions or situations simulating those within a distribution center may also be critical in determining the degree to which fresh produce is at risk in these environments.

APPENDICES

Publications and Presentations

Townsend, A., Strawn, L. K., Chapman, B. J., Dunn, L. L. 2021. A Systematic Review of *Listeria* Species and *Listeria monocytogenes* Prevalence, Persistence, and Diversity throughout the Fresh Produce Supply Chain. *Foods* 10(6), 1427; https://doi.org/10.3390/foods10061427.

Townsend, A., Strawn, L. K., Chapman, B. J., Dunn, L. L. Adenosine Triphosphate (ATP) Bioluminescence as an Indicator of *Listeria* spp. in Distribution Centers that Handle Fresh Produce (*in preparation*).

A. Townsend, L. Dunn, L. Strawn, B. Chapman, C. Murphy. 2021. Environmental Microbial Risks Associated with Fresh Produce Distribution Centers. International Association for Food Protection. July 18-21, Phoenix, AZ.

Budget Summary

The award to University of Georgia (UGA) was further divided into two subawards to support the Co-PIs at Virginia Tech and North Carolina State University. The Virginia Tech subaward covered student employee costs to process microbial samples that were shipped directly from each distribution center, and for supplies necessary for the work (e.g., biological media, extraction kits, pipet tips, loops, spreaders, petri plates). The NC State subaward covered employee time necessary for survey development and dissemination, and data compilation. Prior to COVID-19 pandemic related travel restrictions in 2020, the award also covered travel for the NC State group to travel to each distribution center.

UGA funding supported a PhD student who was responsible for sample collection, further sample processing, data analysis, and manuscript preparation. During the pandemic, the student was tasked with completing a systematic review of literature describing *Listeria* throughout the fresh produce supply chain (Townsend et al., 2021; *Foods* 10(6):1427). Funding covered the travel costs (e.g., airfare, hotels, per diem, rental cars, fuel) necessary for sample collection as well as shipping costs associated with sending samples to the Virginia Tech laboratory. Laboratory supplies necessary for sample collection were also purchased, including sponge sticks, biological media, vortex adapter (for extractions), pipettes, and ATP tests. Additionally, CPS allowed the addition of a metagenomic study, which required the purchase of DNA extraction kits, both at Virginia Tech and UGA (because both groups were involved). CPS approved the team's request to include Dr. Henk den Bakker (UGA Center for Food Safety) in this portion of the study and approved funding to cover costs associated with Dr. den Bakker's contribution; this included two invoices as the team determined to send additional samples after submission of the first invoice.

Figures 1–6: see below

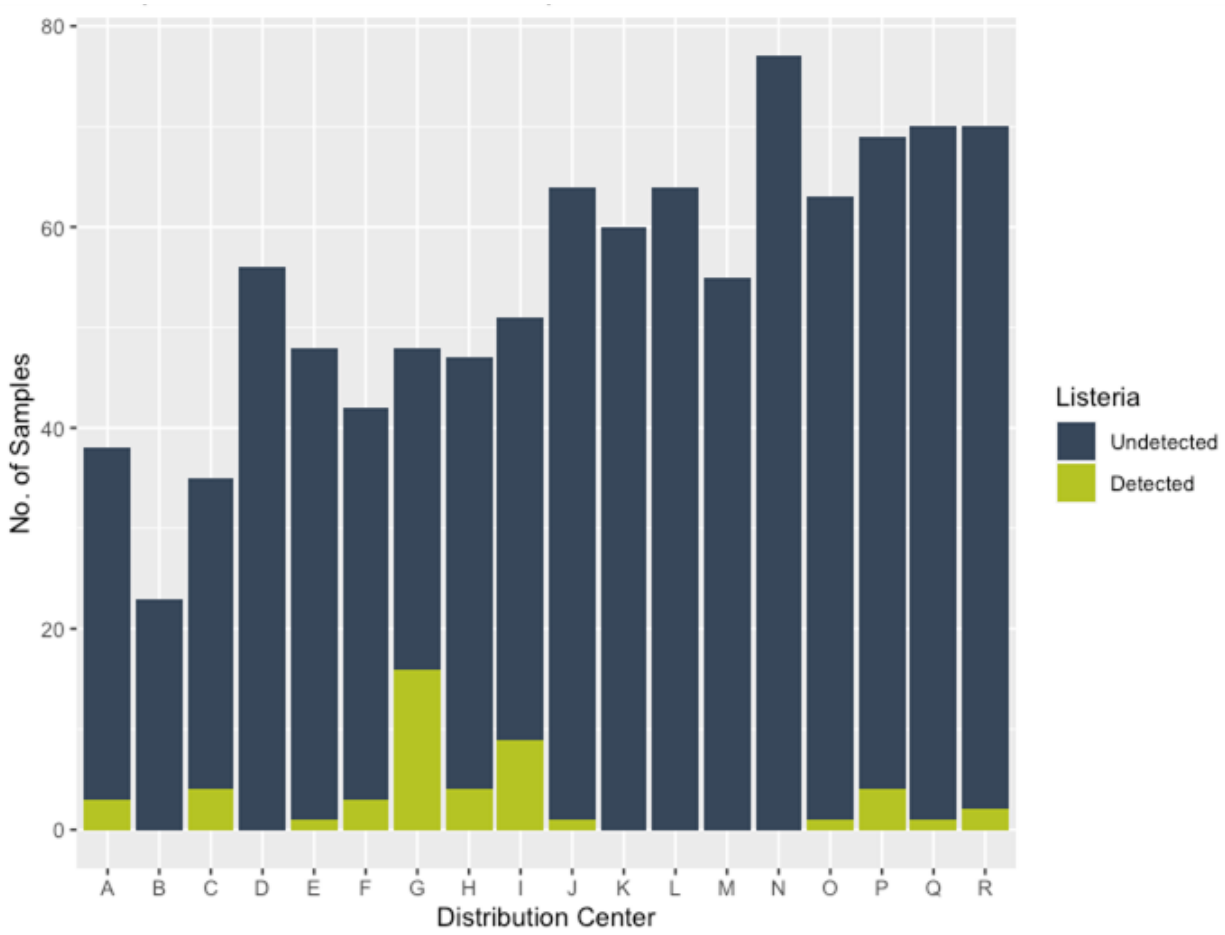

Figure 1. Stacked bar and table exhibiting total samples with undetectable and detectable *Listeria* per distribution center (DC).

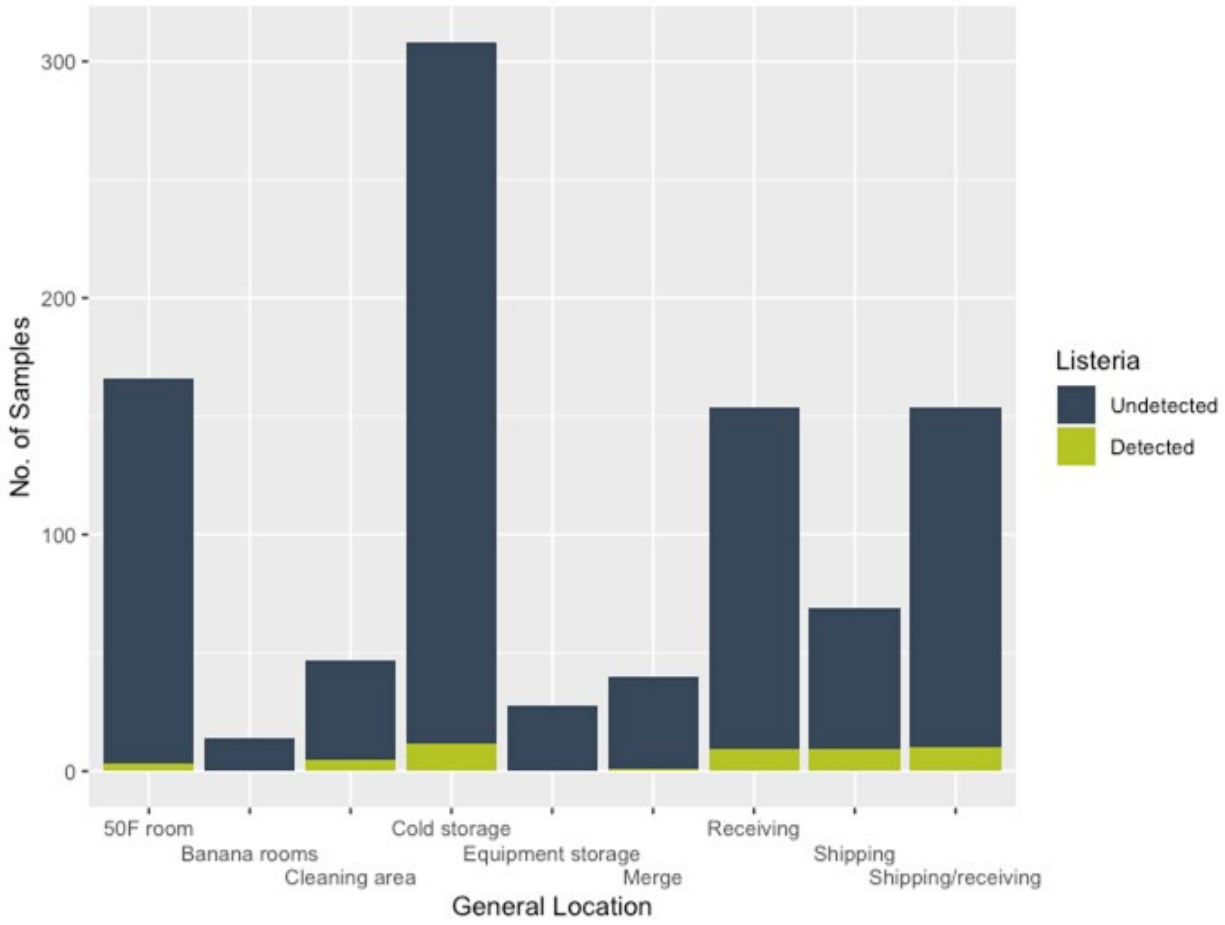

Figure 2. Stacked bar exhibiting total samples with undetectable and detectable *Listeria* per general location within DCs.

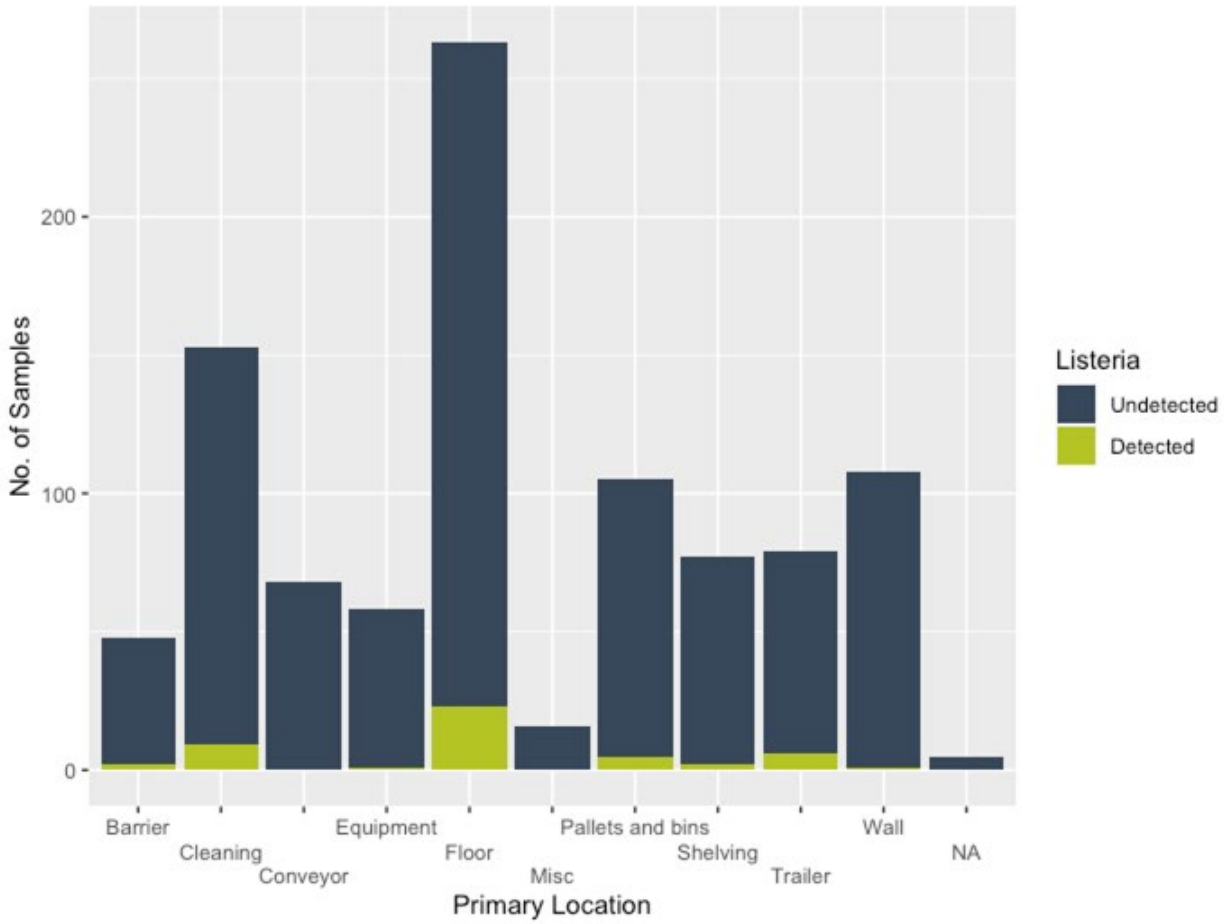

Figure 3. Stacked bar exhibiting total samples with undetectable and detectable *Listeria* per primary location within DCs.

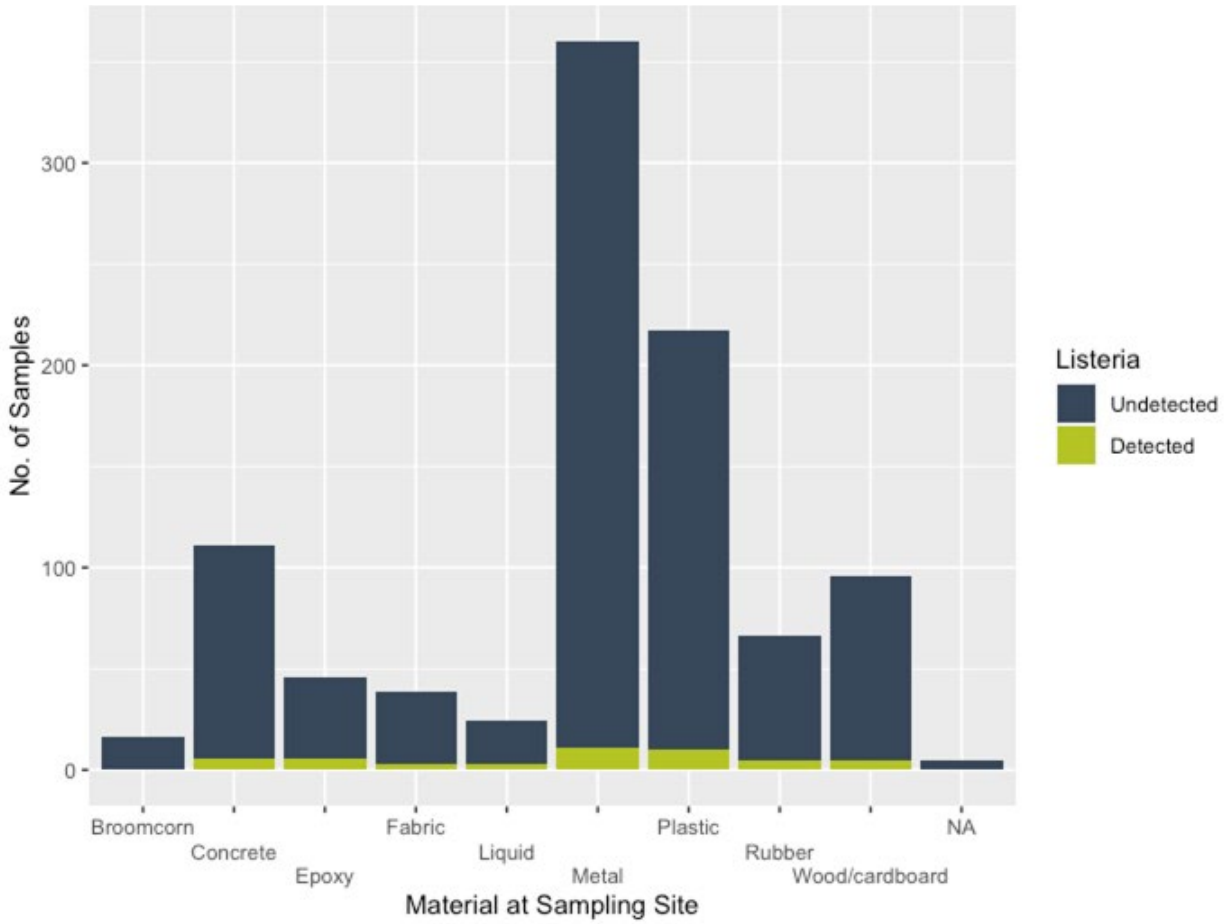

Figure 4. Stacked bar exhibiting total samples with undetectable and detectable *Listeria* per sample site material within DCs.

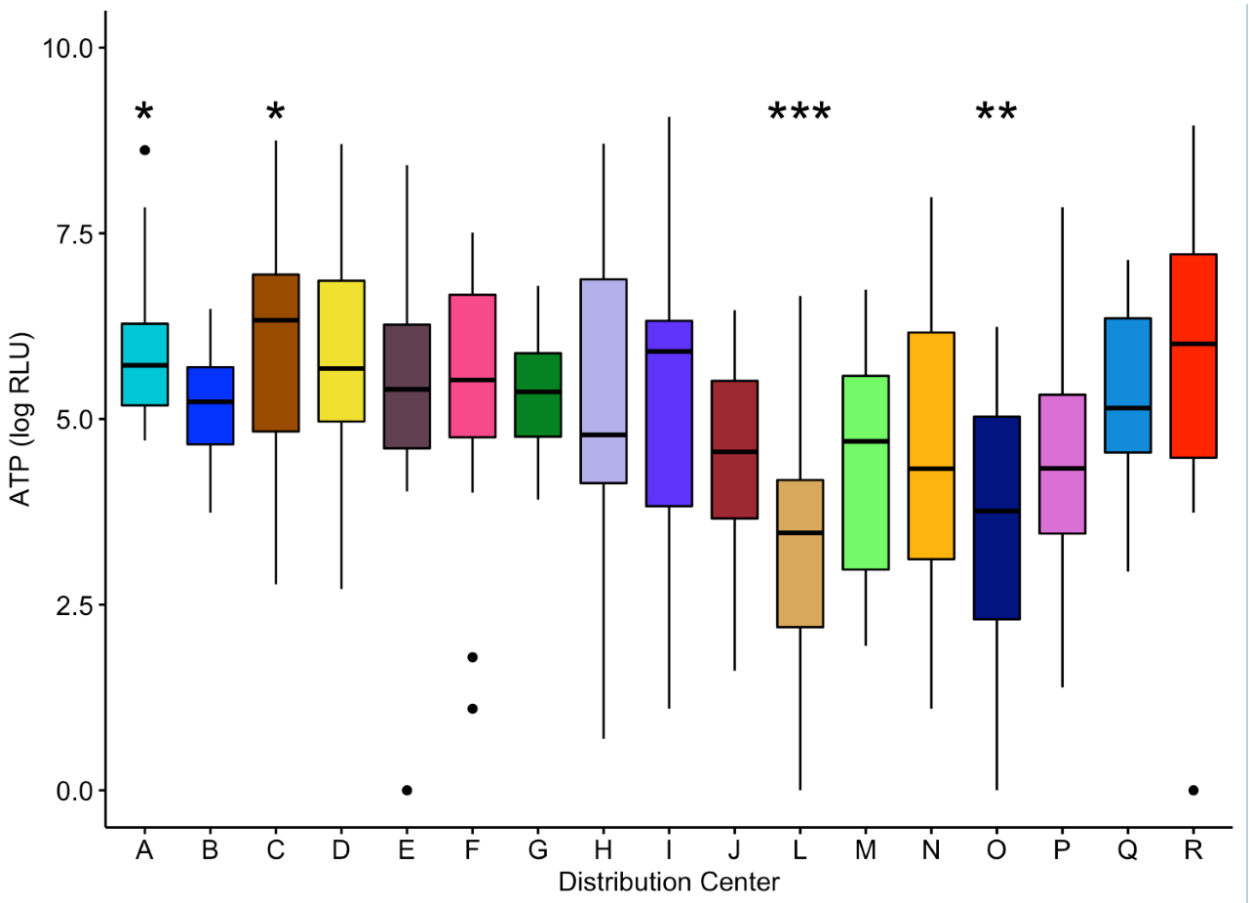

Figure 5. Significant differences between mean log RLUs across all distribution centers are noted by asterisks (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$). ATP data not available for distribution center K.

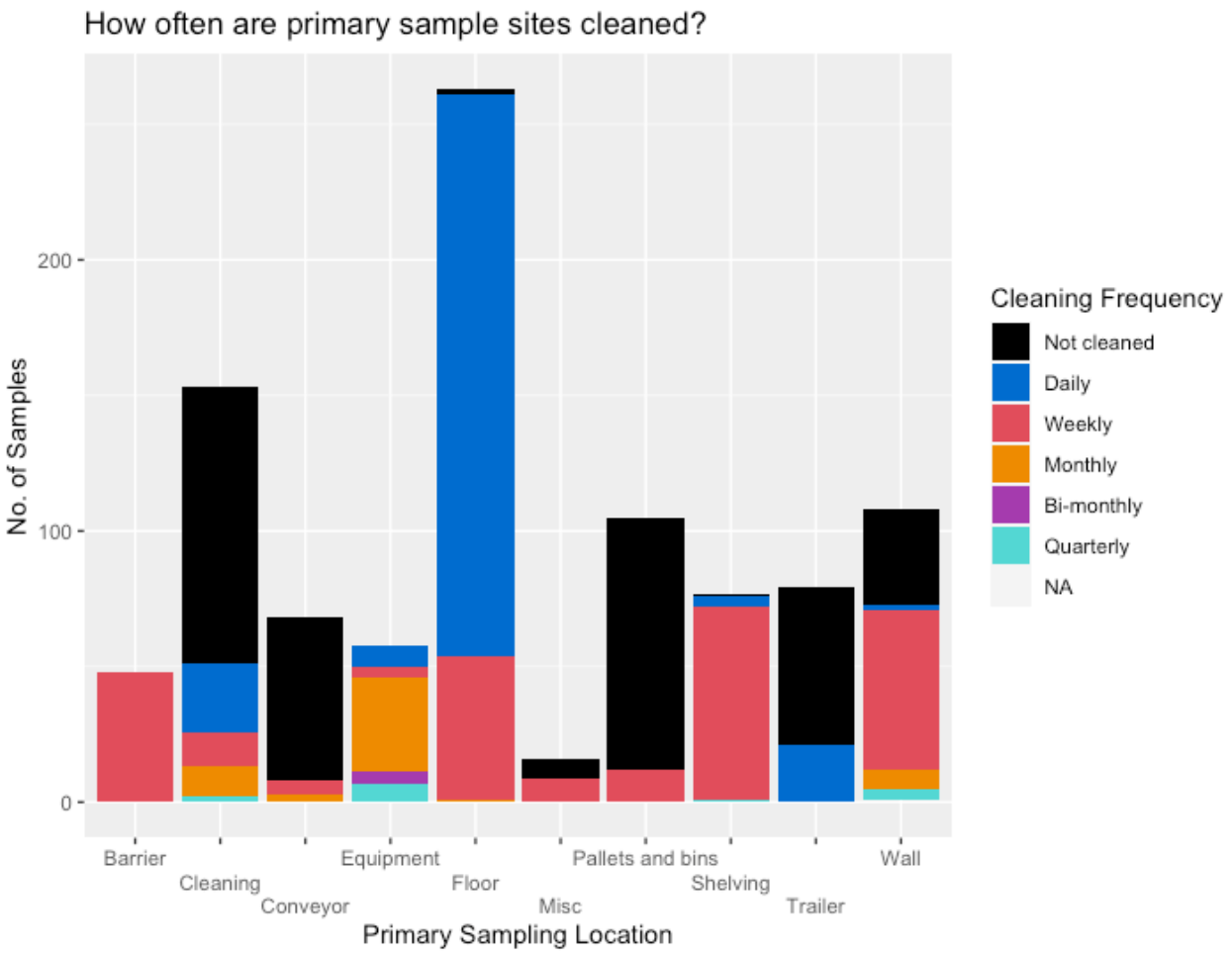

Figure 6. Frequency of cleaning protocols on primary surfaces within 18 distribution centers.