Decision Making Tools for Optimizing Environmental Sampling Plans for *Listeria* in Poultry Processing Plants

Abdullah Al Wahaimed

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Food Science and Technology

Joseph Eifert, Committee Chair

Monica Ponder

Jian Wu

(June 2022)

Blacksburg, VA

Keywords: listeriosis, *Listeria monocytogenes,* environmental monitoring, contamination, sampling, processing plants

Abstract

Meat and poultry slaughtering and processing practices have been associated with the microbial contamination with *Listeria* spp. Ready-to-eat poultry products have been considered as a primary agent associated with *Listeria monocytogenes* illness outbreaks. Developing environmental monitoring programs (EMPs) that are based on product and/or process risk level analysis is a useful approach to reduce contamination in poultry processing plants and enhance food safety. Sampling criteria that is based on product risk levels and process control in ready-to-eat poultry processing facilities was developed to allow users to design and conduct appropriate sampling plans to target *Listeria* spp. After developing the criteria, an internet-based environmental monitoring program ("EZSafety") was developed to allow poultry producers to enhance their sample collection and analysis of test results over time and conduct appropriate sampling plans for *Listeria* spp. and other microbiological indicators. The frontend of the program website was built using React Native (an open-source JavaScript library for building user interfaces). The backend of the program website was built using Node.js which executes JavaScript code outside a web browser. MongoDB was used as a document-oriented database for the website. The program was evaluated by 20 food safety professionals to assess its ability to develop appropriate sampling plans to target *Listeria* spp. The majority of these participants believed that EZSafety has several tools that are effective for targeting *Listeria* spp. and other indicators and enhancing environmental monitoring. Additionally, most participants agreed that EZSafety is organized and user-friendly. EMPs can play a significant role in improving the detection rate and the prevention of *Listeria* spp. and other indicators in poultry processing plants.

Public Abstract

Meat and poultry slaughtering and processing practices have been associated with the microbial contamination with a bacterium known as *Listeria*. Cooked poultry products during the manufacturing process have been considered as a primary agent associated with *Listeria monocytogenes (*disease causing type of bacteria*)* sickness outbreaks. Developing environmental monitoring plans to detect and prevent this bacterium in poultry processing establishments is a useful approach to reduce contamination and enhance food safety. Several guidelines and baselines were developed to allow users to design and conduct appropriate environmental monitoring plans to target this bacterium. After developing these guidelines and baselines, an internet-based environmental monitoring program ("EZSafety") was developed to allow poultry processors to enhance their sample collection and analysis of test results over time. The program was developed using several kinds of computer platforms (JavaScript, React Native, and MongoDB) . These open-source platforms were used to design, develop, and store the program over the internet. In order to validate its usefulness, the program was evaluated by 20 users who are majored in food safety and familiar with poultry processing plants hygiene to assess its ability to suggest appropriate monitoring plans. Most of the participants believed that EZSafety has several tools that are effective for targeting *Listeria* and other kinds of bacteria and enhancing environmental monitoring plans. Additionally, most participants agreed that EZSafety is organized and user-friendly. Such automated monitoring programs can play a significant role in enhancing the detection rate and the prevention of *Listeria* and other organisms in poultry processing facilities.

Acknowledgements

Words cannot express my deep gratitude and appreciation toward my academic advisor, Professor Joe Eifert. This project and the research behind it would not have been achievable without his exceptional support. His passion, expertise, and attention to detail have helped me tremendously to ask critical questions, think as a researcher, and improve my academic skills. I would like to extend my thanks and gratitude toward my committee members Dr. Monica Ponder and Dr. Jian Wu for their time, advice, and support during this study.

Additionally, this project would not have been possible without the generous support from the Saudi Arabian Cultural Mission (SACM) and King Faisal University, who financed my scholarship and provided me with all the resources I needed. I also want to thank Mihir Patel for his great technical efforts and for helping me to develop this environmental monitoring program, “EZSafety”.

Finally, I wish to thank my parents for their love, prayers, and support and for being patient with me when I decided to leave my country and seek my graduate degrees. I also want to thank my wife Norah Alwisali and my son Saud for giving me the hope, love, and support. Also, I cannot forget my other family members, friends, classmates, colleagues, and the FST family of Virginia Tech for their love and positive energy.

Table of Contents

Abstract……………………………………………………………………………..…..… ii

Public Abstract………………………………………………………………………..…….. iii

Acknowledgements……………………………………………………………..……….. iv

Table of Contents…………..……………………………………………………..……... v

I. Introduction……………………………..1

II. Literature Review…………………………………………………........................ 4

A. Microbial Contamination during Poultry Processing……………………..……... 4

1. US Poultry Production

2. Product Contamination in Poultry Processing Plants

3. Sources of *Listeria* in Poultry Processing Plants

4. Listeriosis

B. The Significance of Environmental Monitoring Plans (EMPs)……………….………..7

1. Microbial Monitoring for Qualitative and Quantitative Organism

2. Acceptable Criteria for Quantitative and Quantitative Organisms

3. Monitoring the Presence of *Listeria* and *Listeria monocytogenes*

C. Strategies for Organizing Environmental Monitoring in Food Industry…..……11

1. Sampling Procedures for *Listeria* spp.

2. Sampling Frequency of *Listeria* spp. and Other Indicator Organisms

3. Environmental Monitoring for Poultry Processing Plants

D. Responding to EMP Test Results………………………………………….…..14

1. *Listeria spp./L. monocytogenes* Corrective Actions

2. Sanitation of Meat and Poultry Processing Plants

3. Evaluation of Environmental Monitoring Programs

III. Materials and Methods………………………………………………………..….20

A. Software Design and Development…………………………………….………20

B. User Inputs for "EZSafety" Software…………………………….…….…...…21

1. Accessing the Program

2. Categorize Sample Types by Environmental Zones

3. Select Sample Sites
4. Select Type of Microbiological Tests
5. Initiating the Sampling Plan
5.1 Select number of samples based on size of processing facility
5.2 Select sampling frequency
C. Acceptable Limits and Rejection Criteria for Sample Test Results………….25
D. Analytical Reports……………………………………………………………27
E. Corrective Actions……………………………………………………………27
F. Evaluation of the “EZSafety” Program…………………………………….....28

IV. Results……………………………………………………………………………..….30
A. The Outcomes of the Program…………………………………………….….…..30
1. Storage Abilities
2. Sampling Plan Determination
3. Unacceptable Limits Detection
B. Evaluation of the Sampling Program “EZSafety”……………………………..……32

V. Discussion…………………………………………………………………………..34

VI. References……………………………………………...39

VII. Figures and Tables ……………………………………………………………48
Figure 1: Create an account page in the main webpage of the program
Figure 2: Users sign in page on the program’s main page
Figure 3: Explanation of the types of environmental zones with examples
Figure 4: A pop-up window built in the Data entry page explaining the types of environmental zones with examples of surfaces in each zone
Figure 5: Definitions of environmental zones with examples of surfaces for each zone in the Guidelines page

Figure 6: Common food and non-food contact surfaces contaminated with *Listeria* spp. in poultry processing plants shown in the Guidelines page of the program
Figure 7: The types of target indicators included in the program in the Create New Plan page
Figure 8: A dropdown menu displaying the size of establishment based on the daily production volume in the Create New Plan page
Figure 9: A screenshot of the Guidelines page showing the recommended number of samples in each size of poultry processing establishments
Figure 10: A screenshot displaying the suggested sampling frequency for *Listeria* in each zone in the Create New Plan page
Figure 11: A pop-window was displayed after a test result above acceptable limits was entered in the Add New Data page
Figure 12: A screenshot of the data summary page showing the program's ability to respond to unacceptable findings
Figure 13: A screenshot of the Report page showing the type of indicator and the time frame to be displayed on the chart
Figure 14: A screenshot of the Corrective Actions page showing several sets of recommended activity to troubleshoot issues
Figure 15: A screenshot of the Corrective Actions page showing several corrective actions specific to each type of zones
Figure 16: A screenshot showing the questionnaire's instructions and start button
Figure 17: Summary of 20 participant responses to the questionnaire's statement regarding the usefulness of EZSafety to enhance *Listeria* environmental monitoring
Figure 18: Summary of 20 participant responses to the questionnaire's statement regarding the usefulness of the monitoring program to enhance poultry processors' decision making
Figure 19: Summary of 20 participant responses to the questionnaire's statement regarding the effectiveness of EZSafety to detect unacceptable limits of indicator organisms

Figure 20: Summary of 20 participant responses to the questionnaire’s statement regarding the usefulness of the program’s guidelines and recommendations to enhance *Listeria* environmental sampling
Figure 21: Summary of 20 participant responses to the questionnaire’s statement regarding the usefulness of EZSafety to suggest corrective actions to prevent *Listeria* contamination events
Figure 22: Summary of 20 participant responses to the questionnaire’s statement regarding the ease of using the monitoring program
Table 1: A sample of one of the 20 previous data sets that were given to the participants showing *Listeria* spp. and APC sample test results
Table 2: a screenshot showing the feedback left by the participants during their evaluation of the program

VIII. Appendices…………………………………………………………………….....…72
Appendix A: Survey Questions and Responses for User Evaluations………..…72
Appendix B: Screenshots of Displays of File Structure for Data Storage and Program Files…..……………………………………………………….……..90
Appendix C: An example showing some of the codes used for EZSafety…...…92

I. Introduction

The consumption of unsafe food accounts for the loss of 33 million healthy lives globally every year, and this is likely underestimated. About one in 10 individuals are affected by foodborne illnesses every year, and unsafe food causes 600 million illnesses and 420,000 deaths each year worldwide. People under the age of five years are inordinately affected by foodborne illnesses by which 125,000 children die every year. Several major consequences of foodborne illnesses include arthritis, nervous system disorders, liver and kidney failure, cancer, and death (World Health Organization, 2020). Nevertheless, it is estimated that one in six Americans (~48 million people) becomes ill, 128,000 hospitalized, and 3,000 die as a result of foodborne illnesses annually (Centers for Disease Control and Prevention, 2018).

Foodborne illnesses have a major impact on the economy of the United States. Around $7 billion is spent yearly due to food safety incidents such as the costs of recalls and paying damages resulted from lawsuits, litigation, and firm closures (Hussain & Dawson, 2013). Moreover, the economic impact of foodborne illness outbreaks may also include product removal from the market, costs of investigations to detect causative agents, costs of increased insurance, and loss of consumer confidence (Zacharski et al., 2018). Several preventive measures are needed to improve food safety and prevent such economic losses.

A food recall is defined when a manufacturer or distributor is voluntarily asked to protect the public from products that may lead to foodborne illnesses or death. A recall is intended to remove a product from the market when there is a belief that this product is unsafe or misbranded (Food Safety and Inspection Service, 2015). Between 2017 to

2021, there was a total of 47 USDA recalls (accounting for 16,120,484 pounds) due to the contamination of food products with *Listeria monocytogenes*. The majority of these recalls were associated with the contamination of ready-to-eat poultry products with this pathogen (Food Safety and Inspection Service, 2022). This high proportion of recalls reveals how often food products are delivered to the consumers with a potential to cause a serious health hazard (The Food Industry Association, 2021).

Product contamination can occur at any stage of the food chain. Food processing plants are considered as a significant source of contamination. Improper cleaning and sanitation and transfer during production are common causes of this contamination. Hence, developing environmental monitoring programs (EMPs) is a useful approach to reduce contamination from the processing environment and enhance food safety (Mota et al., 2021). The term EMP is defined as a program that validates the efficacy of cleaning, sanitation, and other environmental pathogens control programs (3M & Cornell University, 2019). EMPs usually specify sampling locations, testing methodology, sampling frequency, corrective actions, and acceptable criteria. Furthermore, environmental monitoring programs often include various methodologies such as ATP bioluminescence testing, indicator organism counts, spoilage organism counts, allergen detection, and qualitative or quantitative microbial pathogens. Therefore, they can serve as a useful tool for validation or verification of specific prerequisite programs or can work as an approach to monitor the environment for unsanitary conditions that may affect food safety and/or quality (3M & Cornell University, 2019). If a trend analysis of environmental sample test results can be conducted, then the corresponding sampling plan can be adjusted to support food processor decisions to enhance food safety. Unfortunately, many food processors may not select appropriate environmental sample locations or test at appropriate frequencies. Additionally, an ongoing analysis of sample test results over time and adjustments to sampling plans is widely variable in processed food industries.

The aim of the current study is to develop guidance for initiating, evaluating, and modifying an environmental monitoring plan for *Listeria* spp. in poultry processing plants based on validated, scientific approaches. This project developed an internet-based interactive tool for designing an environmental sampling monitoring plan to target *Listeria* (qualitative) and Aerobic Plate Counts (quantitative) in poultry processing plants. This tool was designed to record and sort qualitative and quantitative sample test results, and to alert the user to modify the monitoring plan, if necessary, based on data entry and test results summaries. The sampling tool can suggest an appropriate environmental sampling plan (e.g., sampling frequency and number of samples in each zone) based on product and process risk levels and production volume and provide a rationale and guidance for adjusting these plans. Specific project objectives include the following:

1) Develop criteria for designing environmental sampling plans based on product and process risk and baseline test results.
2) Develop an internet-based program for recording and sorting environmental sample data to detect important trends.
3) Develop guidance and recommendations for environmental sampling plans in poultry processing plants.
4) Validate the recording and reporting tools of the internet-based program.

II. Literature Review

A. Microbial Contamination during Poultry Processing

1. U.S. Poultry Production

The United States is considered as the world's largest producer and second largest trader of poultry meat. The consumption of poultry meat (broilers, chickens, and turkeys) in the USA is markedly greater than beef or pork, but less than overall consumption of red meat (USDA, 2022). Chickens ranked as the second most important category of fresh meat with sales of 13.4 billion U.S. dollars in 2020. In 2020, around 224 million turkeys were produced in the United States (Shahbandeh, 2021). The total value of turkeys produced in the United States in 2020 was 5.19 billion U.S. dollars. A total of 7.32 billion pounds of turkey were produced in the USA in 2020 (USDA, 2021).

2. Product Contamination in Poultry Processing Plants

Food products can be contaminated through one of the following: 1) the addition of contaminated ingredients after the slaughter, or 2) transfer from the processing plant. Recontamination can result from contact with contaminated raw materials, transfer from processing equipment, improper sanitation, and poor manufacturing practices (Almond Board of California, 2010). Various pathogens including *Listeria monocytogenes, Campylobacter jejuni, Salmonella* spp*, Yersinia enterocolitica,* and enteropathogenic strains of *Escherichia coli* can survive from several hours to days on different kinds of surfaces (Martinon et al., 2012). In poultry processing plants environmental samples are generally tested for *Listeria* spp. However, *L. monocytogenes* is typically tested when swabs are collected from finished products (Cornell University, 2022).

One of the most common factors of cross-contamination events is the formation of biofilms on food contact surfaces (Giaouris et al., 2014). Biofilms are defined as a group of cells that adhere to surface, substrate, or other biofilms, which are held together by a self-produced matrix (Donlan & Costerton, 2002). Biofilms generally persist when cleaning and sanitation are improperly performed. The residual food on the unclean food contact surfaces serves as a source of nutrients for other microorganisms that may be present (Srey et al., 2013). A crucial step to prevent contamination in processing environments is maintain high hygienic standards, especially in food contact surfaces (Osimani et al., 2014).

3. Sources of *Listeria* in Poultry Processing Plants

Improper or inadequate cleaning and sanitation may support the growth of *L. monocytogenes* on food contact surfaces which will increase the risk of contamination of ready-to-eat products. There are various possible product sources of L. *monocytogenes* such as uncooked product and ingredients, brine solutions, loose product, reworked products, and returned product (Cutter, 2016). In meat and poultry processing environments, food-contact surfaces and non-food contact surfaces can become contaminated with *Listeria* spp*.* and/or *L. monocytogenes* during production*.* Common contaminated food contact sites include tables, trays, mixers, slicers, utensils, wipers, blades, saws, casing peelers, racks, shelves, containers, plastic wraps, knives, product carts, gloves, and aprons. Possible contaminated non-food contact surfaces can involve control buttons, drains, doors, cooling units, equipment framework, sinks, hoses, walls, floors, wheels of carts, lifters, mobs, squeegees, standing water, walkways, boots, air blower, and fans (Food Safety and Inspection Service, 2014).

4. Listeriosis

Listeriosis is often associated with consumption of contaminated milk, meat, fish, and vegetables, or their products (Chen et al., 2014). Ready-to-eat poultry products have been considered as a primary source of *L. monocytogenes* outbreaks (Elmali et al., 2015). Slaughtering and processing practices have been associated with the contamination of poultry meat (Aury et al., 2011). In humans, consumption of products contaminated with the bacterium can lead to listeriosis. This intracellular bacterium is capable of infecting various cell types and travel across intestines, brain, and placenta. The symptoms may range from flu-like illness to febrile gastroenteritis in healthy individuals. The pathogen is also capable of leading to septicemia in vulnerable individuals (Rahimi et al., 2014). Infants, young children, elderly, pregnant women, and people with weakened immune systems are considered the major risk group for listeriosis which often leads to meningitis, convulsions, abortion, or even death (Cutter et al., 2017). The mortality rate of listeriosis can reach 20% and the case-fatality may upsurge in these risk groups (Jalali & Abedi, 2008).

Globally, listeriosis resulted in 23,150 illnesses, 5463 deaths, and 172,823 disability-adjusted life years (DALYs) in 2010. The proportion of perinatal illnesses was 20,7% (de Noordhout, et al., 2014). In the USA, it is estimated that 1600 people are infected with listeriosis each year, and around 260 die (Centers for Disease Control and

Prevention, 2021). The hospitalization rate of the disease is 94%, indicating that 1,500 people of the known 1600 cases will be hospitalized (Food and Drug Administration, 2020). People with listeriosis often require treatment in an intensive-care-unit, making *L. monocytogenes* the third most costly foodborne pathogen after *Clostridium botulinum and Vibrio vulnificus* (Scharff, 2012).

B. The Significance of Environmental Monitoring Plans (EMPs)

The primary role of environmental monitoring is to ensure that food handlers are working in a hygienic environment. Environmental monitoring plans (EMPs) are useful when they focus on the frequency and efficacy of cleaning and sanitation, zoning, training of food handlers, and eradication of causative agents (International Dairy Federation, 2020). EMPs may be considered as an obligatory tool under food safety programs (Zacharski et al., 2018). Testing end-products is not adequate to ensure their safety because a negative test result for a given pathogen does not mean its absence in the whole product. Therefore, EMPs can be used as a primary warning indicator combined with ready-to-eat product control to prevent product contamination (Mota et al., 2021). An effective EMP, frequent environmental sampling, and a controlled process are more efficient than end-product control alone (Muhterem-Uyar et al., 2015).

To assure an effective microbial control, food establishments must develop an EMP (Zacharski et al., 2018). Automating EMPs decrease the time required to utilize and analyze sampling data, increase testing efficacy, improve production performance, and reduce product waste (Heinzelmann, 2021). Likewise, an effective EMP can prove that food safety plans are contributing to the producer’s ability to produce safe products.

Several factors can affect EMPs including the strategy used to detect and eliminate pathogens of concern and the efficacy of corrective actions that follow any positive findings (Tompkin, 2002).

Cleaning and sanitation practices are performed to remove food residues and to inhibit or destroy microorganisms that may be present on food contact surfaces in the processing environment. However, the presence of microorganisms such as *Listeria* spp. on the food contact surface can indicate inadequate cleaning and sanitation practices (Chmielewski & Frank, 2003). There are several factors other than cleaning and sanitation that may increase the risk of product contamination including poor personal hygiene, poorly designed processing facility and equipment, and lack of validated risk control processes (Keller et al., 2002). Due to the intricacy of factors that can lead to product contamination in food production facilities, the strategy of creating a well-designed EMP is a critical approach (Magdovitz et al., 2019).

1. Microbial Monitoring for Qualitative and Quantitative Organisms

Utilizing microbial testing is a major component of an effective food safety process management. Scientific research suggests that all animal products such as meat and poultry may naturally contain bacteria including pathogenic bacteria. Therefore, reducing bacterial contamination including pathogens on products is a necessary step to enhance food safety (Foundation for Meat and Poultry Research and Education, n.d.). Routine environmental microbial monitoring in food industry aims mainly to determine the levels of indicator organisms (e.g., *Listeria* spp.) and tests for the presence or absence of specific pathogens in the processing line. Indicator organisms are regularly tested within each type of processing zones to ascertain whether contamination is present, absent, or exceeds acceptable limits (Manju & Mishra, 2021). Indicator organisms can include aerobic plate count (APC), total coliform, and *Enterococcus* spp. (Channaiah, 2013).

USDA requires a variety of different microbiological tests in each poultry processing plant which can be quantitative or qualitative. Quantitative microbiological tests can include aerobic plate count (APC), total coliform, generic *E. coli,* and Enterobacteriaceae which are common indicator organisms investigated by poultry processors to monitor the presence of contaminants in the production line (Cutter, 2016). On the other hand, qualitative bacteriological tests in poultry facilities are conducted to monitor the presence/absence of *Listeria, Salmonella*, *E. coli,* and *Campylobacter*. Several microbiological tests such as Aerobic Plate Counts (APC), *Listeria* spp., or ATP bioluminescence can be used to verify the efficacy of cleaning and sanitation program in meat and poultry processing plants (Cutter, 2016). Likewise, such microbiological tests should include appropriate sample frequency and analysis to ensure the establishment's ability to prevent contamination and to maintain its process control system (Food Safety and Inspection Service, 2015).

2. Acceptable Criteria for Qualitative and Quantitative Organisms

Records of contamination by indicator organisms may assist food processors to determine the source of *Listeria* contamination and the efficacy of the sanitation program. The level of contamination with aerobic plate count (APC) on cleaned and sanitized surfaces should be less than 100 CFUs/in^2 (Food Safety and Inspection

Service, 2014). Similarly, the acceptable limits of *Enterobacteriaceae* should be less than 100 CFUs per swab from food contact and non-food contact surfaces (Dosland, 2018). Manju & Mishra (2021) summarize other published recommended acceptable limits for microbiological indicators of cleaned and disinfected surfaces in food industries.

Alternatively, since there is no specific acceptable limit for ATP bioluminescence counts, manufacturers can specify their acceptable limits based on their facility and the type of surface. However, a common starting baseline for food processors is to set 150 relative light units (RLUs) as pass and 300 RLUs as fail (3M, 2016). ATP tests can be used as a warning system to verify the effectiveness of cleaning; microbial tests can be used to verify the efficacy of the sanitation process (3M, 2016).

3. Monitoring the Presence of *Listeria* and *Listeria monocytogenes*

Previous reports have shown that listeriosis outbreaks were linked to the consumption of contaminated RTE turkey deli meat, soft cheese, raw milk, cantaloupe, ice cream, and packaged and frozen vegetables (Rothrock et al., 2017).The global distribution of *Listeria monocytogenes* in contaminated food products and the high case-fatality rate have made this pathogen as a major public health concern (Vázquez-Boland et al., 2001). There are at least six common species of *Listeria*: *L. welshimeri, L. seeligeri, L. grayi, L. ivanovii, L. innocua,* and *L. monocytogenes. Listeria ivanovii* is only pathogenic to animals while *Listeria monocytogenes* is the only *Listeria* species that is considered a human pathogen (Rothrock et al., 2017). This Gram-positive, non-spore forming bacterium is considered a facultative anaerobic microorganism which grows at a broad range of pH 4.5-9 and a temperature from 0-45°C. Its optimum growth temperature varies between 30-37°C. *L. monocytogenes* can grow and multiply at low refrigeration temperatures, high salt concentrations (10% NaCl), and acidic pH. It also shows a remarkable resistance to a variety of disinfectants (Sebastián et al., 2019). *L. monocytogenes* is considered as a significant etiological agent of foodborne diseases (Reiter et al., 2005). In food processing environments, this pathogen can colonize and form biofilms on food contact surfaces on which it can persist for several months to years leading to cross-contamination events (Lakicevic et al., 2015).

Planned and written environmental monitoring procedures are recommended when *Listeria* spp. and/or *L. monocytogenes* are targeted in the processing facility. Written procedures should be scientifically valid, identify sample collection sites during routine monitoring, specify whether *Listeria* spp. or L. *monocytogenes* are targeted, indicate the frequency and timing of sampling, determine which type of tests are conducted, and include corrective actions to be implemented when *Listeria* spp. or *L*. *monocytogenes* are detected (Food and Drug Administration, 2017).

C. Strategies for Organizing Environmental Monitoring in Food Industry

The “Zone Concept” is a term which is used to divide and classify food processing environments into certain areas based on the risk level of contamination that may occur. The use of the Zone concept is a crucial step to determine whether cleaning and sanitation are efficient in the food industry. Zone 1 includes product contact surfaces such as tables, conveyor belts, knives, sinks, containers, storage bins, rollers, and workers’ hands or gloves. Zone 1 may also involve indirect product contact surfaces such as overhead structures that may become in contact with the food or food contact surfaces (Spanu & Jordan, 2020). Zone 2 includes non-food contact surfaces that are in close proximity to food contact surfaces such as framework, cooling units, carts' wheels, tools, pumps, exterior parts of machines, and walkways. And, Zone 3 includes non-food contact surfaces that are farther from Zone 2, for example, walls, floor, drains, trash cans, storage areas, restrooms, and catwalks (The Pennsylvania State University, 2019).

1. Sampling Procedures for *Listeria* spp.

The time of environmental swabbing may vary depending on the purpose of the sampling. In most cases, sampling is carried out in order to detect *Listeria* spp./*L. monocytogenes.* In other situations, sampling is used to verify the efficacy of cleaning and sanitation (Spanu & Jordan, 2020). If the purpose of EMP is to verify the effectiveness of cleaning and sanitation, samples should be collected from cleaned surfaces prior to production (Ministry for Primary Industries, 2017). In contrast, if the aim of the EMP is to assess whether Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP) are preventing the risk of product contamination or not, then samples should be collected during production (Tompkin, 2002). Samples collected prior to processing may fail to detect *Listeria* spp. that are hidden within biofilms or injured as a result of using chemical agents used for cleaning and sanitation. In such situations, *Listeria* spp. may persist in a viable but nonculturable state (Overney et al., 2017). Carpentier & Barre (2012) recommend monitoring food processing plants before cleaning and sanitation at least two or three hours after the start or at the end of production to improve the probability of detecting *Listeria* spp. from biofilms or niches.

2. Sampling Frequency of *Listeria* spp. and other Indicator Organisms

Identifying sampling frequency (e.g., daily, weekly, bi-weekly, monthly, etc.) and the time of sampling (e.g., preoperational, first shift, during production, etc.) are crucial elements of EMP. The number of samples collected from processing lines depends largely on the number of processing lines and the purpose of sampling (e.g., validation, verification, investigation, etc.). Additionally, the number of samples can depend on the characteristics of the processing plant, resource availability, records of contamination, and risk-based practices (Zoellner et al., 2018). Samples taken from Zone 1 should represent 10% to 20% of the total number of samples (Channaiah, 2015). The occurrence of *Listeria* spp. contamination should be considered as a warning indicator for a potential *L. monocytogenes* hazard, which may lead to product recalls and immediate corrective actions (Spanu & Jordan, 2020). The amount of sampling is greater when samples are collected from Zone 2 and Zone 3 which will approximately be 40% to 50% and 30% to 40%, respectively (Channaiah, 2015). Furthermore, the Grocery Manufacturers Association (2014) indicates that most of the sampling should be conducted from Zones 2 and 3 for the aim of obtaining an early indicator for contamination. On the contrary, the Food and Drug Administration (2017) suggests that environmental sampling should be generally greater in Zone 1 and Zone 2 because of the higher risk of product contamination if the pathogen is present in these zones. EMPs should be verified for their effectiveness after they are fully implemented. If sampling plans fail to meet anticipated findings, then the frequency and number of samples in each zone should be adjusted to accomplish the desired results (Channaiah, 2015).

According to the United States Department of Agriculture, Food Safety and Inspection Service (FSIS), one sample unit should be collected from very small establishments, two sample units from small establishments, and three sample units from large establishments. Processing establishments can be categorized based on their daily production volume as follows: very small (1-6,000 pounds), small (6,001-50,000 pounds), and large (50,001,>600,000 pounds). Furthermore, establishments can be classified based on the number of employees in the processing facility as the following: large establishments (500 or more employees), small establishments (10-499 employees), very small establishments (<10 employees), respectively (Food Safety and Inspection Service, 2014). A sample unit includes 10 food contact surfaces swabs, 5 non-food contact surfaces swabs, and 5 intact product swabs. The minimum routine sampling frequency for testing food contact surfaces for *Listeria* spp. should be at least 3-5 samples per production line each time (weekly, biweekly, monthly, every 6 months), (Food Safety and Inspection Service, 2014).

D. Responding to EMP Test Results

1. *Listeria spp./L. monocytogenes* Corrective Actions

<u>Initiating Corrective Actions</u>

The occurrence of *Listeria* spp. contamination could be considered as an early warning indicator and corrective actions can be implemented before *L. monocytogenes* becomes a major concern (Food and Drug Administration, 2017). Positive findings for

Listeria spp./*L. monocytogenes* on food contact surfaces or in finished products should instigate corrective actions. Corrective actions should be implemented as quickly as possible to destroy the potential hazard. The primary goal of initiating corrective actions is “seek and destroy” *Listeria* spp. when it is present in the processing plant. The major aim is to ensure that the root cause has been eradicated as determined by repeated negative samplings (Grocery Manufacturers Association, 2014).

The type of corrective actions will mainly depend on the situation, size, and type of processing plant (Grocery Manufacturers Association, 2014). An EMP for *Listeria* spp./*L. monocytogenes* can be used to verify the efficacy of *L. monocytogenes* control programs, or to evaluate the effectiveness of corrective actions in eradicating *L. monocytogenes* from the environment (Food and Drug Administration, 2017).

For meat and poultry processing plants, corrective actions in response to detection of *L. monocytogenes* or *Listeria* spp. are required. If environmental sample tests are positive for *Listeria* spp. or *L. monocytogenes*, then appropriate corrective actions must be carried out as required by regulation 9CFR 417.3(a) if environmental sampling is included in their Hazard Analysis and Critical Control Point (HACCP) plan or as required by 9CFR 417.3(b) if environmental sampling is incorporated in a Sanitation Standard Operating Procedure (SSOP). Corrective actions will ensure that the cause of the contamination is identified and eliminated, and that contamination will not occur after the corrective action is taken; and that measures to prevent recurrence of the contamination are established. Additionally, processors must not release into commerce a product that contains *L. monocytogenes*, or that has been in contact with a food contact surface contaminated with *L. monocytogenes* without first reworking the product using a process that is destructive of *L. monocytogenes* as described in 9CFR 430.4 (Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products).

<u>General Corrective Actions to Prevent *Listeria* Recontamination</u>

Once a positive *Listeria* result is found in ready-to-eat processing plants it is recommended to: initiate an investigation to detect the root cause or source for the contamination, determine whether *Listeria* spp. has moved to next zone by collecting samples from the nearby area (e.g., contamination may spread from Zone 2 to Zone 1), review previous data and verify if there is any patterns or trends that can lead to the root cause, implement repeated cleaning and sanitation of the affected area, increase sampling frequency (e.g., from weekly to daily to daily in Zone 2, from weekly to once every two days in Zone 3), ensure that three consecutive follow-up samples that show negative results are collected from the contaminated site in order to return to routine monitoring (Grocery Manufacturers Association, 2014). It is recommended to initiate product testing to prevent delivery of contaminated products to the consumers once a positive *Listeria* spp. result is detected, (Cutter, 2016). The recommended sampling plan for Zone 1 product testing is n=20 according to case 12 from the International Commission on Microbiological Specifications for Foods (ICMSF), (2016).

<u>Corrective Actions for a Single *Listeria* Contamination in Zone 1</u>

If a single positive *Listeria* result is found in the Zone 1, it is advised to: 1) hold the production until necessary corrective actions are implemented, 2) initiate product testing if the processing line has a history of Zone 1 *Listeria* contamination, 3) release products only if they only show negative *Listeria* results, 4) perform intensified cleaning and sanitation of the affected area, and 5) increase sampling frequency of the affected zone (Grocery Manufacturers Association, 2014).

Corrective Actions for a Single *Listeria* Contamination in Zone 2

If a single contamination of *Listeria* spp. is found in the processing environment, it is recommended to: stop production and initiate sampling, take additional samples from the nearby Zone 1, 2, and 3 sites, implement intensified cleaning and sanitation, and increase Zone 2 sampling frequency (e.g., from weekly to daily), (Grocery Manufacturers Association, 2014).

Corrective Actions for Reoccurring Positive *Listeria* Results in Zone 1

If a recontamination of *Listeria* spp*./ L. monocytogenes* is found in Zone 1, several activities can be followed to resolve the main issue, these activities may include, re-certifying the line by initiating pre-operational sampling, testing the processed products of the affected line, reassessing the sanitary design by disassembling the equipment, and initiating increased sampling, and reprocessing or destroying, if necessary, the products that show positive *Listeria* results, (Grocery Manufacturers Association, 2014).

Corrective Actions for Reoccurring Positive *Listeria* Results in Zone 2

If reoccurring positive *Listeria* results are found in Zone 2, these activities can be followed to troubleshoot the main problem, the production line should be shut down until the problem is resolved, the equipment in the affected line should be disassembled for sanitation, additional samples should be collected from the suspected areas of the contamination, the nearby Zone 1 sites should be tested to ensure that no contamination has spread to food contact surfaces, the frequency of sampling should be increased if repeated positive samples persist, and the use of molecular subtyping of isolates and review of other quantitative microbiological criteria may be used to detect the source of contamination, (Grocery Manufacturers Association, 2014).

Corrective Actions for Reoccurring Positive *Listeria* Results in Zone 3

If additional *Listeria* contamination is detected in Zone 3, it is suggested to map the positive sites on a layout so that the source of contamination could be discovered or, at minimum, suggest further sites to sample, reexamine sites that may support the growth of contaminants, initiate intensified cleaning and sanitation, review Good Manufacturing Practices (GMP), review the efficacy of the HACCP program, keep the processing line as dry as possible during production, and implement any necessary actions based on findings, (Grocery Manufacturers Association, 2014).

2. Sanitation of Poultry Processing Plants

Written sanitation procedures are recommended to ascertain that food contact surfaces and framework close to that area are cleaned and sanitized in a manner that prevents the risks of *Listeria* contamination. To determine the frequency of cleaning and sanitation, a full review of the sanitary design of the plant and equipment, the level of product exposure to the line and environment, the microbiological profiles during production, and the history of *Listeria* spp. in the processing line are needed (Food and Drug Administration, 2017).

On the other hand, food processors can determine the effectiveness of cleaning and sanitation and recognize contaminated sites by developing baseline microbiological testing of food contact surfaces and the environment. These tests include Aerobic Plate

Counts (APC), generic *Listeria* identifications, or ATP bioluminescence. Such tools can be employed to obtain information about the efficiency of cleaning and sanitation practices (Cutter, 2016). The sanitation program should be reevaluated once a positive result of *Listeria* spp. is found in the processing line. This may also suggest reassessment of the record-keeping tools and critical limits for a HACCP critical control point (Cutter, 2016).

3. Evaluation of Environmental Monitoring Programs

Environmental monitoring programs (EMPs) must be evaluated regularly as environmental conditions may change in the processing facility as a result of renovations and maintenance, deterioration of the equipment, introduction of new equipment and tools, employment of new staff, production of new products, and change in seasons. Research and guidance recommend assessing the efficacy of an EMP at least every 6 months (Spanu & Jordan, 2020). Several trends can indicate that the environmental monitoring program needs to be evaluated and modified, such as the increase of positive test results of samples in particular sites or locations, the increase of *Listeria* spp. findings in the same area for multiple occasions, and the increase of the overall positive sample results in the processing facility (Food and Drug Administration, 2017).

III. Materials and Methods

A. Software Design and Development

An internet-based environmental monitoring program that can suggest appropriate *Listeria* spp. sampling plans was developed to help poultry producers to enhance their sample collection and analysis of test results over time. The program was named "EZSafety" and was stored on the internet. The program is based on product and process risk analysis and can provide poultry processors several guidelines to enhance the detection and control of target *Listeria spp.* in their processing plants. The program can suggest the appropriate time (before production or during production) for sampling and direct processors to the contact surfaces that could be contaminated with *Listeria* spp. to increase the likelihood of finding this contaminant. It can also help poultry processors to maintain their record keeping, analyze their test results, and improve their sample-taking over time. The users of this system can target other organisms that may be indicators of pathogens or indicators of hygiene and sanitation such as *Salmonella* spp., Aerobic Plate Count (APC), Enterobacteriaceae (Eb), and ATP bioluminescence count. Hence, the program can help poultry processors to respond to qualitative and quantitative detection of important microbial contaminants in the environment and take the necessary steps and corrective actions to avoid potential risks. This automated platform can provide guidance to processors so that they ensure that their processing plants are hygienic enough to process and deliver products to the consumers.

AdobeXD (Adobe, San Jose, CA) was used to design the rough and final designs of the program. Adobe XD is a vector-based user experience design tool for web apps and mobile apps. A JavaScript format was used to develop both the frontend (user interface) and backend (data access and storage, business logic, resides on a server) of the platform. The frontend of the program website was built using React Native (an open-source JavaScript library for building user interfaces). The backend of the program website was built using Node.js which executes JavaScript code outside a web browser. The program is primarily based on the coding language in which numerous codes were developed to set its reporting tools, acceptable limits, and rejection criteria. React Native software (Meta Platforms, Inc., Menlo Park, CA) was employed to implement the codes into the program (Appendix C). The program is cloud-based, and its data is stored on the internet. The MongoDB Compass tool (MongoDB, New York City, NY) was used to store the data temporary while developing the program. MongoDB was used as a document-oriented database for the website. After developing and designing the program, it was stored on Amazon Web Services, a provider of on-demand cloud computing platforms and web services. The key features of the program include, but are not limited to, easy login using any smart device, user privacy keeping, simple data entry, effective tracking of existing data, efficient plan creating tool, graphical representation of data, and capability to troubleshoot problems.

B. User Inputs for "EZSafety" Software

1. Accessing the Program

To use the program, users must create personal accounts by visiting the "Sign Up" page seen on the program webpage (https://www.ezsafety.net). The required personal information for any account includes the following: first name, last name, email account, password, and birthday (Figure 1). Thereafter, users can “Sign In” to their account (Figure 2). Requiring users to have personal accounts will save their data in the program’s database and allow them to keep their personal privacy. Saving their sampling history in the database will also allow users to retrieve their data in case it is lost or accidentally deleted.

2. Categorize Sample Types by Environmental Zones

The zoning concept was implemented into the program in which users will be able to categorize the sampling environment into three distinct types based on product and process risk levels (Figure 3). Three kinds of environmental zones were built in the program. Zone 1 includes food contact surfaces (e.g., worktables, slicers, conveyor belts, peelers, air blowers, fillers, screens, hoppers, knives, racks, and employee hands). Zone 2 comprises non-food contact surfaces that are close to Zone 1 (e.g., cooling units, equipment exterior, control panels, switches, framework, etc.). Zone 3 involves non-food contact surfaces that are farther from Zone 1 and 2 (e.g., floors, drains, walls, other equipment and structures, hand trucks, forklifts, carts, wheels, air return covers, hoses, etc.). The program can help the user to determine the type of zone during data entry by displaying a pop-window during zone selection in the Add New Data page (Figure 4). The definitions and examples of zones can be accessed by visiting the Guidelines page (Figure 5).

3. Select Sample Sites

Several guidelines for common sampling sites contaminated with *Listeria* spp. were implemented in the program to help users to target this organism in their processing facilities. Users can browse these sites before conducting their sampling by clicking on the “Guidelines” icon in the main page of the program. By clicking on this icon, users can view several useful details related to *Listeria* environmental sampling. These details include possible product sources of *Listeria*, potential food contact contaminated with *Listeria*, and potential non-food contact surfaces contaminated with *Listeria* (Figure 6). This tool can play a major role to enhance the user’s decision to target the common sites contaminated with *Listeria* spp. and increase the probability of detecting it.

4. Select Type of Microbiological Tests

Other microbiological test options and different indicators were included in the software to allow users to target a specific contaminant before starting the sampling plan. These tests include *Listeria* spp. and *Salmonella* spp. (qualitative) and Aerobic Plate Count (APC), Enterobacteriaceae, and ATP bioluminescence (quantitative). Users can select the type of target indicator by visiting the Create New Plan page (Figure 7). Users not only can target one type of contaminant per plan but also can combine two or more indicator organisms while creating their plans. For instance, they can target *Listeria* spp., Aerobic Plate Count (APC), and ATP bioluminescence count all together in the same plan and that will eventually allow them to enter the test result for each category and alert them if actions are needed to reduce or prevent microbial contamination.

5. Initiating the Sampling Plan

5.1. Select number of samples based on size of processing facility

The “Create New Plan” option allows users to initiate their sampling plans and decide which kind of tests they need. Before initiating the sampling plan, the program can help the user to decide on an appropriate number of samples to collect based on the size of their operation. Users can classify their processing facility by the number of employees in the processing facility or by the daily production volume. Based on the number of employees, processing facilities were categorized into very small (<10 employees), small (10-499 employees), and large (500 or more employees). Based on the daily production volume, processing plants were classified into very small (1-6,000 pounds/day), small (6,001-50,000 pounds/day), and large (50,001,>600,000 pounds/day), as shown in Figure 8. The program can also recommend the minimum number of samples that should be collected from each size of establishment as the following: one sample unit from very small establishments, two sample units from small establishments, and three sample units from large establishments. A sample unit consists of 20 samples collected from food contact surfaces swabs (Zone 1), non-food contact surfaces swabs, and intact product swabs (Figure 9). The recommended percentage of sampling that were suggested for users to target *Listeria* spp. were 10-20% for Zone 1, 40-50% for Zone 2, and 20-30% for Zone 3 (Figure 10).

5.2. Select sampling frequency

After determining the size of the processing facility, users can choose a certain organism to target and that will be followed by deciding the intended number of samples and the frequency of sampling in each zone. EZSafety can recommend the minimum routine sampling frequency for the plan. The lowest sampling frequency that was suggested to target *Listeria* spp. is 3-5 samples from Zone 1, 8-10 samples from Zone 2, and 3-5 samples from Zone 3 per plan each time (daily, weekly, biweekly, or monthly). However, EZSafety was also designed to allow users to choose the total number of samples and sampling frequency for the monitoring plan based on their preferences. After adding the frequency of sampling into the program, users can choose the start date and end date of the plan. After initiating the plan, the anticipated number of samples that should be collected each day/week from each zone will be displayed in the Plan page. This tool will allow users to feed certain information into the program and that will be a useful approach to initiate appropriate environmental sampling plans to target and prevent *Listeria* contamination events.

C. Acceptable Limits and Rejection Criteria for Sample Test Results

To provide guidance to the user to initiate appropriate plans to target *Listeria* spp. and/or other indicators, certain test result limits were implemented into the program. These limits were employed by using certain codes to allow the program to respond if unacceptable test results are found. The goal of using coding was to allow the program to react to any unacceptable test result and alert the user to adjust the sampling plan, if necessary, based on their data entry. *Listeria* spp. and *Salmonella* spp. test result categories were assigned to be qualitative (positive or negative) when new data is entered by users. The coding language was translated to allow the program to differentiate between positive and negative test results. As shown in Figure 11, the program will only respond if positive *Listeria* results are entered by users and will display a pop-up window to alert them that actions are needed. After adding test results into the program, the text of unwanted findings was defined with distinct colors in which the text of the word “Negative” was assigned to be green (Good) whereas the word “Positive” was specified with a red color (Bad). These colors can be seen in data summary page of the plan and will help the user to determine unwanted test results and take necessary actions to resolve problems (Figure 12).

In contrast, Aerobic Plate Count (APC), Enterobacteriaceae, and ATP bioluminescence test results were developed to be quantitative. The acceptable and rejection limits for Aerobic Plate Count (APC) and Enterobacteriaceae were designated as the following: a test result below 10 CFUs/in^2 was assigned as a passing result, a test result between 10-100 CFUs/in^2 was translated as an unsatisfactory result, and a test result above 100 CFUs/in^2 as a failing result (<10=pass, 10-100= Not satisfactory, >100=fail). For ATP bioluminescence, a result showing 150 relative light units (RLUs) and below was set as pass, and a result with 300 RLUs and above was set as fail. The program can also suggest several corrective actions specific to each zone when unacceptable results are entered so that users can troubleshoot problems and prevent further contaminations.

D. Analytical Reports

As shown in Figure 13, an analytical report tool was developed to allow users to have a visual representation of their data. The report tool can allow users to have a basic overview of the history of sampling. This tool can translate qualitative test results (e.g., *Listeria* spp. and *Salmonella* spp. test results) and quantitative test results (e.g., Aerobic Plate Count, Enterobacteriaceae, and ATP test results) into graphs, charts, and tables. This tool allows users to keep track of their monitoring over time and allow them to detect important trends and correlations. Eventually, after choosing an indicator organism, users can select a specific period (day, month, and year) and show the data summary within the selected period. The report option can be a useful method to summarize and download data, and it allows the user to take necessary actions to prevent unwanted findings.

E. Corrective Actions

As shown in Figure 14, a corrective actions tool was created to provide users with suggested actions that can be undertaken in response to any positive findings or test result above acceptable limits. Several kinds of corrective actions specific to each kind of zone (Zone 1, Zone 2, and Zone 3) and case scenarios (e.g., first contamination, recurring contamination) were added into the program (Figure 15). General corrective actions with different attributions for each kind of error or failure in the production line were also added to provide the user with numerous solutions to resolve issues. The corrective actions' general guide can be accessed by clicking on the "Corrective Actions" icon in the main page. The specific corrective actions were also implemented into the program by coding in which the program will respond to any unwanted test result by displaying a pop-up window that alerts the user and suggests several necessary actions to resolve the problem. Seven types of corrective actions category were added to the program, and they are categorized as the following:

1. General Corrective Actions to Prevent Recontamination.
2. General Corrective Actions for a Single Positive *Listeria* Result in (Zones 1, Zone 2, and Zone 3).
3. Corrective Actions for Single *Listeria* Detection in Zone 1.
4. Corrective Actions for Single *Listeria* Detection in Zone 2.
5. Corrective Actions for Reoccurring Positive *Listeria* Results in Zone 1.
6. Corrective Actions for Reoccurring Positive *Listeria* Results in Zone 2.
7. Corrective Actions for Reoccurring Positive *Listeria* Results in Zone 3.

F. Evaluation of the "EZSafety" Program

A permission from the Institutional Review Board (IRB) was obtained before surveying the participants. A total of 20 food safety professionals or individuals with a background in food safety/microbiology and environmental monitoring were asked to evaluate the program. They were given a set of instructions, specific usernames, and passwords to access the program and assess its ability to initiate appropriate sampling plans, report sampling test results, sort data, and suggest changes to the plan if unwanted results are found. They were assigned to add 400 previously collected *Listeria* and APC sample test results (200 *Listeria* spp. and 200 APC test results) into the program. As shown in Table 1, each participant was given a data set comprising 10

Listeria and 10 ATP sample test results previously collected from 6 turkey processing plants. The participants were asked to initiate sampling plans based on the test results summarized in their data sheets. Before creating the plan, the participants were asked to check the Guidelines and the Corrective Actions pages so that they have a basic overview about the sampling criteria for *Listeria* spp. in poultry processing plants. After that, they were asked to create a sampling plan for *Listeria* and APC by visiting the Data page, and they were asked to report the 20 sample test results to the program. After feeding the data into the program, the participants were asked to adjust their sampling plans in response to any positive finding based on the corrective actions provided by the program. After using the program, the participants were asked to evaluate it by answering a short questionnaire designed in "QuestionPro" survey software to provide their feedback (Figure 16). Users' feedback was used to resolve issues and validate the data collection and decision-making tools of the program. The survey included 18 statements that covered the following topics:

1. Ease of use and privacy keeping ability.
2. Ability to record quantitative and qualitative data.
3. Ability to detect unacceptable limits of indicator organisms.
4. Ability to improve poultry processors environmental monitoring plans.
5. Ability to enhance poultry processors decision making.
6. Ability to provide guidelines and corrective actions to enhance environmental monitoring.

All 18 questions and summarized responses are included in Appendix A. Selected responses are discussed in the Results.

IV. Results

A. The Outcomes of the Program

1. Storage Abilities

After developing the monitoring program (EZSafety) and its various tools, the program's data was successfully stored in a protected web-cloud using a private domain and a server. Storage of the program's data on a protected webpage was essential to protect users' data especially when they initiate their sampling plans and start entering *Listeria* and/or indicators test results. EZSafety showed a noticeable ability to allow a large number of users to create personal accounts and start using the program in which more than 60 users were created, including the participants of the evaluation, and it did not feature any errors or malfunctions. The sampling program showed a capacity to record a huge amount of data and test results (up to 5GB) which can be stored over the platform's cloud.

2. Sampling Plan Determination

Regardless of the type of plan and indicator organism selected, the program successfully suggested a sampling plan that can be followed to enhance the environmental sampling based on the contact surface and process risk level. EZSafety was able to allow the user to initiate sampling plans by providing various kinds of guidelines and instructions which are necessary to conduct appropriate environmental monitoring for *Listeria* spp. and other indicators. To be more specific, these detailed instructions and tools can allow the user to determine the size of the processing establishment (very small, small, and large), the type of zone (food contact surface or non-food contact surface), the most common sites contaminated with *Listeria* spp., the recommended sampling frequency in each zone for *Listeria* spp., and the minimum number of *Listeria* spp. samples needed in each zone per the plan. Furthermore, the program demonstrated an ability to create multiple sampling plans for the same user to target several indicators or include multiple indicators in the same plan. The program with its various tools can support users to have a strategic sampling plan and detect or prevent negative outcomes.

3. Unacceptable Limits Detection

After initiating sampling plans, the program showed a 100% accuracy to record more than 1000 samples, sort data, and respond to unacceptable limits. The critical limits seen on the Guidelines page are an essential element of the program to enhance users' decision to conduct appropriate sampling plans and modify their sampling, if needed. Additionally, EZSafety was able to differentiate between acceptable findings and findings above critical limits. Followed by each entry of unwanted test results, the program was able to highlight these results and suggest modifying the monitoring plan. The coding language of unwanted findings allowed the program to display pop-windows and categorize the text of unwanted findings with specific colors (green "acceptable findings" and red "unacceptable findings"). The program was also able to provide a list of corrective actions specific to each zone (Zone 1, Zone 2, and Zone 3) and situation (first contamination, recurring contamination) to allow users to troubleshoot their issues and prevent their recurrence. EZSafety can suggest that the user modify the frequency and number of samples and increase them after each detection of indicator organism. The key functionalities of the sampling program can be summarized to:

- Allows users to initiate a sampling plan based on the size of their processing establishments.
- Enables users to conduct qualitative and quantitative test results (*Listeria* spp., *Salmonella* spp., APC, EB, and ATP).
- Allows users to target the common contaminated sites with *Listeria* spp. in Zone 1, Zone 2, and Zone 3.
- Assists users to determine the optimal time to target *Listeria* and other indicators.
- Suggests a sampling frequency and number of samples based on the size of the plant.
- Recommends adjusting the monitoring plan if unwanted test results are found.
- Provides a list of corrective actions specific to each zone to troubleshoot issues and prevent future contaminations.

B. Evaluation of the Sampling Program “EZSafety”

An evaluation of the environmental monitoring program “EZSafety” was conducted through users’ experiences followed by answering a questionnaire produced in QuestionPro survey software (QuestionPro, Inc., Dallas, TX). The survey platform enabled users (20) to submit their responses securely online. After completing the questionnaires, the survey statements were analyzed using the data analysis tools found in QuestionPro. The majority of the participants believed that EZSafety has several tools that are effective to enhance environmental monitoring. Additionally, most of the participants had an agreement that EZSafety is organized and user-friendly. Listed below are the key findings of the survey.

As Figure 17 shows, all of the 20 participants agreed (70% strongly agreed and 30% agreed) that EZSafety can enhance environmental monitoring plans to target *Listeria* spp. in poultry processing plants. 18 out of 20 respondents reported that EZSafety can improve poultry processors' decision making to initiate appropriate sampling plans to target *Listeria* (60% strongly agreed and 30% agreed, as shown in Figure 18). Furthermore, 90% of the participants agreed (17 respondents strongly agreed and 1 respondent agreed) that this monitoring program can detect unacceptable limits of indicator organisms when data is administered into the program by users (Figure 19). All of the 20 participants agreed (65% strongly agreed and 35% agreed) that the guidelines and recommendations provided by EZSafety are useful to enhance environmental monitoring plans to target *Listeria* spp. and other indicators (Figure 20). As shown in Figure 21, the majority of the participants agreed that EZSafety can recommend appropriate corrective actions to resolve issues resulting from *Listeria* contamination events. Finally, 19 out 20 respondents (95%) reported that EZSafety is organized and easy to use to conduct proper environmental monitoring plans (Figure 22). The complete set of questions and response summaries are found in Appendix A.

V. Discussion

This study emphasized the significance of using environmental monitoring programs (EMPs) to enhance environmental monitoring plans and prevent contamination in poultry and other food processing establishments. EMPs not only can assist poultry processors to target indicator organisms but also, they can provide several effective tools to enhance their environmental monitoring plans which are achieved by determining important trends, associating various patterns based on data recording, and preventing the recurrence of unwanted findings. If developed based on product and process risk levels and control measures, EMPs can play a significant role in improving the detection rate and prevention of *Listeria* spp. and indicators in poultry processing plants.

There are several commercially available environmental monitoring programs which are used to target indicator organisms and/or pathogens in various types of processing plants including meat and poultry, produce, and milk. Enviromap (bioMérieux, Inc., Durham, NC), is a cloud-based environmental monitoring program used to target indicator organisms and pathogens in various food processing plants including meat, poultry, and produce. This monitoring platform has a variety of tools that can enhance the environmental monitoring. Another commercially available environmental monitoring program is CONTROL-PRO (Corvium, Inc., Reston, VA), an internet-based program, used to conduct environmental monitoring plans for food processors in their processing plants. Similar to EZSafety, Enviromap and CONTROL-PRO can suggest sampling plans and allow users to sort and record their data over time. However, both Enviromap and CONTROL-PRO have additional features that can support the data collection and record keeping tools. For instance, Enviromap can allow users to conduct automatic sample scheduling, classify their collected samples by mapping them on the floor plan, notify users about important trends via text or email, and print custom sample barcodes or labels for identification. Likewise, CONTROL-PRO platform can allow users to visualize their sampling plants by creating a 3D design that can highlight contaminated sites and risk points which can be stored in the program's databases. This platform is also capable of randomly selecting sites, either for routine monitoring or for contaminated spot testing.

In spite of the above, a key difference between EZSafety and the above platforms is that EZSafety can be accessed without the need for a subscription or monthly/yearly service fees which are seen on the platforms' websites. The cost of environmental monitoring programs may become a barrier that may not allow food processors to automate their environmental monitoring plans. Moreover, the monitoring program of the current study was carried out with an approach of simplicity in which its various tools can be implemented to monitor the environment of any kind of poultry processing plants.

EZSafety has various tools that can improve environmental monitoring in poultry processing plants. Employing the zoning concept into the program is one of the useful approaches that can provide a broad overview of the types of sites and surfaces that can be categorized based on the risk levels of product contamination. Processors can use this concept to improve *Listeria* detection rate and focus on a particular area (zone) when contamination is present. The zoning concept may also help poultry processors to prevent the spread of a contaminant from one zone to another by allowing users to verify their cleaning and sanitation practices in the affected zone. Nonetheless, EZSafety can provide users with numerous *Listeria* monitoring guidelines and baselines, including, the optimal time to target *Listeria* spp. and other indicators, the common contact surfaces contaminated *Listeria* spp., the recommended sampling frequency in each zone to target *Listeria* spp., the minimum number of samples necessary to be collected based on the size of the production plant, and the suggested corrective actions used to overcome undesirable findings. These various tools can be a crucial element of an effective environmental monitoring program that can allow poultry processors to enhance their monitoring plans and prevent contamination of ready-to-eat or finished products.

Based on participants' experiences and feedback, certain issues and limitations of the monitoring program were determined (Table 2). Most of the issues were resolved during the evaluation process and that included system malfunctions such as plan data entry errors, incorrect number of Zone 2 samples after dividing the total number of samples by the selected percentage of the same zone, and inability to adjust the number of samples in response to unwanted test results. Yet, EZSafety has a variety of limitations that could be overcome to further improve its data collection and record keeping tools. Some of the limitations can be summarized as follows: no export or print option for data, the program does not randomly suggest sampling sites prior to swabbing, positive test results shown in the data summary page should be hyperlinked, and the report tool should be based on individual plans and not only the time frame of sampling. Implementing these features into the program will aid in improving its efficacy to target indicators and enhance environmental monitoring.

Regardless, EZSafety can be further improved to expand its abilities and features to enhance environmental monitoring in processing plants and prevent outbreaks. Future modifications could include adding other indicator organisms beside *Listeria* spp. *Salmonella* spp., APC, EB, and ATP (e.g., total coliform and fecal coliform). EZSafety can be also modified to conduct environmental monitoring plans specific to the type of product (raw or ready-to-eat), surface (e.g., rough or smooth; stainless-steel or wood), and processing facilities (e.g., meat, milk, or produce). The program can be designed to work as a pathogen environmental monitoring program (PEMP) and target pathogens (e.g., *Listeria monocytogenes*, pathogenic *E. coli*, *Campylobacter jejuni,* etc.). Such indicators and pathogens can be targeted using quantitative and qualitative measures based on users' preferences. Moreover, EZSafety can be developed to allow users to choose and modify their pass, fail, and marginal limits based on their products and processing plants. This feature will allow the program to respond if their test results are higher than the desired limits. Instead of having colony-forming units (CFUs) implemented in the program with square inch only (CFUs/inch^2), other units such as CFUs per milliliter (CFUs/mL) or CFUs per gram (CFUs/g) could be also developed to allow users to select the unit based on their preferences. Zone 4 could be added to the program beside the three types of zones (Zone 1, Zone 2, and Zone 3) which will provide users with a choice to test for this area if they aim to conduct farther environmental monitoring. Flowcharts and diagrams that display suggested sampling plans or lists of corrective actions based on the type of product and zone can be developed to provide the user with useful guidelines to follow and therefore improve the environmental monitoring.

A data entry tool can be created to allow users to enter existing datasets into the program which could be sorted to highlight significant trends. A notification tool can be developed to alert users to take necessary actions and follow up with important trends. If achieved, this tool can allow the program to send text messages and emails to users so that they can be reminded if the sampling plan is not operating appropriately. More codes could be added to the program to support its reporting tools and alert the user if issues are found. For instance, no codes are currently employed in the program to alert the user if too many unsatisfactory results are found. Therefore, developing more codes to send alerts and pop-up windows to users will be a useful approach to predict unwanted findings and enhance the environmental monitoring. Algorithm and machine learning can also be implemented so that the program can detect certain patterns or findings and provide users with feedback and suggestions to enhance their environmental monitoring.

Automated environmental monitoring plans can have a positive impact on food safety and public health. Poultry processors can enhance the detection rate of indicator organisms and prevent the transfer of contaminated products to consumers. Ultimately, EMPs such as EZSafety can provide poultry processors with effective tools to answer the following monitoring questions: where to sample?, when and how often to sample?, and how to sample?. Environmental monitoring programs can enhance the monitoring plans of not only poultry products but other commodities as well.

VI. References

3M & Cornell University. (2019). *Environmental monitoring handbook*. Retrieved July 12, 2021, from https://multimedia.3m.com/mws/media/1684575O/environmental-monitoring-handbook.pdf

3M. (2016). *How clean is it? What ATP can tell you about the safety of your food handling areas*. 3M Food Safety News. Retrieved March 30, 2022, from https://food-safety-news.3m.com/fsn/how-clean-is-it-what-atp-can-tell-you-about-the-safety-of-your-food-handling-areas/

Almond Board of California. (2010). *Pathogen environmental monitoring program (PEM)*. Retrieved July 30, 2021, from https://www.almonds.com/sites/default/files/content/attachments/pem_book.pdf

Aury, K., le Bouquin, S., Toquin, M. T., Huneau-Salaün, A., le Nôtre, Y., Allain, V., Petetin, I., Fravalo, P., & Chemaly, M. (2011). Risk factors for *Listeria monocytogenes* contamination in french laying hens and broiler flocks. *Preventive Veterinary Medicine*, *98*(4), 271–278. https://doi.org/10.1016/j.prevetmed.2010.11.017

Carpentier, B. C., & Barre, L. B. (2012). Guidelines on sampling the food processing area and equipment for the detection of *Listeria monocytogenes*. *French Agency for Food, Environmental and Occupational Health Safety*, *3*, 6–7. https://www.researchgate.net/publication/281600684_Guidelines_on_Sampling_the_Food_Processing_Area_and_Equipment_for_the_Detection_of_*Listeria*_monocytogenes

Centers for Disease Control and Prevention. (2018, November 5). *Estimates of foodborne illness in the United States*. https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html

Centers for Disease Control and Prevention. (2021). *Listeria*. Retrieved August 2, 2021, from https://www.cdc.gov/*Listeria*/index.html.

Channaiah, L.H. (2015, February). *Improving environmental controls*. Food Quality & Safety. Retrieved March 22, 2022, from https://www.foodqualityandsafety.com/article/improving-environmental-controls/2/

Chen, M., Wu, Q., Zhang, J., Yan, Z., & Wang, J. (2014). Prevalence and characterization of *Listeria monocytogenes* isolated from retail-level ready-to-eat foods in south china. *Food Control*, *38*, 1–7. https://doi.org/10.1016/j.foodcont.2013.09.061

Chmielewski, R., & Frank, J. (2003). Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety*, *2*(1), 22–32. https://doi.org/10.1111/j.1541-4337.2003.tb00012.x

Cornell University. (2022). *Control of Listeria in produce food safety laboratory and milk quality improvement program*. Retrieved June 28, 2022, from https://foodsafety.foodscience.cornell.edu/links/control-listeria-produce/

Cutter, C. N. (2016, June 28). *Control of Listeria monocytogenes in meat and poultry*. Penn State Extension. Retrieved March 29, 2022, from https://extension.psu.edu/control-of-Listeria-monocytogenes-in-meat-and-poultry

Cutter, C. N., McElroy, D., & Penn, S. (2017, November 5). *Control of Listeria monocytogenes in retail establishments*. Pennsylvania State University. https://extension.psu.edu/control-of-Listeria-monocytogenes-in-retail-establishments

de Noordhout, C. M., Devleesschauwer, B., Angulo, F. J., Verbeke, G., Haagsma, J., Kirk, M., Havelaar, A., & Speybroeck, N. (2014). The global burden of listeriosis: A systematic review and meta-analysis. *The Lancet Infectious Diseases*, *14*(11), 2–3. https://doi.org/10.1016/s1473-3099(14)70870-9

Donlan, R. M., & Costerton, J. W. (2002). Biofilms: Survival mechanisms of clinically relevant microorganisms. *Clinical Microbiology Reviews*, *15*(2), 167–193. https://doi.org/10.1128/cmr.15.2.167-193.2002

Dosland, O. L. (2018, April 18). *Monitoring microorganisms in a food plant*. Quality Assurance & Food Safety. Retrieved March 29, 2022, from https://www.qualityassurancemag.com/article/monitoring-microorganisms-in-a-food-plant/

Elmali, M., Can, H. Y., & Yaman, H. (2015). Prevalence of *Listeria monocytogenes* in poultry meat. *Food Science and Technology*, *35*(4), 672–675. https://doi.org/10.1590/1678-457x.6808

Food and Drug Administration. (2017, January). *Control of Listeria monocytogenes in ready-to-eat foods: Guidance for industry. Draft guidance.* Food and Drug Administration. Retrieved March 22, 2022, from https://www.fda.gov/media/102633/download-monocytogenes-in-ready-to-eat-foods-part-3-monitoring-activities

Food and Drug Administration. (2020, August 3). *Get the facts about Listeria*. https://www.fda.gov/animal-veterinary/animal-health-literacy/get-facts-about-listeria#statistics

Food Safety and Inspection Service. (2022, January 28). *Annual recall summaries*. Retrieved June 28, 2022, from https://www.fsis.usda.gov/food-safety/recalls-public-health-alerts/annual-recall-summaries/summary-recall-cases-calendar-8

Food Safety and Inspection Service. (2022). *Listeria* Rule Verification Activities, FSIS Directive 10,240.4 Rev. 4 (3/25/22). Retrieved May 22, 2022 from https://www.fsis.usda.gov/sites/default/files/media_file/2020-08/10240.4.pdf

Food Safety and Inspection Service. (2014, January). *Controlling Listeria monocytogenes in post-lethality exposed Ready-to-Eat meat and poultry products*. Retrieved March 25, 2022, from https://www.fsis.usda.gov/guidelines/2014-0001

Food Safety and Inspection Service. (2015, June). *Microbiological sampling of raw poultry*. https://www.fsis.usda.gov/sites/default/files/import/Microbiological-Testing-Raw-Poultry.pdf

Food Safety and Inspection Service. (2015, October 16). *Understanding FSIS food recalls*. https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/food-safety-basics/understanding-fsis-food-recalls

Foundation for Meat and Poultry Research and Education. (n.d.). *Microbiological testing*. Retrieved September 21, 2021, from https://www.meatpoultryfoundation.org/fact-sheets/microbiological-testing

Giaouris, E., Heir, E., Hébraud, M., Chorianopoulos, N., Langsrud, S., Møretrø, T., Habimana, O., Desvaux, M., Renier, S., & Nychas, G. J. (2014). Attachment and biofilm formation by foodborne bacteria in meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel methods. *Meat Science*, *97*(3), 298–309. https://doi.org/10.1016/j.meatsci.2013.05.023

Grocery Manufacturers Association. (2014, July 7). *Listeria monocytogenes guidance on environmental monitoring and corrective actions in at-risk foods*. Ucfoodsafety. Retrieved March 15, 2022, from https://ucfoodsafety.ucdavis.edu/sites/g/files/dgvnsk7366/files/inline-files/208833.pdf

Heinzelmann, J. (2021, April 7). *Quantifying the ROI of environmental monitoring program automation*. FoodSafetyTech. Retrieved March 29, 2022, from https://foodsafetytech.com/feature_article/quantifying-the-roi-of-environmental-monitoring-program-automation/

Hussain, M., & Dawson, C. (2013). Economic impact of food safety outbreaks on food businesses. *Foods*, *2*(4), 585–589. https://doi.org/10.3390/foods2040585

International Commission on Microbiological Specifications for Foods (ICMSF). (2016). *Microorganisms in foods 8: Use of data for assessing process control and product acceptance* (Softcover reprint of the original 1st ed. 2011 ed.) [E-book]. Springer. https://doi.org/10.1007/978-1-4419-9374-8

International Dairy Federation. (2020, June). *Processing environment monitoring*. https://fil-idf.org/wp-content/uploads/2020/06/IDF-factsheet-Processing-Environment.pdf

Jalali, M., & Abedi, D. (2008). Prevalence of *Listeria* species in food products in isfahan, iran. *International Journal of Food Microbiology*, *122*(3), 336–340. https://doi.org/10.1016/j.ijfoodmicro.2007.11.082

Keller, S.E., Merker, R.I., Taylor, K.T., Tan, H.L., Melvin, C.D., Chirtel, S.J., & Miller, A.J. (2002). Efficacy of sanitation and cleaning methods in a small apple cider mill. *Journal of Food Protection*, *65*(6), 911–917. https://doi.org/10.4315/0362-028x-65.6.911

Lakicevic, B., Nastasijevic, I., & Raseta, M. (2015). Sources of *Listeria monocytogenes* contamination in retail establishments. *Procedia Food Science*, *5*, 160–163. https://doi.org/10.1016/j.profoo.2015.09.046

Leclercq, A., Oevermann, A., Danguy-des-Déserts, R., Granier, S., Hammack, T., Jinneman, K., Chen, Y., Rathbone, R. and Riegler, G. (2014). *Listeria monocytogenes*. Chapter 2.9.7 in OIE (World Organisation for Animal Health) Terrestrial Manual. Retrieved from https://www.researchgate.net/publication/266858383_Listeria_monocytogenes

Magdovitz, B.F., Gummalla, S., Thippareddi, H., & Harrison, M. A. (2019). Evaluating environmental monitoring protocols for *Listeria* spp. And *Listeria monocytogenes* in frozen food manufacturing facilities. *Journal of Food Protection*, *83*(1), 172–187. https://doi.org/10.4315/0362-028x.jfp-19-190

Martinon, A., Cronin, U., Quealy, J., Stapleton, A., & Wilkinson, M. (2012). Swab sample preparation and viable real-time PCR methodologies for the recovery of escherichia coli, staphylococcus aureus or *Listeria monocytogenes* from artificially contaminated food processing surfaces. *Food Control*, *24*(1–2), 86–94. https://doi.org/10.1016/j.foodcont.2011.09.007

Ministry for Primary Industries. (2017, February 13). *Guidance for the control of Listeria monocytogenes in ready-to-eat foods part 3: Monitoring activities*. Ministry for Primary Industries. Retrieved March 22, 2022, from https://www.mpi.govt.nz/dmsdocument/16306-guidance-for-the-control-of-listeria-monocytogenes-in-ready-to-eat-foods-part-3-monitoring-activities

Manju, G. & Mishra, S.K. (2021). Microbiological environmental monitoring in food processing. *Indian Food Industry Mag*, *3*(2), 48–49.

Mota, J. M., Boué, G., Prévost, H., Maillet, A., Jaffres, E., Maignien, T., Arnich, N., Sanaa, M., & Federighi, M. (2021). Environmental monitoring program to support food microbiological safety and quality in food industries: A scoping review of the research and guidelines. *Food Control*, *130*, 1–2. https://doi.org/10.1016/j.foodcont.2021.108283

Mota, J., Boué, G., Prévost, H., Maillet, A., Jaffres, E., Maignien, T., Arnich, N., Sanaa, M., & Federighi, M. (2021). Environmental monitoring program to support food microbiological safety and quality in food industries: A scoping review of the research and guidelines. *Food Control*, *130*, 1–2. https://doi.org/10.1016/j.foodcont.2021.108283

Muhterem-Uyar, M., Dalmasso, M., Bolocan, A.S., Hernandez, M., Kapetanakou, A.E., Kuchta, T., Manios, S.G., Melero, B., Minarovičová, J., Nicolau, A.I., Rovira, J., Skandamis, P.N., Jordan, K., Rodríguez-Lázaro, D., Stessl, B., & Wagner, M. (2015). Environmental sampling for *Listeria monocytogenes* control in food processing facilities reveals three contamination scenarios. *Food Control*, *51*, 94–107. https://doi.org/10.1016/j.foodcont.2014.10.042

Osimani, A., Garofalo, C., Clementi, F., Tavoletti, S., & Aquilanti, L. (2014). Bioluminescence ATP monitoring for the routine assessment of food contact surface cleanliness in a university canteen. *International Journal of Environmental Research and Public Health*, *11*(10), 10824–10837. https://doi.org/10.3390/ijerph111010824

Overney, A., Jacques-André-Coquin, J., Ng, P., Carpentier, B., Guillier, L., & Firmesse, O. (2017). Impact of environmental factors on the culturability and viability of *Listeria monocytogenes* under conditions encountered in food processing plants. *International Journal of Food Microbiology*, *244*, 74–81. https://doi.org/10.1016/j.ijfoodmicro.2016.12.012

Rahimi, E., Momtaz, H., Behzadnia, A., & Baghbadorani, Z. T. (2014). Incidence of *Listeria* species in bovine, ovine, caprine, camel and water buffalo milk using cultural method and the PCR assay. *Asian Pacific Journal of Tropical Disease*, *4*(1), 50–53. https://doi.org/10.1016/s2222-1808(14)60313-3

Reiter, M.G.R., Bueno, C.M.M., Lopez, C., & Jordano, R. (2005). Occurrence of *Campylobacter* and *Listeria monocytogenes* in a poultry processing plant. *Journal of Food Protection*, *68*(9), 1903–1904. https://doi.org/10.4315/0362-028x-68.9.1903

Rothrock, M.J., Davis, M.L., Locatelli, A., Bodie, A., McIntosh, T.G., Donaldson, J.R., & Ricke, S.C. (2017). *Listeria* occurrence in poultry flocks: Detection and potential implications. *Frontiers in Veterinary Science*, *4*, 1–2. https://doi.org/10.3389/fvets.2017.00125

Scharff, R.L. (2012). Economic burden from health losses due to foodborne illness in the united states. *Journal of Food Protection*, *75*(1), 123–131. https://doi.org/10.4315/0362-028x.jfp-11-058

Sebastián, M., Arilla-Luna, S., Bellalou, J., Yruela, I., & Medina, M. (2019). The biosynthesis of flavin cofactors in *Listeria monocytogenes*. *Journal of Molecular Biology*, *431*(15), 2762–2776. https://doi.org/10.1016/j.jmb.2019.05.029

Shahbandeh, M. (2021, April 14). Poultry industry in the united states- statistics & facts. Statista. Retrieved March 30, 2022, from https://www.statista.com/topics/6263/poultry-industry-in-the-united-states/

Spanu, C., & Jordan, K. (2020). *Listeria monocytogenes* environmental sampling program in ready-to-eat processing facilities: A practical approach. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 2843–2861. https://doi.org/10.1111/1541-4337.12619

Srey, S., Jahid, I. K., & Ha, S.D. (2013). Biofilm formation in food industries: A food safety concern. *Food Control*, *31*(2), 572–585. https://doi.org/10.1016/j.foodcont.2012.12.001

The Food Industry Association. (2021, January 13). *FDA recalls 2017–2020*. https://www.fmi.org/docs/default-source/food-safety/fs21--fda-recall-report_1-13-21_v2_.pdf?sfvrsn=19c8d6d_2

The Pennsylvania State University. (2019, October 17). *Food safety modernization act: The zone concept*. Penn State Extension. Retrieved March 21, 2022, from https://extension.psu.edu/food-safety-modernization-act-the-zone-concept

The Pew Charitable Trusts. (2016, September). *Emerging pathogens in meat and poultry*. https://www.pewtrusts.org/en/research-and-analysis/reports/2016/09/emerging-pathogens-in-meat-and-poultry

Tompkin, R.B. (2002). Control of *Listeria monocytogenes* in the food processing environment. *Journal of Food Protection*, *65*(4), 709–725. https://doi.org/10.4315/0362-028x-65.4.709

USDA. (2021, April). *Poultry - production and value 2020 summary*. National Agricultural Statistics Service. Retrieved March 30, 2022, from https://www.nass.usda.gov/Publications/Todays_Reports/reports/plva0421.pdf

USDA. (2022, March 10). *USDA ERS - poultry & eggs*. Economic Research Service U.S. Department of Agriculture. Retrieved March 30, 2022, from https://www.ers.usda.gov/topics/animal-products/poultry-eggs/

Vázquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., & Kreft, J. (2001). *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews*, *14*(3), 584–640. https://doi.org/10.1128/cmr.14.3.584-640.2001

World Health Organization. (2020, April 30). *Food safety*. https://www.who.int/NEWS-ROOM/FACT-SHEETS/DETAIL/FOOD-SAFETY

Zacharski, K.A., Southern, M., Ryan, A., & Adley, C.C. (2018). Evaluation of an environmental monitoring program for the microbial safety of air and surfaces in a dairy plant environment. *Journal of Food Protection*, *81*(7), 1108. https://doi.org/10.4315/0362-028x.jfp-17-464

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. *Comprehensive Reviews in Food Science and Food Safety*, *17*(5), 1156–1171. https://doi.org/10.1111/1541-4337.12366

VII. Figures and Tables

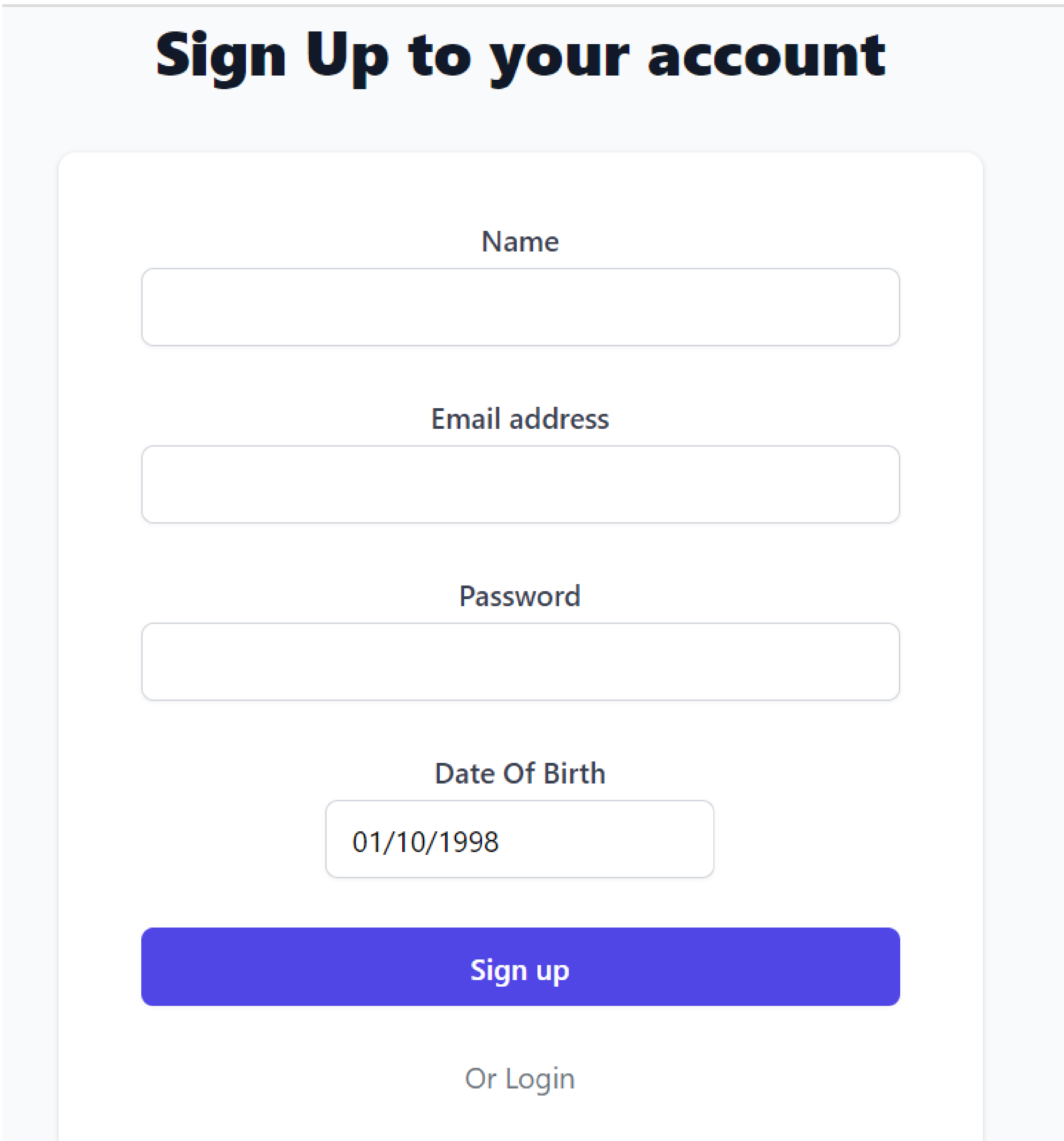


Figure 1: Create an account page in the main webpage of the program.

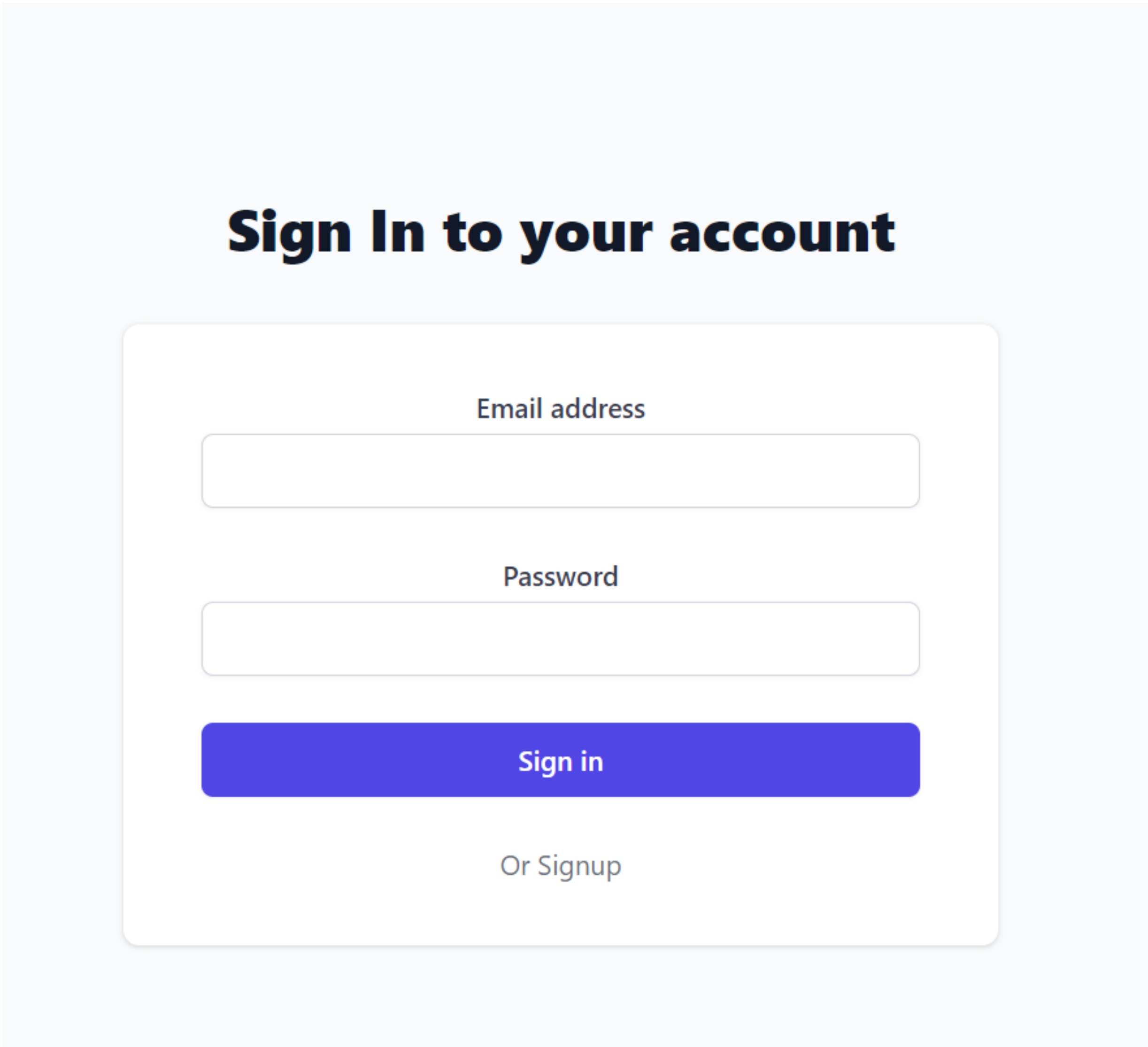


Figure 2: Users sign in page on the program’s main page.

Figure 3: Explanation of the types of environmental zones with examples of surfaces in each zone that was designed for the program.

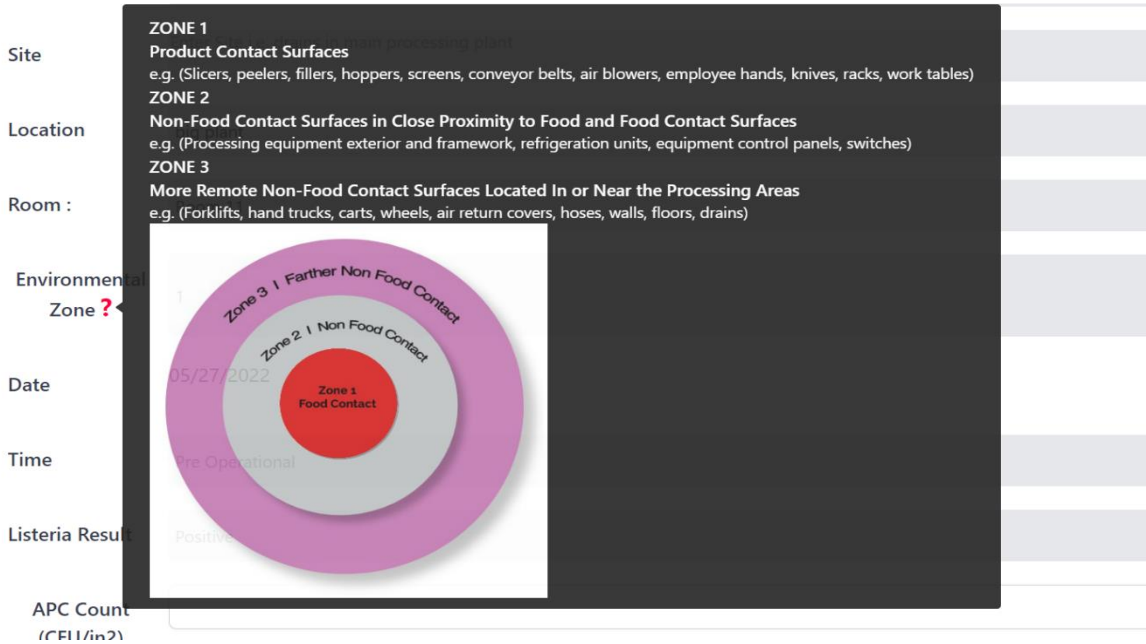
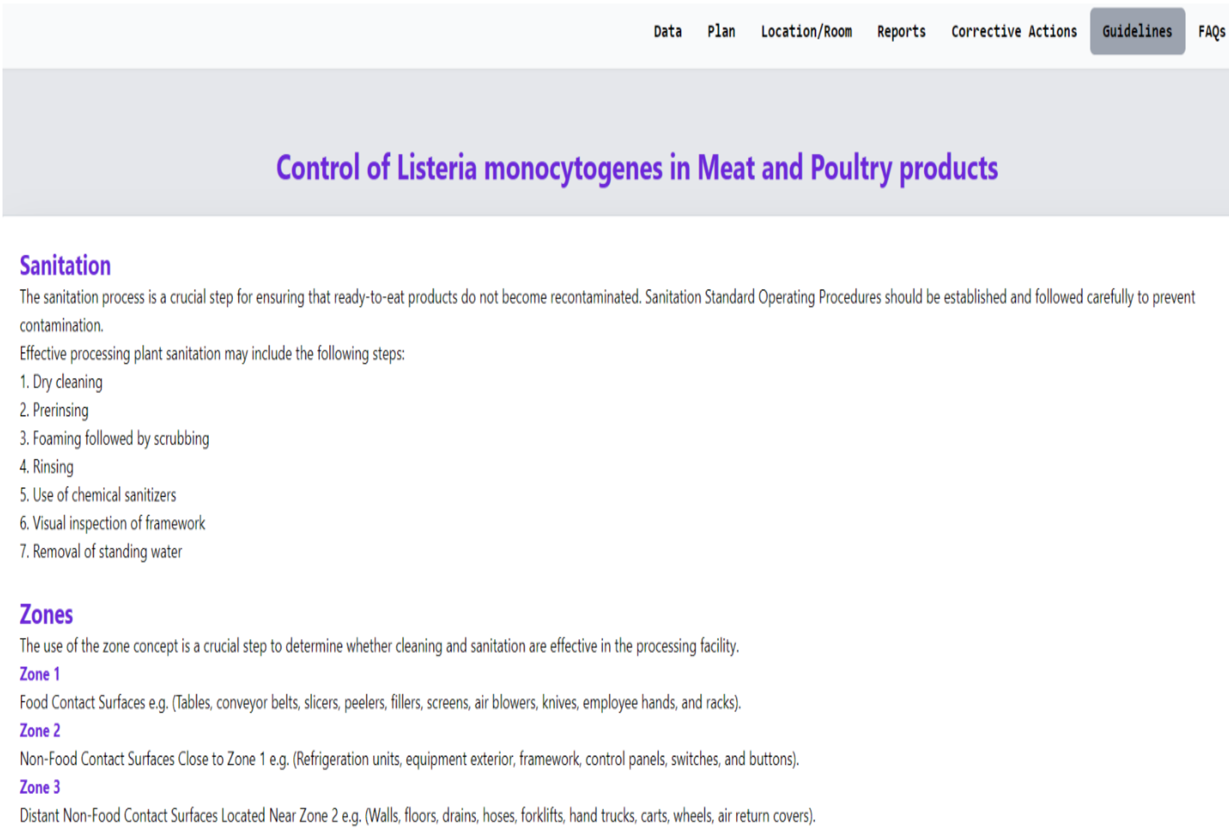


Figure 4: A pop-up window built in the Data entry page explaining the types of environmental zones with examples of surfaces in each zone.



Data Plan Location/Room Reports Corrective Actions Guidelines FAQs

Control of Listeria monocytogenes in Meat and Poultry products

Sanitation

The sanitation process is a crucial step for ensuring that ready-to-eat products do not become recontaminated. Sanitation Standard Operating Procedures should be established and followed carefully to prevent contamination.

Effective processing plant sanitation may include the following steps:

1. Dry cleaning
2. Prerinsing
3. Foaming followed by scrubbing
4. Rinsing
5. Use of chemical sanitizers
6. Visual inspection of framework
7. Removal of standing water

Zones

The use of the zone concept is a crucial step to determine whether cleaning and sanitation are effective in the processing facility.

Zone 1

Food Contact Surfaces e.g. (Tables, conveyor belts, slicers, peelers, fillers, screens, air blowers, knives, employee hands, and racks).

Zone 2

Non-Food Contact Surfaces Close to Zone 1 e.g. (Refrigeration units, equipment exterior, framework, control panels, switches, and buttons).

Zone 3

Distant Non-Food Contact Surfaces Located Near Zone 2 e.g. (Walls, floors, drains, hoses, forklifts, hand trucks, carts, wheels, air return covers).

Figure 5: Definitions of environmental zones with examples of surfaces for each zone in the Guidelines page.

Potential Food-Contact Surfaces Contaminated with Listeria

1. Tables
2. Slicers
3. Dicers
4. Saws
5. Casing peelers
6. Racks & Shelves
7. Containers
8. Ligs & tubs
9. Gloves & aprons
10. Packaging materials & equipment
11. Conveyors belts
12. Cleaning materials(sponges & brushes)

Possible Reservoirs of Listeria in Small Processing Plants

1. Drains and Floors
2. Ceilings
3. Pipes
4. Cooling units
5. Standing water
6. Wet areas
7. Cleaning materials (brushes, sponges, and squeegees)
8. Trolleys and rails

Figure 6: Common food and non-food contact surfaces contaminated with *Listeria* spp. in poultry processing plants shown in the Guidelines page of the program.

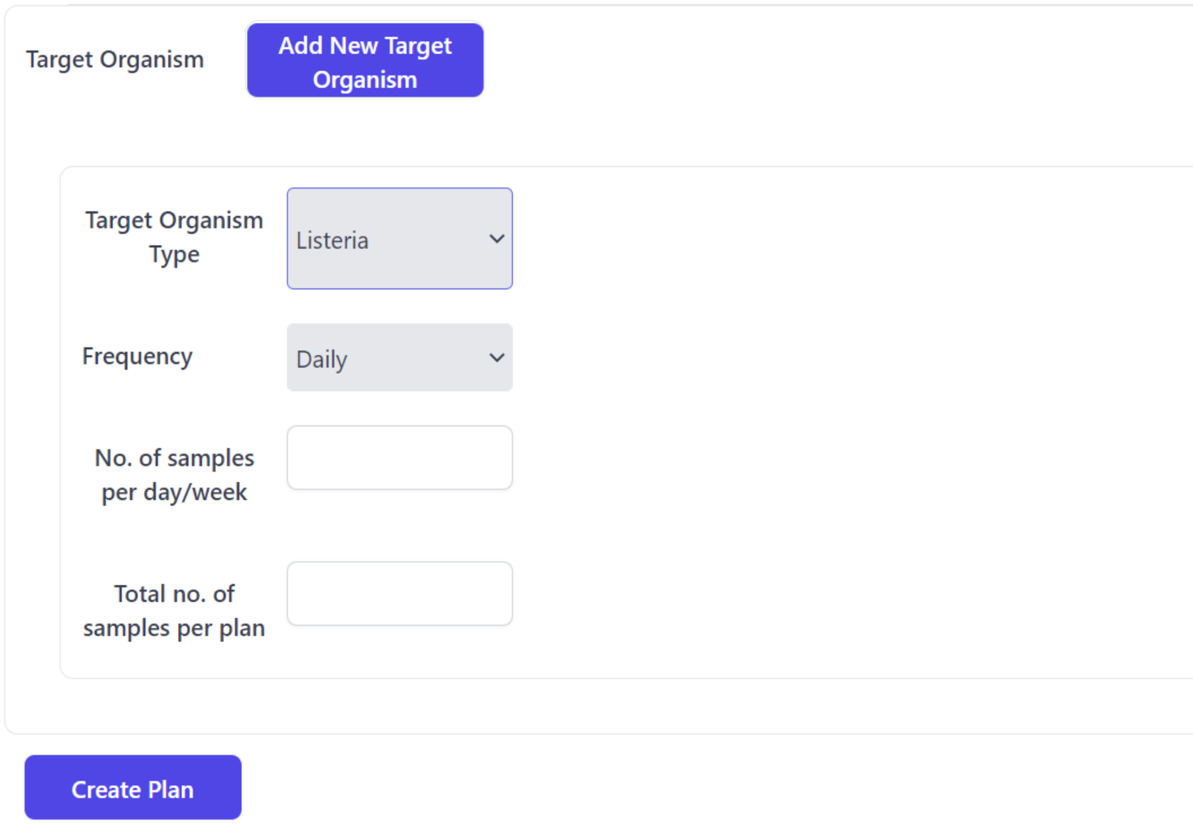


Figure 7: The types of target indicators included in the program in the Craete New Plan page.

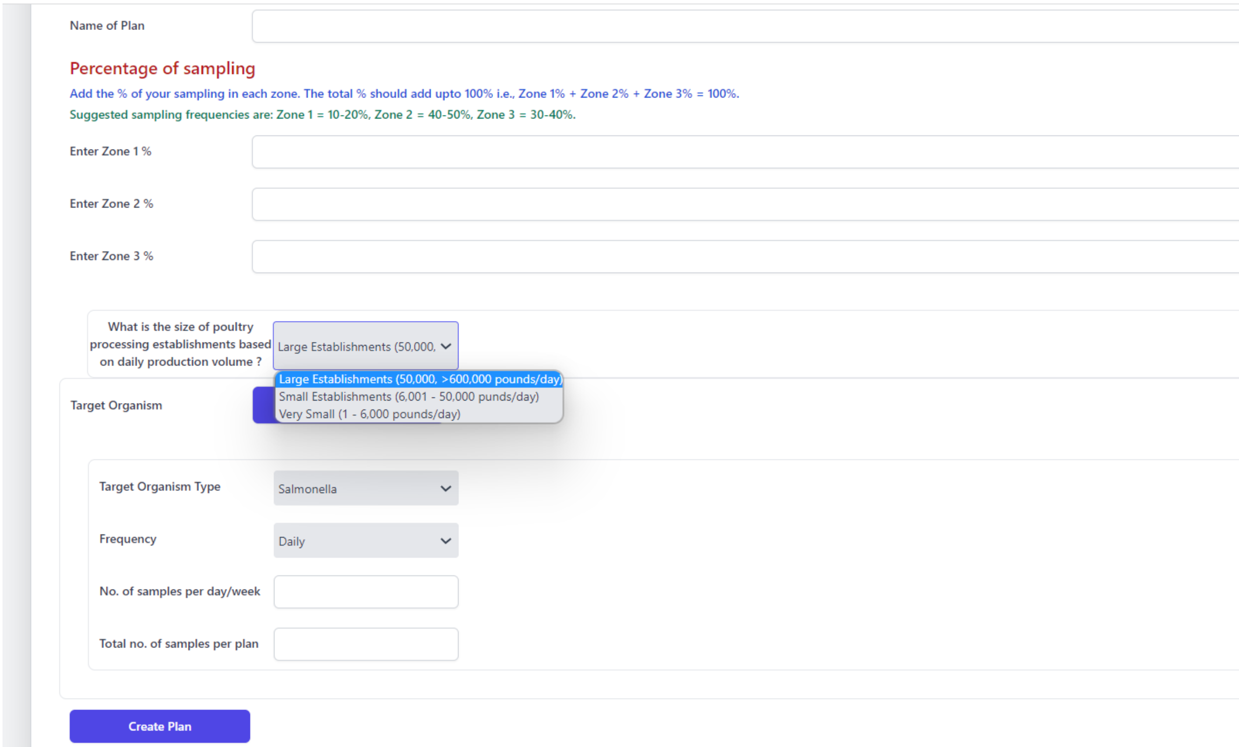


Figure 8: A dropdown menu displaying the size of establishment based on the daily production volume in the Create New Plan page.

Other Areas to check for possible Listeria Contamination

1. Light switches
2. Hollow object e.g. switch boxes, rollers, etc.
3. Rusted tools and equipment (frames, pipes, shelves)
4. Cracked objects (walls, doors, hoses, etc.)
5. Ice machines
6. Air filters
7. Wheels and bearings

Possible Product Sources of L. monocytogenes

1. Uncooked product and ingredients (meat and poultry)
2. Solutions used for chilled foods (ex: brine solutions)
3. Loose product
4. Rework products
5. Returned product

Size classification of poultry processing establishments based on daily production volume

1. Very small establishments (1-6,000 pounds/day)
2. Small establishments (6,001-50,000 pounds/day)
3. Large establishments (50,001, >600,000 pounds/day)

Classification of poultry processing establishments based on the number of employees

1. Very small establishments (>10 employees)
2. Small establishments (10-499 employees)
3. Large establishments (500 or more employees)

Minimum no. of samples collected from poultry processing plants

1. One sample unit* should be collected from very small establishments
2. Two sample units from small establishments
3. Three sample units from large establishments

**A sample unit includes 20 samples collected from food contact surfaces, non-food contact surfaces, and intact product swabs.*

Figure 9: A screenshot of the Guidelines page showing the recommended number of samples in each size of poultry processing establishments.

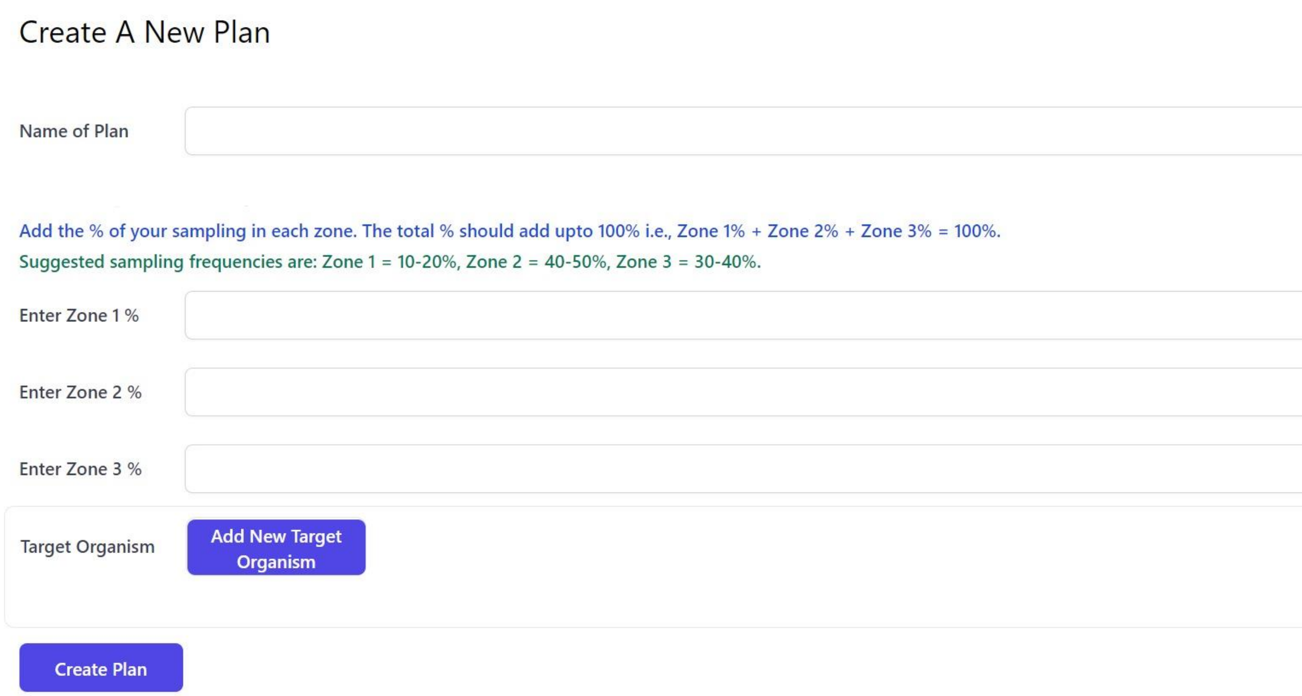


Figure 10: A screenshot dispalying the suggested sampling frequncy for *Listeria* in each zone in the Create New Plan page.

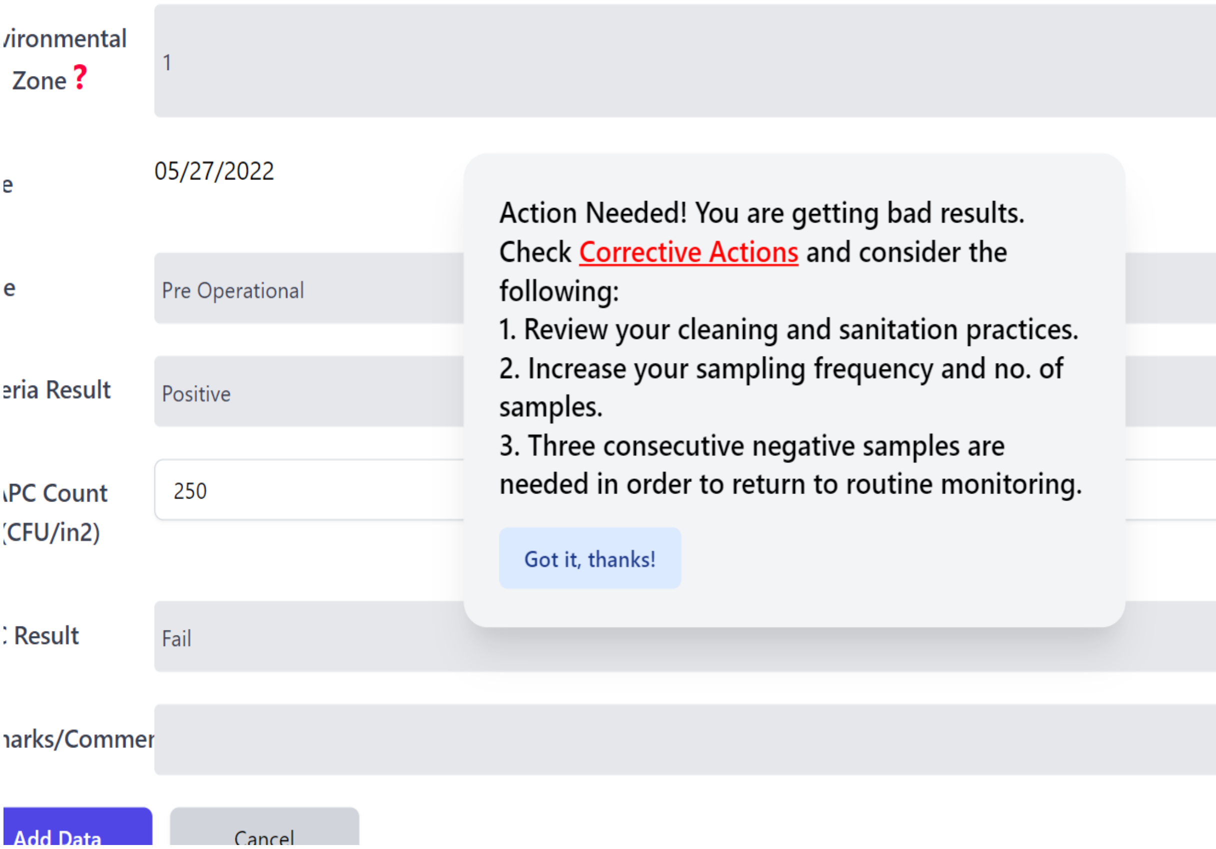
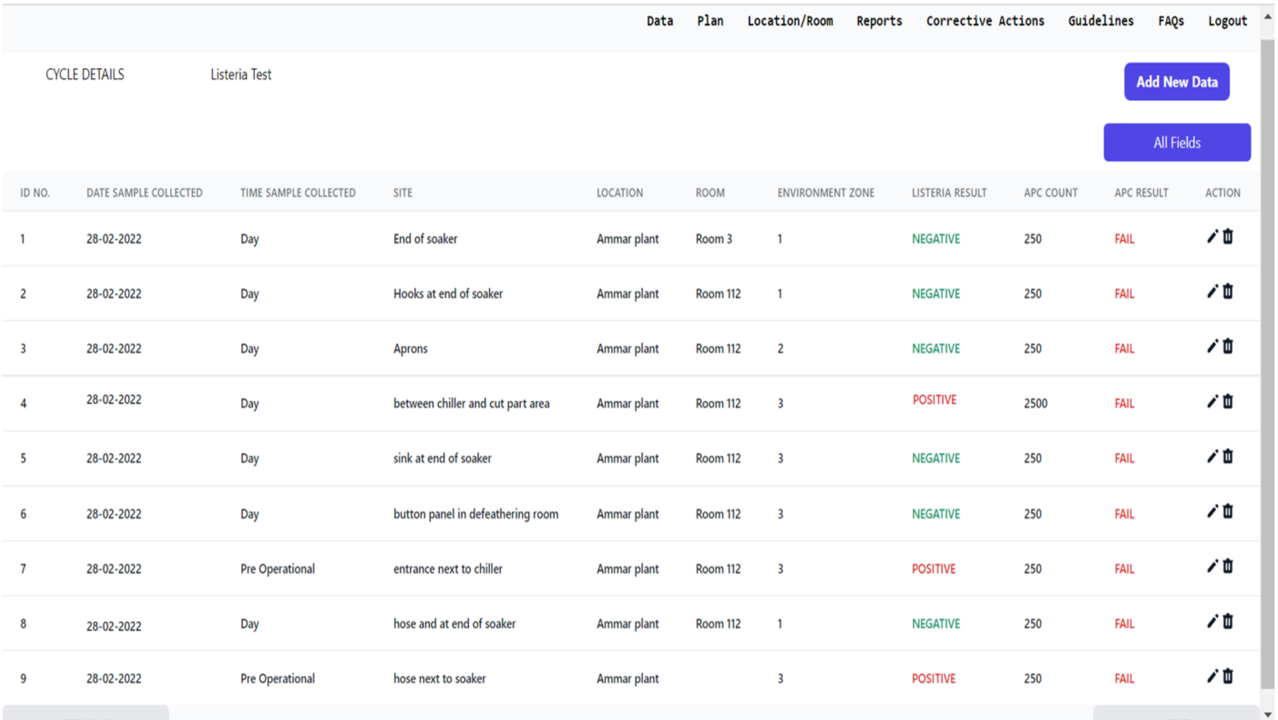


Figure 11: A pop-window was displayed after a test result above acceptable limits was entered in the Add New Data page.



ID NO.	DATE SAMPLE COLLECTED	TIME SAMPLE COLLECTED	SITE	LOCATION	ROOM	ENVIRONMENT ZONE	LISTERIA RESULT	APC COUNT	APC RESULT	ACTION
1	28-02-2022	Day	End of soaker	Ammar plant	Room 3	1	NEGATIVE	250	FAIL	
2	28-02-2022	Day	Hooks at end of soaker	Ammar plant	Room 112	1	NEGATIVE	250	FAIL	
3	28-02-2022	Day	Aprons	Ammar plant	Room 112	2	NEGATIVE	250	FAIL	
4	28-02-2022	Day	between chiller and cut part area	Ammar plant	Room 112	3	POSITIVE	2500	FAIL	
5	28-02-2022	Day	sink at end of soaker	Ammar plant	Room 112	3	NEGATIVE	250	FAIL	
6	28-02-2022	Day	button panel in defeathering room	Ammar plant	Room 112	3	NEGATIVE	250	FAIL	
7	28-02-2022	Pre Operational	entrance next to chiller	Ammar plant	Room 112	3	POSITIVE	250	FAIL	
8	28-02-2022	Day	hose and at end of soaker	Ammar plant	Room 112	1	NEGATIVE	250	FAIL	
9	28-02-2022	Pre Operational	hose next to soaker	Ammar plant		3	POSITIVE	250	FAIL	

Figure 12: A screenshot of the data summary page showing the program's ability to respond to unacceptable finindgs in which it colors the text of the preferred findngs with green and with red for unwanted finindgs.

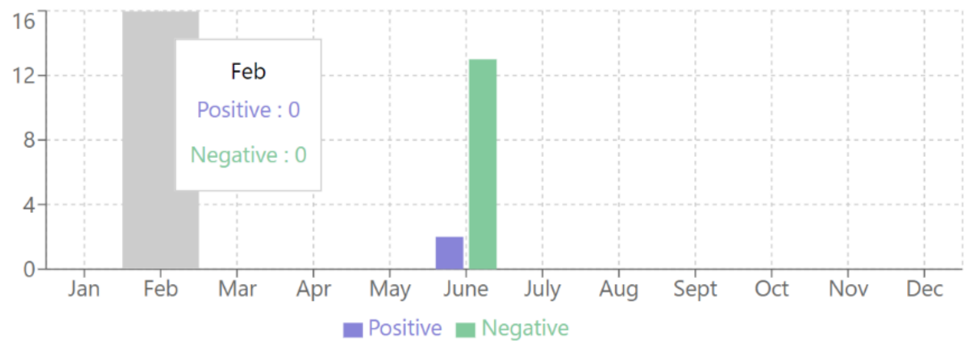
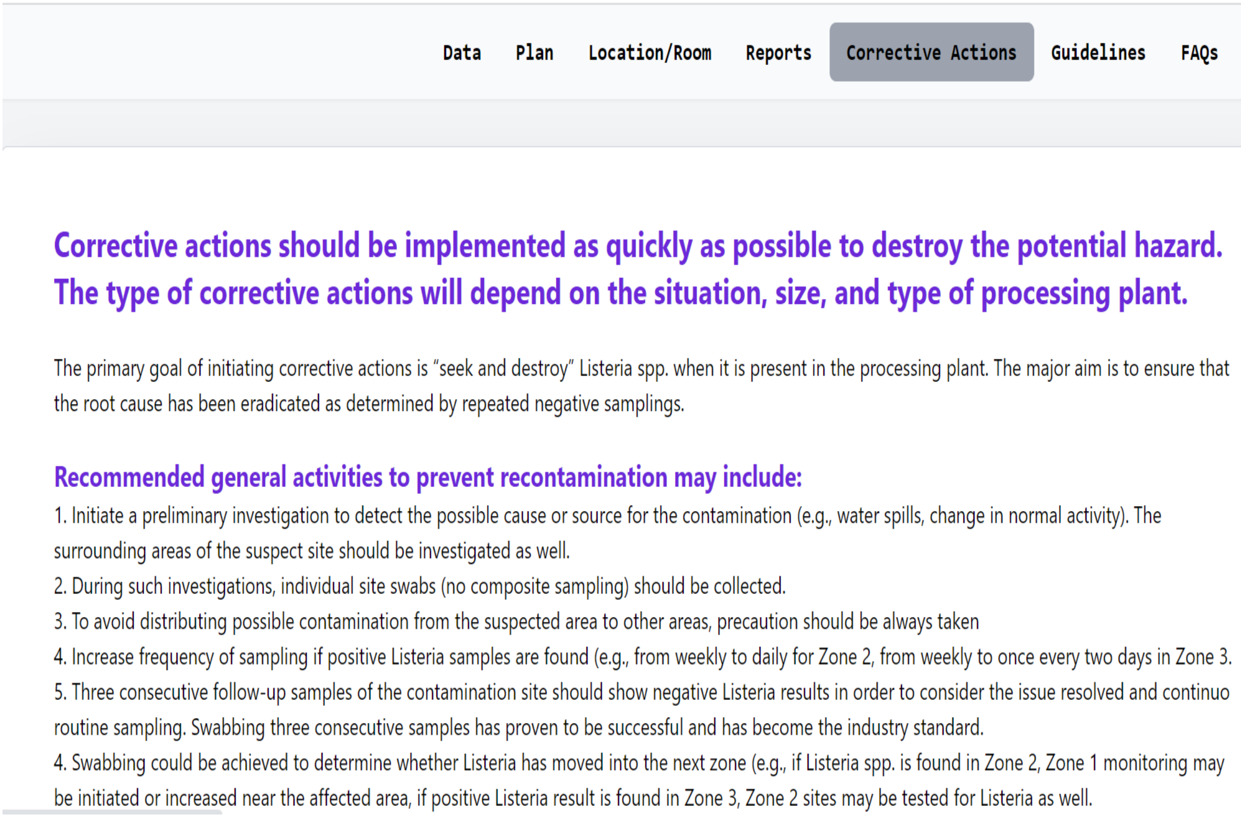


Figure 13: A screenshot of the Report page showing the type of indicator and the time frame to be displayed on the chart.


Data Plan Location/Room Reports Corrective Actions Guidelines FAQs

Corrective actions should be implemented as quickly as possible to destroy the potential hazard. The type of corrective actions will depend on the situation, size, and type of processing plant.

The primary goal of initiating corrective actions is "seek and destroy" Listeria spp. when it is present in the processing plant. The major aim is to ensure that the root cause has been eradicated as determined by repeated negative samplings.

Recommended general activities to prevent recontamination may include:

1. Initiate a preliminary investigation to detect the possible cause or source for the contamination (e.g., water spills, change in normal activity). The surrounding areas of the suspect site should be investigated as well.
2. During such investigations, individual site swabs (no composite sampling) should be collected.
3. To avoid distributing possible contamination from the suspected area to other areas, precaution should be always taken
4. Increase frequency of sampling if positive Listeria samples are found (e.g., from weekly to daily for Zone 2, from weekly to once every two days in Zone 3.
5. Three consecutive follow-up samples of the contamination site should show negative Listeria results in order to consider the issue resolved and continuo routine sampling. Swabbing three consecutive samples has proven to be successful and has become the industry standard.
4. Swabbing could be achieved to determine whether Listeria has moved into the next zone (e.g., if Listeria spp. is found in Zone 2, Zone 1 monitoring may be initiated or increased near the affected area, if positive Listeria result is found in Zone 3, Zone 2 sites may be tested for Listeria as well.

Figure 14: A screenshot of the Corrective Actions page showing several sets of recommended activity to troubleshoot issues.

Corrective Actions for a Single positive Listeria result in Zone 1:

1. Stop production as soon as possible and implement appropriate corrective actions immediately.
2. If the test results of the product are Listeria negative, the product can be released.
3. If the product is positive for Listeria spp., the product should be reprocessed to eliminate Listeria monocytogenes, contrarily, the product should be destroyed.
4. When testing the affected processing line, products in this lot should be held and released if the test results are negative for Listeria spp.
5. When a processing line has a history of Zone 1 positive results and the product type has been linked to listeriosis, products included in that line should be tested for Listeria spp. The recommended sampling plan is n=20 according to case 12 from International Commission on Microbiological Specification for Foods (ICMSF).
6. Repeated cleaning and sanitization of the affected spot may be implemented.
7. When a positive result is found in Zone 1, increase number of samples collected from that zone (e.g., if routine Zone 1 samples are ten, this number could be shifted to 50–100 samples).
8. Three consecutive negative Listeria spp. results are needed in order to return to routine sampling.
9. Additional actions may be implemented based on findings of the above activities.

Corrective Actions for a Single Listeria spp. Finding in Zone 2

1. Stop production to initiate sampling and take additional samples from Zones 1, 2 and 3 sites. These should involve the surrounded areas of the positive site.
2. The original Listeria spp. positive site is considered to be the center point for the problem, so it is recommended to take samples from the area close to that site to prevent any future recontaminations.
3. Repeated cleaning and sanitization of the affected spot may be implemented.
4. Increase frequency of sampling if positive Listeria samples are found (e.g., from weekly to daily for Zone 2, from weekly to once every two days in Zone 3.

Figure 15: A screenshot of the Corrective Actions page showing several corrective actions specific to each type of zones.

Figure 16: A screenshot showing the questionnaire's instructions and start button.

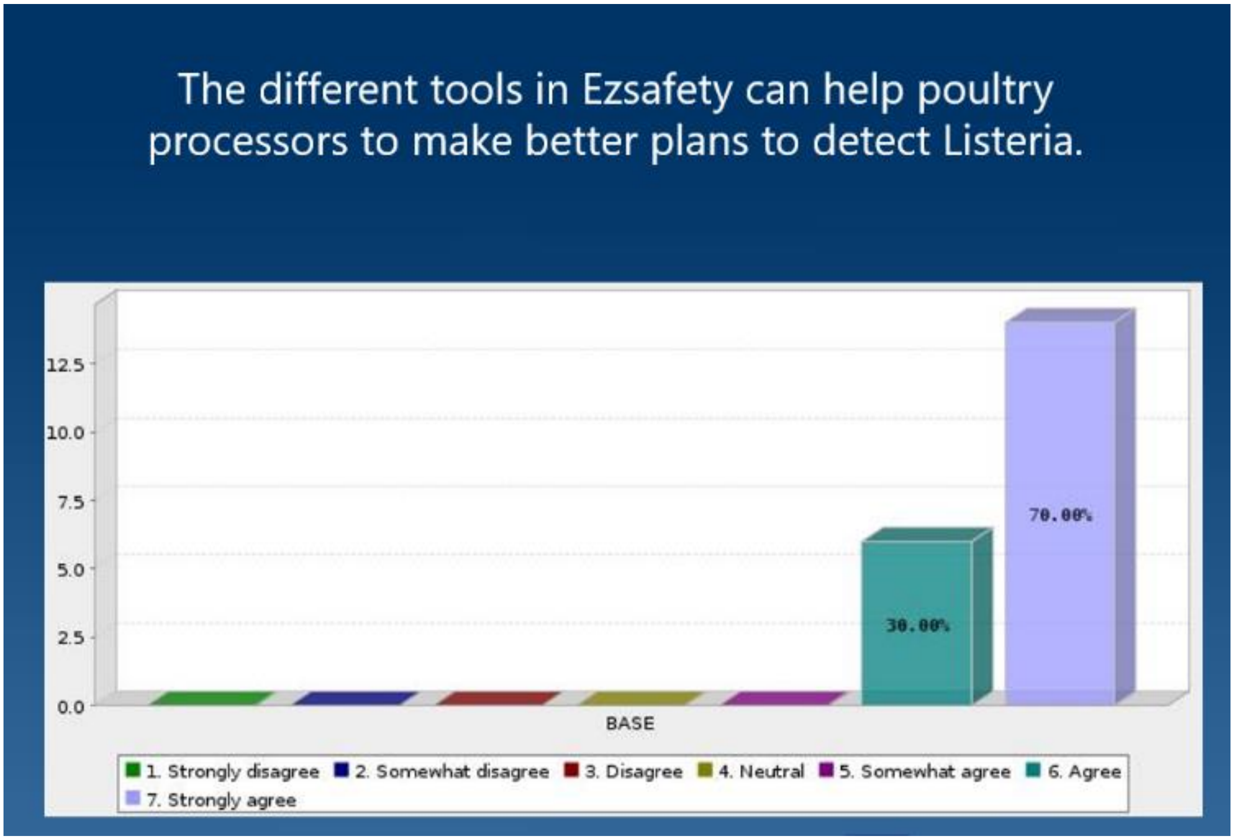


Figure 17: Summary of 20 participant responses to the questionnaire's statement regarding the usefulness of EZSafety to enhance *Listeria* environmental monitoring.

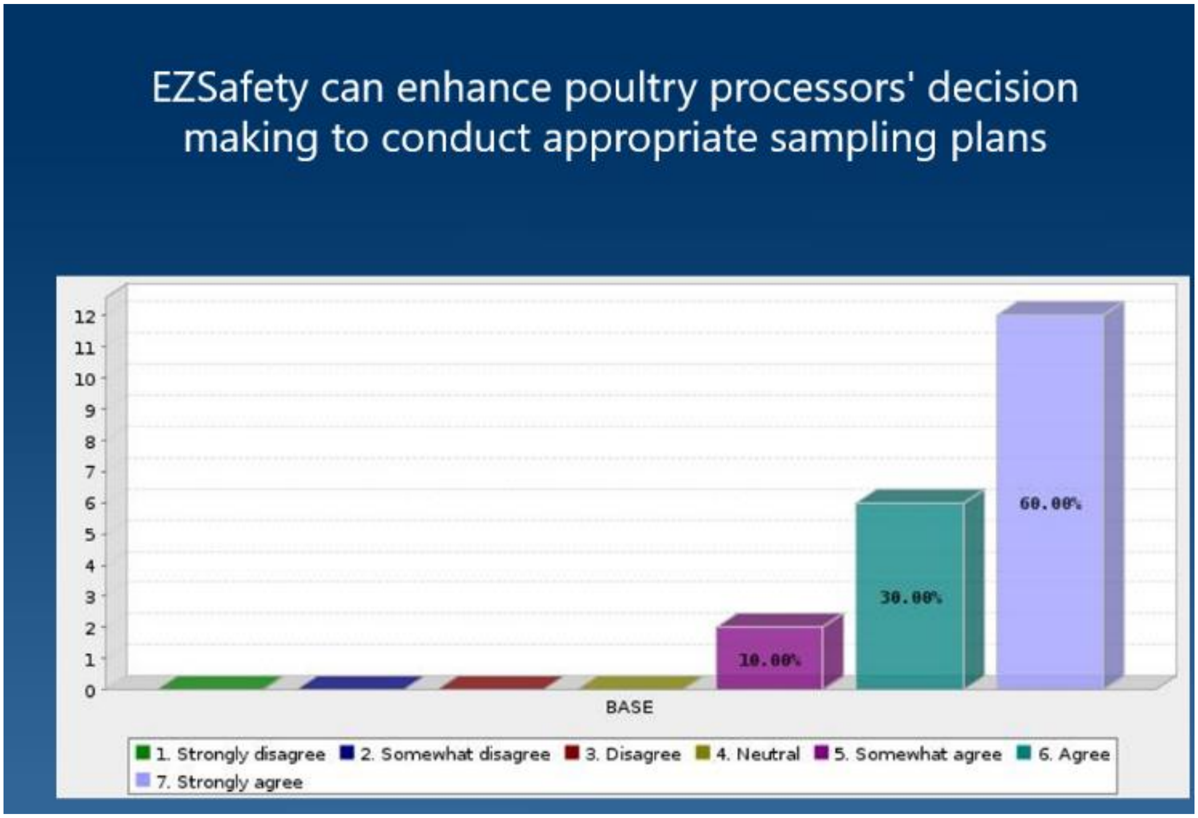


Figure 18: Summary of 20 participant responses to the questionnaire's statement regarding the usefulness of the monitoring program to enhance poultry processors' decision making.

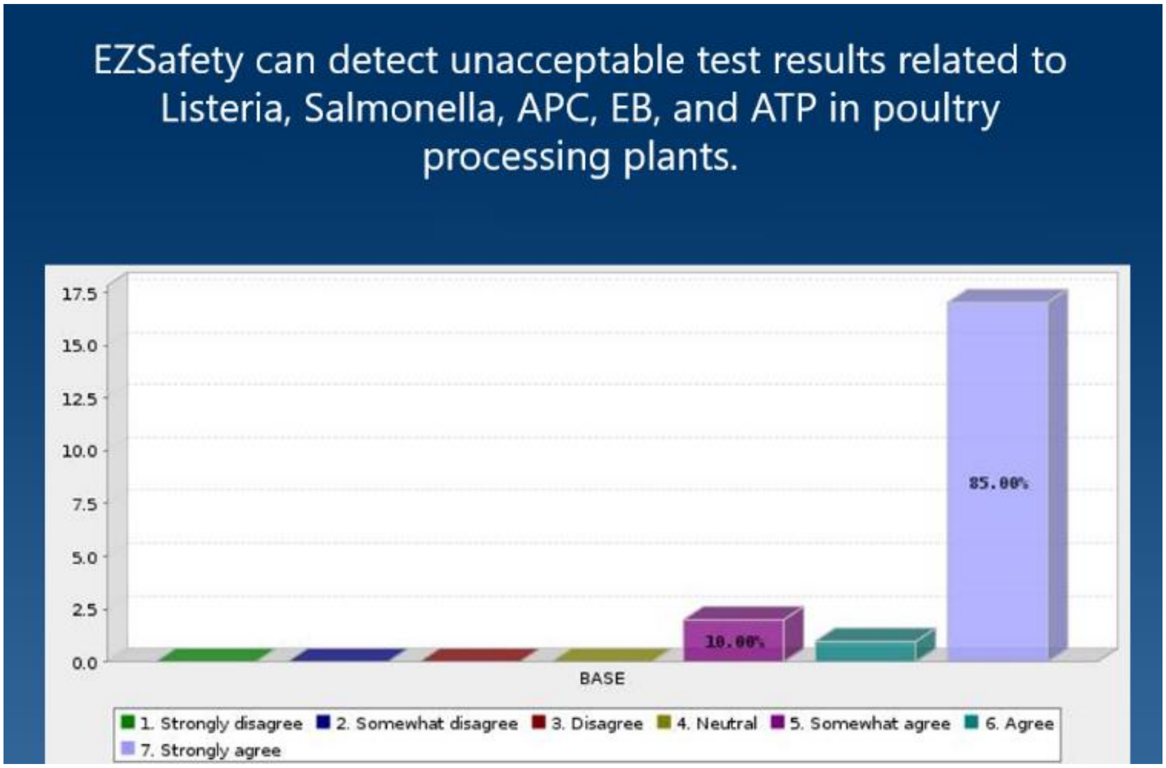


Figure 19: Summary of 20 participant responses to the questionnaire's statement regarding the effectiveness of EZSafety to detect unacceptable limits of indicator organisms.

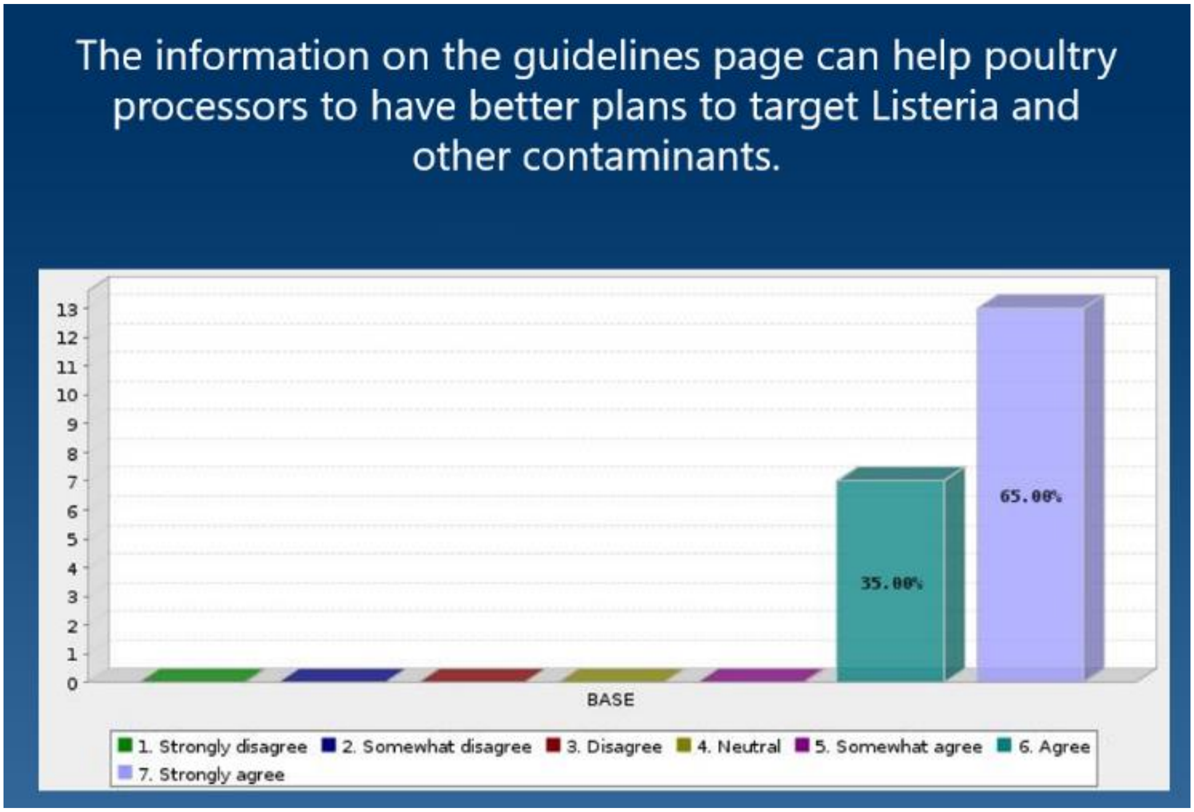


Figure 20: Summary of 20 participant responses to the questionnaire's statement regarding the usefulness of the program's guidelines and recommendations to enhance *Listeria* environmental sampling.

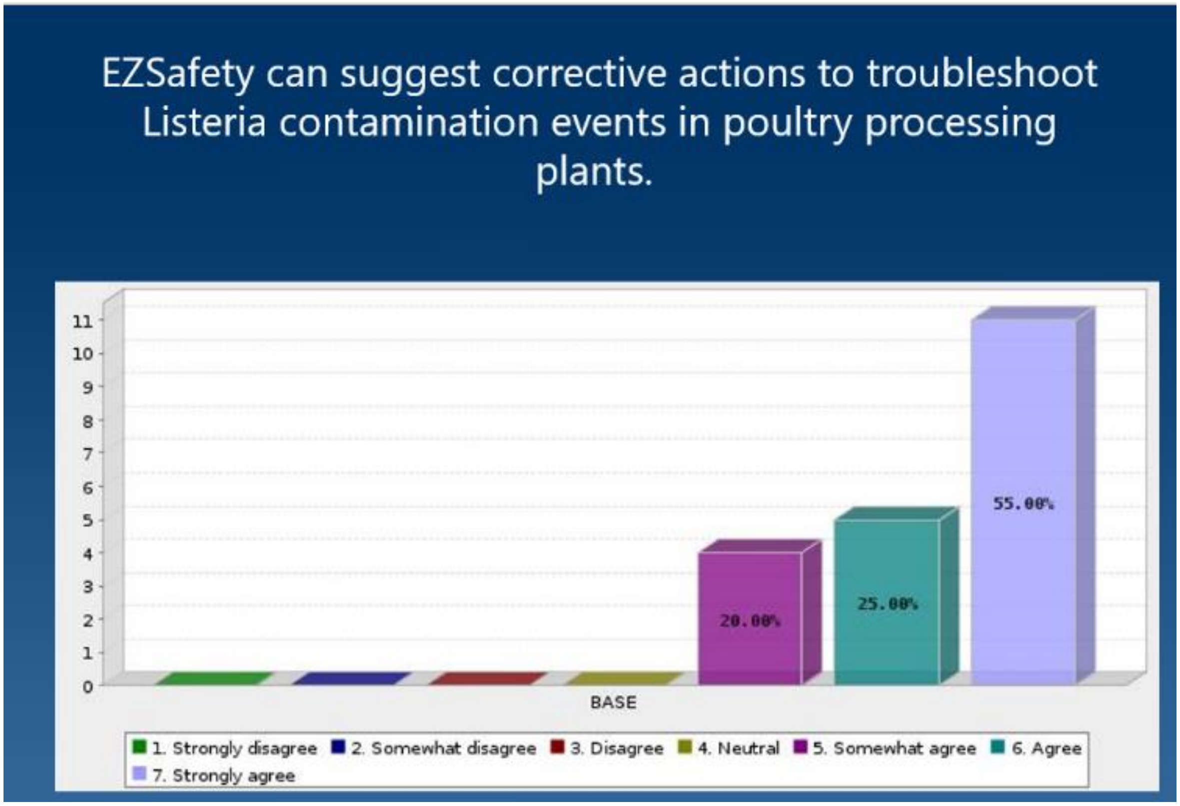


Figure 21: Summary of 20 participant responses to the questionnaire's statement regarding the usefulness of EZSafety to suggest corrective actions to prevent *Listeria* contamination events.

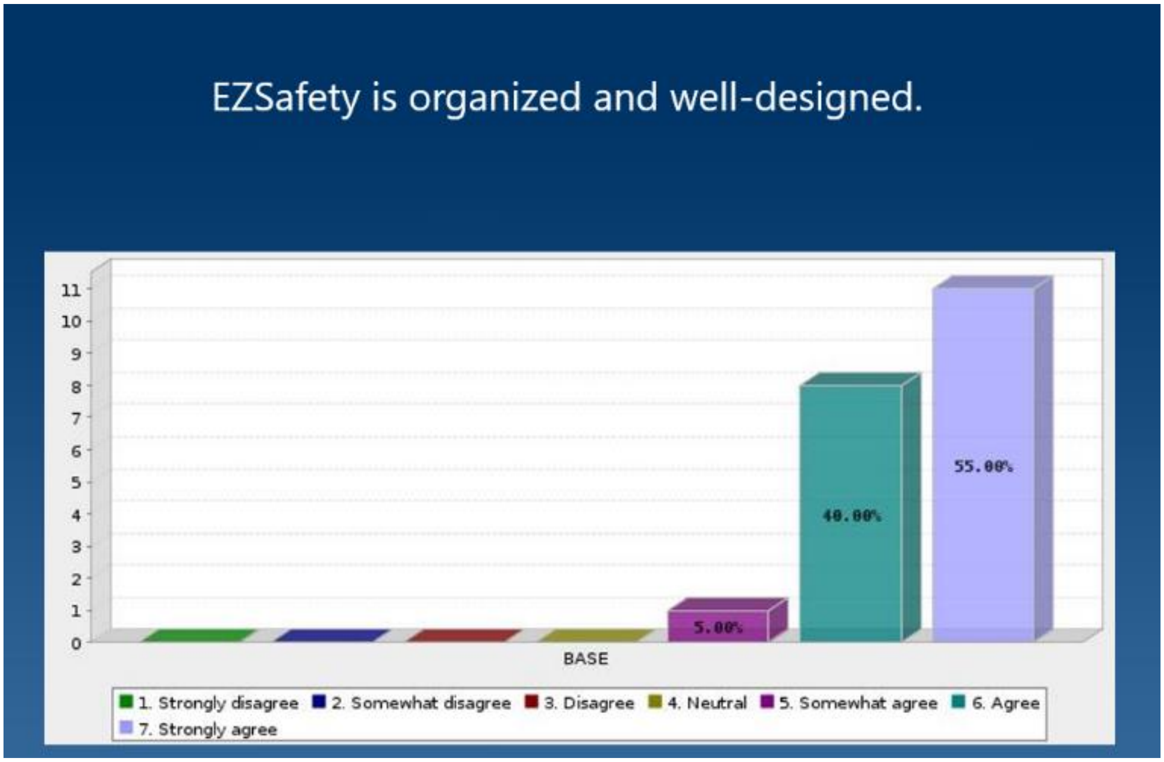


Figure 22: Summary of 20 participant responses to the questionnaire's statement regarding the ease of using the monitoring program.

Sample #	Date Received	Site	Room/ Plant area	Env. Zone	Listeria (+ or -)	Aerobic Plate Count (APC)
121	2/28/20	End of Soaker	Post-evisc. - Chilling	1	-	250
122	2/28/20	Hooks at End of Soaker	Post-evisc. - Chilling	1	-	0
123	2/28/20	Aprons	Post-evisc. - Chilling	2	-	0
124	2/28/20	Between Chiller and Cut Parts Area	Post-evisc. - Chilling	3	+	2,500
125	2/28/20	Sink at End of Soaker	Post-evisc. - Chilling	3	-	0
126	2/28/20	Button panel in Defeathering Room	Post-evisc. - Chilling	3	-	250
127	2/28/20	Entrance Next To Chicken Chiller (Main Entrance)	Post-evisc. - Chilling	3	+	250
128	2/28/20	Hose at End of Soaker	Post-evisc. - Chilling	3	-	0
129	2/28/20	Hose Next to Soaker	Post-evisc. - Chilling	3	+	250
130	2/28/20	Underneath Breast Trimmings Belt	Post-chill - Packaging	1	-	0

Table 1: A sample of one of the data sets that were given to the participants showing *Listeria* spp. and APC sample test results.

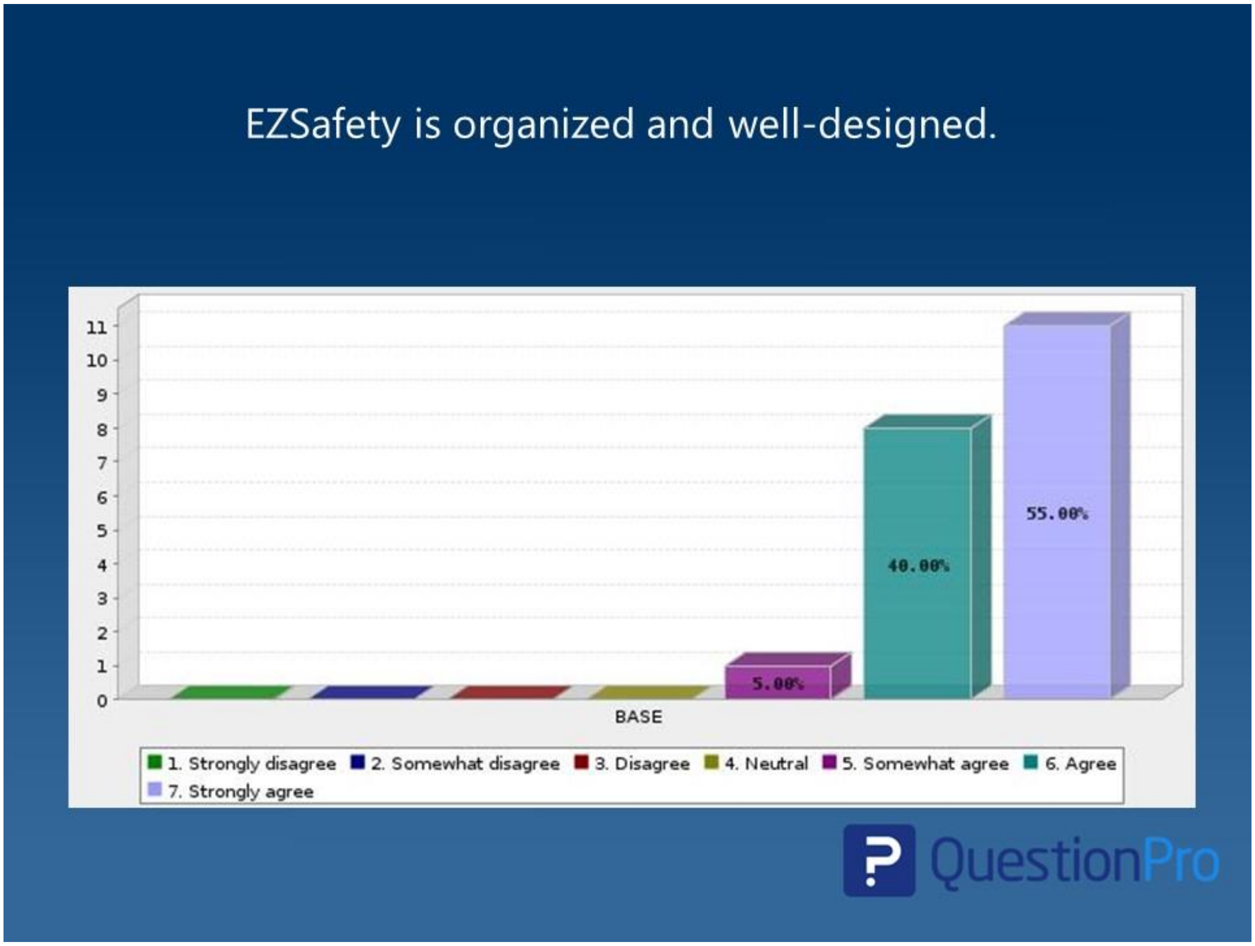
Comments/Suggestions:

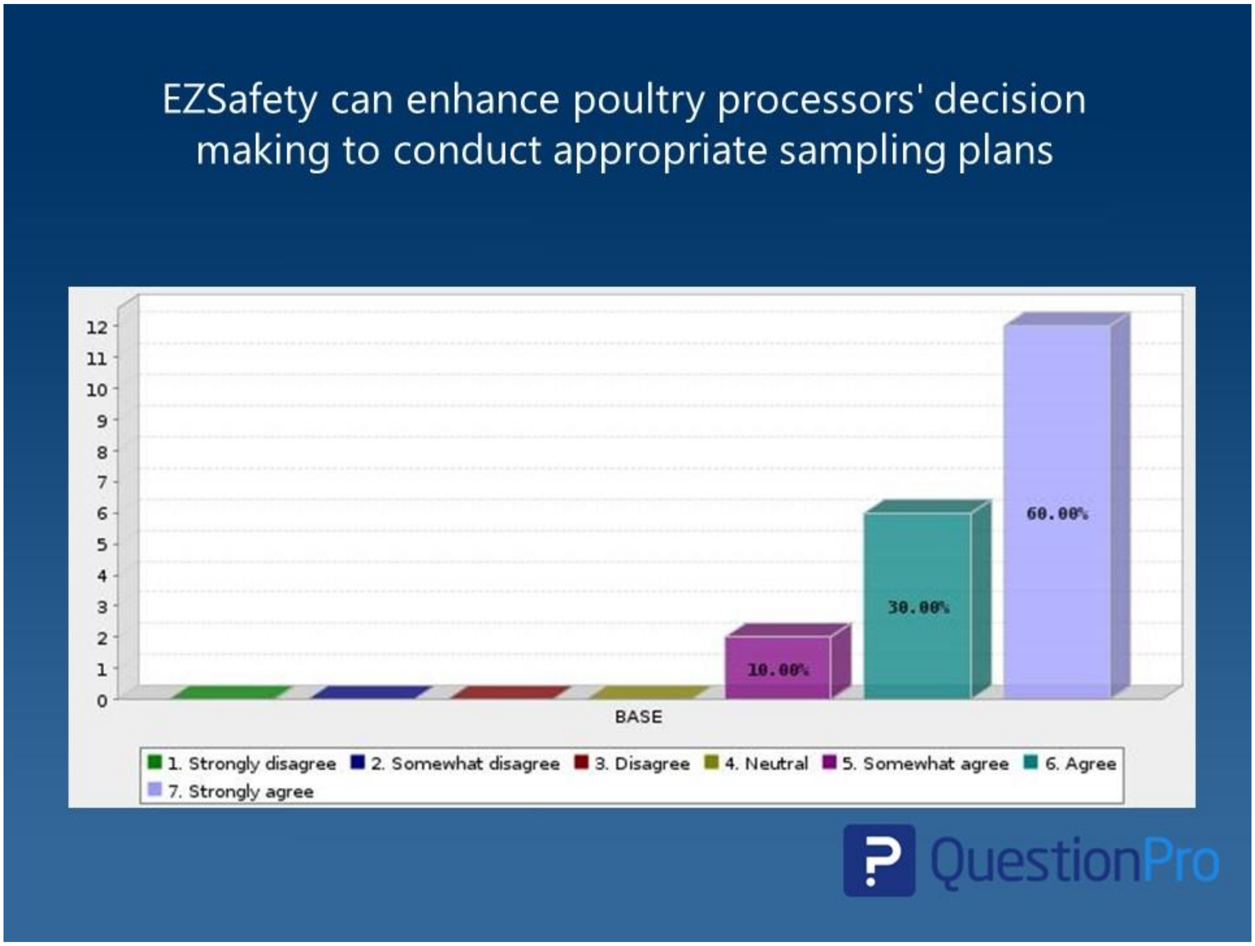
Response ID	Response
76278921	You cannot add a data entry if "Time" is set to "During Production"
76063718	I really like this program. It is pretty clear, easy, straightforward and convenient to use. I believe the application of this program will lead to the increasing efficiency and accuracy for detecting contaminations in a poultry plant.
75987160	Drop down menu for sites/surfaces that can have listeria, and which zone they would be in automatically populating. Also, newer users may not know how long each plan per organism should last, so a suggested plan sampling time may be helpful..
75909624	The program was easy to navigate with excellent guidelines for processors. It has potential to expand to other commodities and could be the tool to create universal sampling plans for processors of all sizes.
75888776	A more streamlined way to put in a bunch of results at one time
75877854	Reports should be made for sampling plans, not for individual tests. Hyperlinks and dropdowns may make the program a little more user-friendly.
75819145	i think this program can help manufacture to determine contamination and easy planning work
75753825	Well done

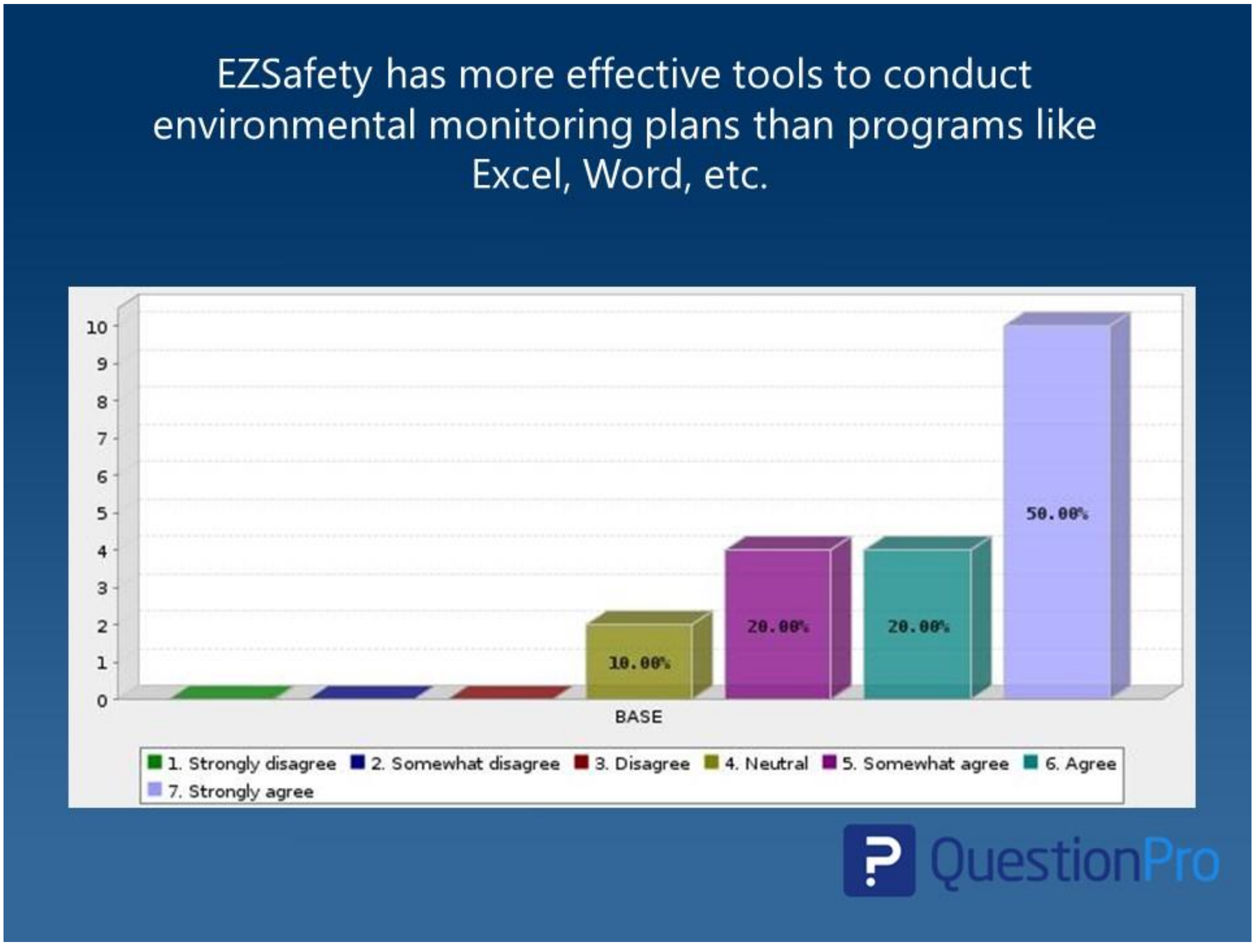
Table 2: The comments left by the participants during their evaluation of the program.

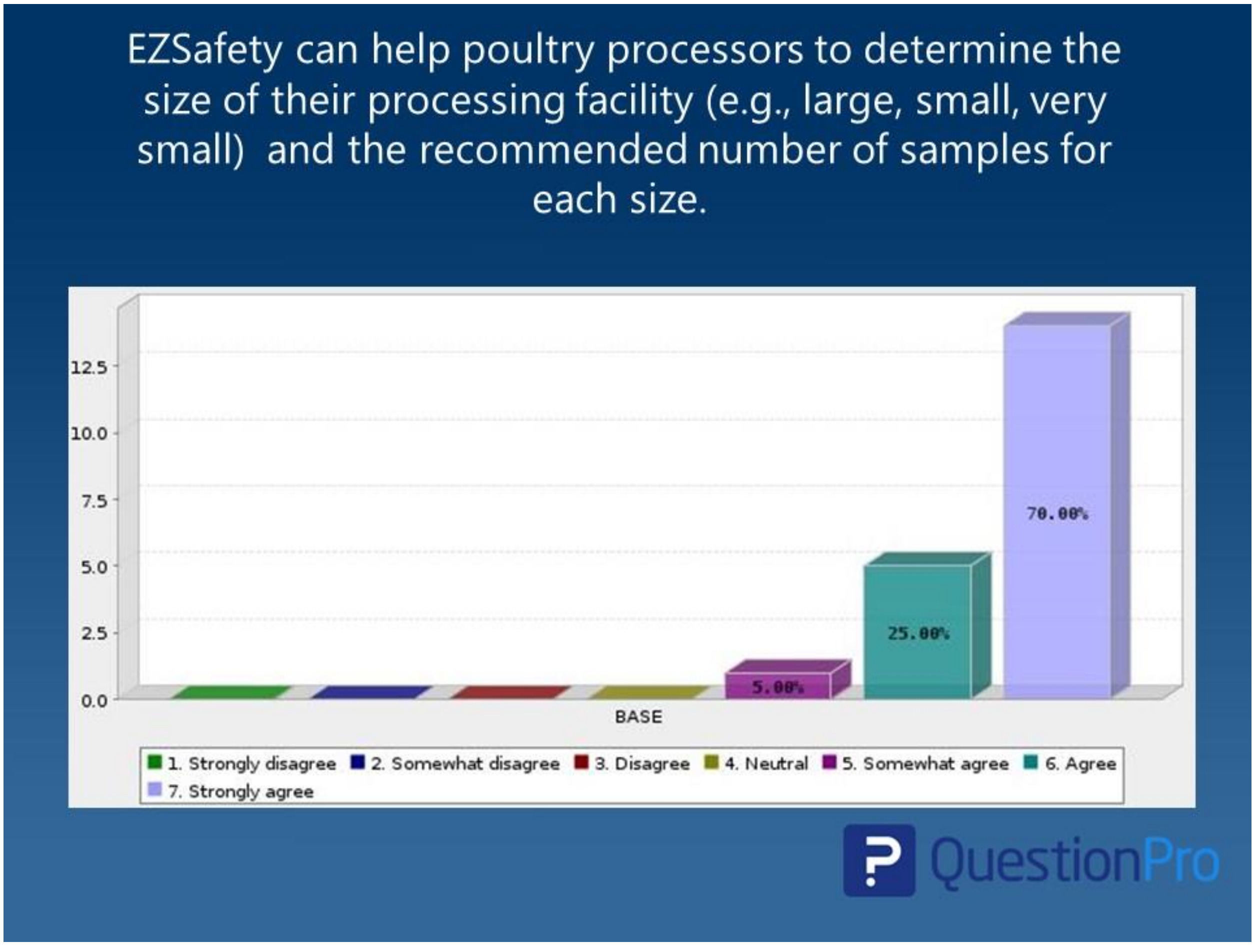
VIII. Appendices

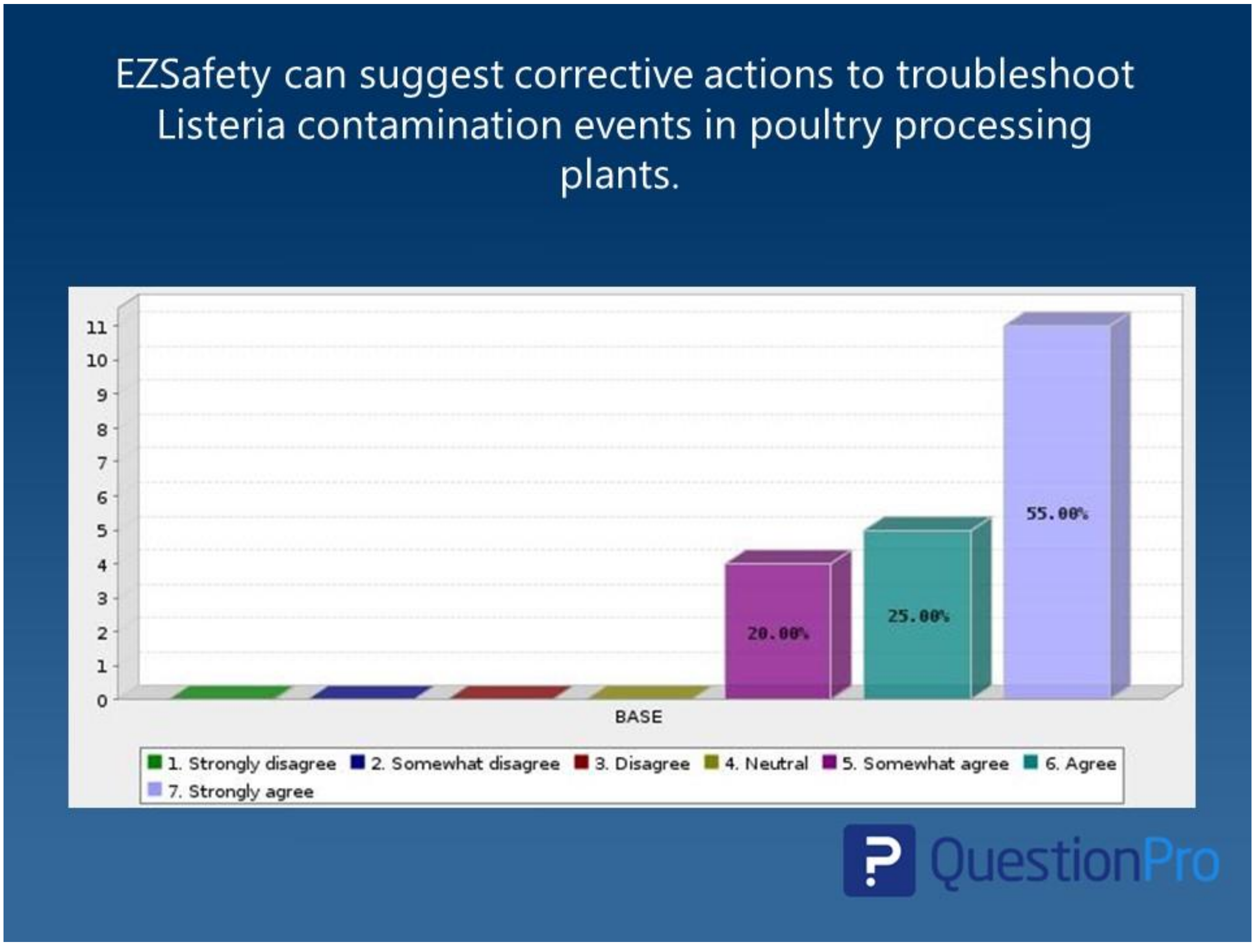
Appendix A: Survey Questions and Responses for User Evaluations

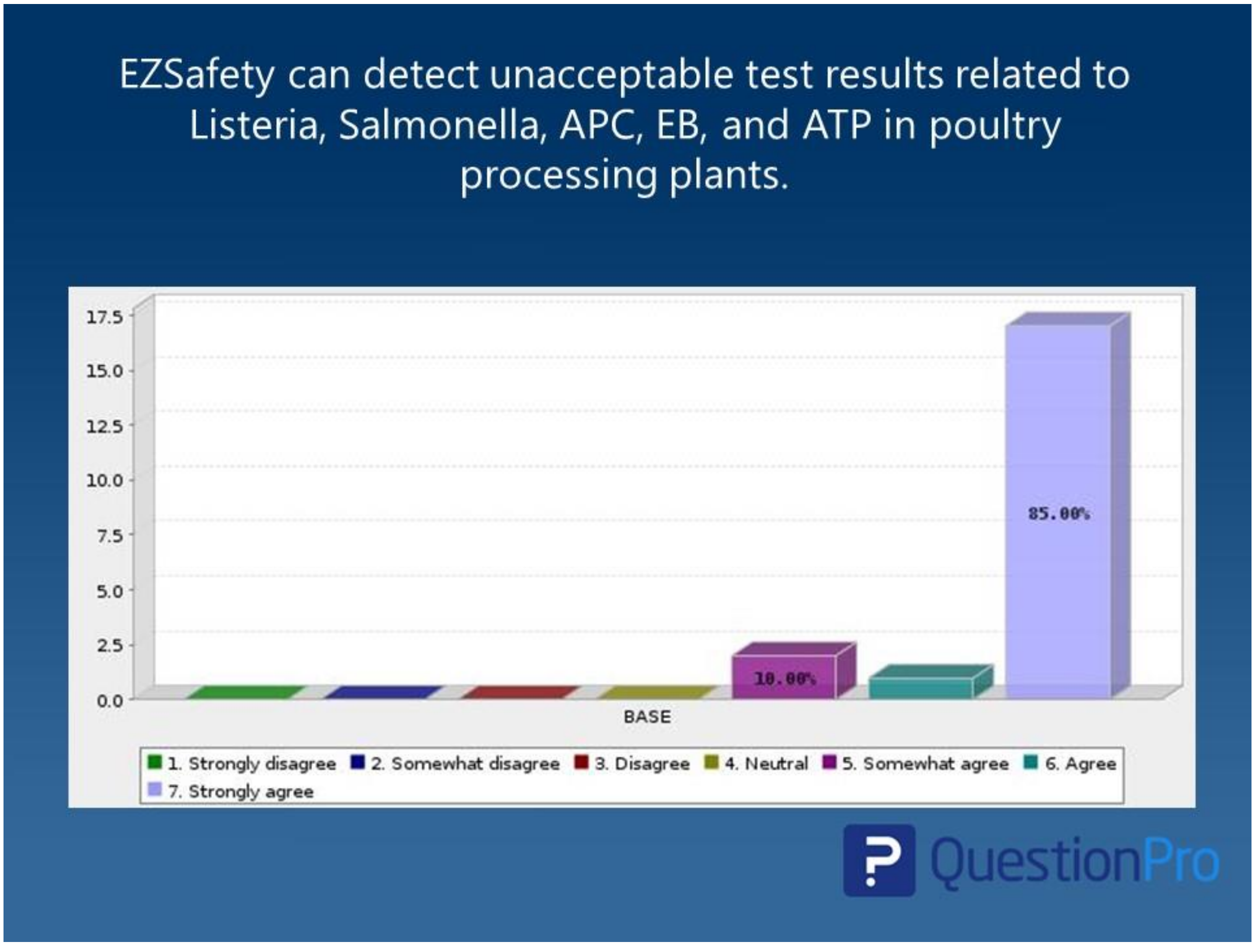


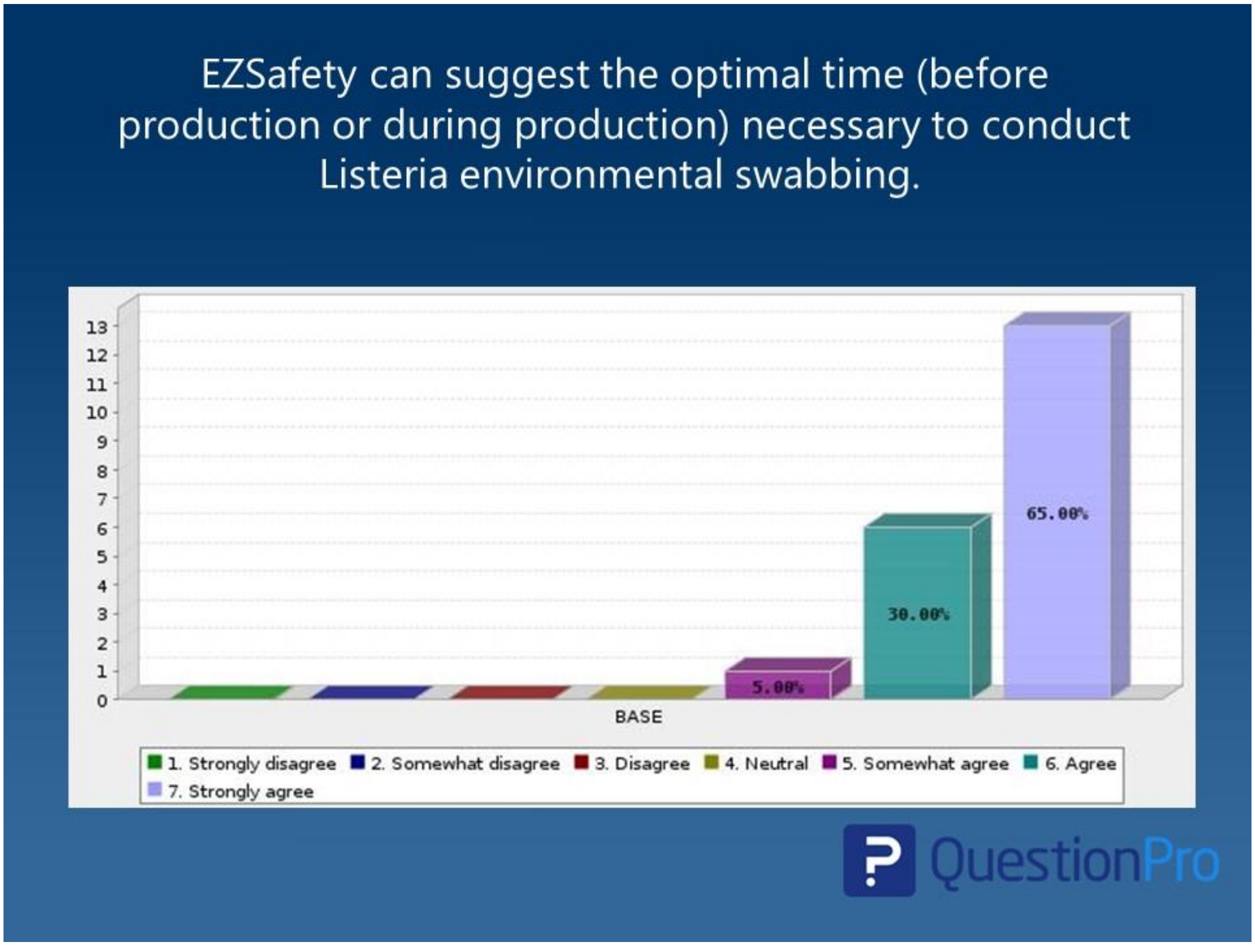

EZSafety can enhance poultry processors' decision making to conduct appropriate sampling plans
12
11
10
9
8
7
6
5
4
3
2
1
0
10.00%
30.00%
60.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

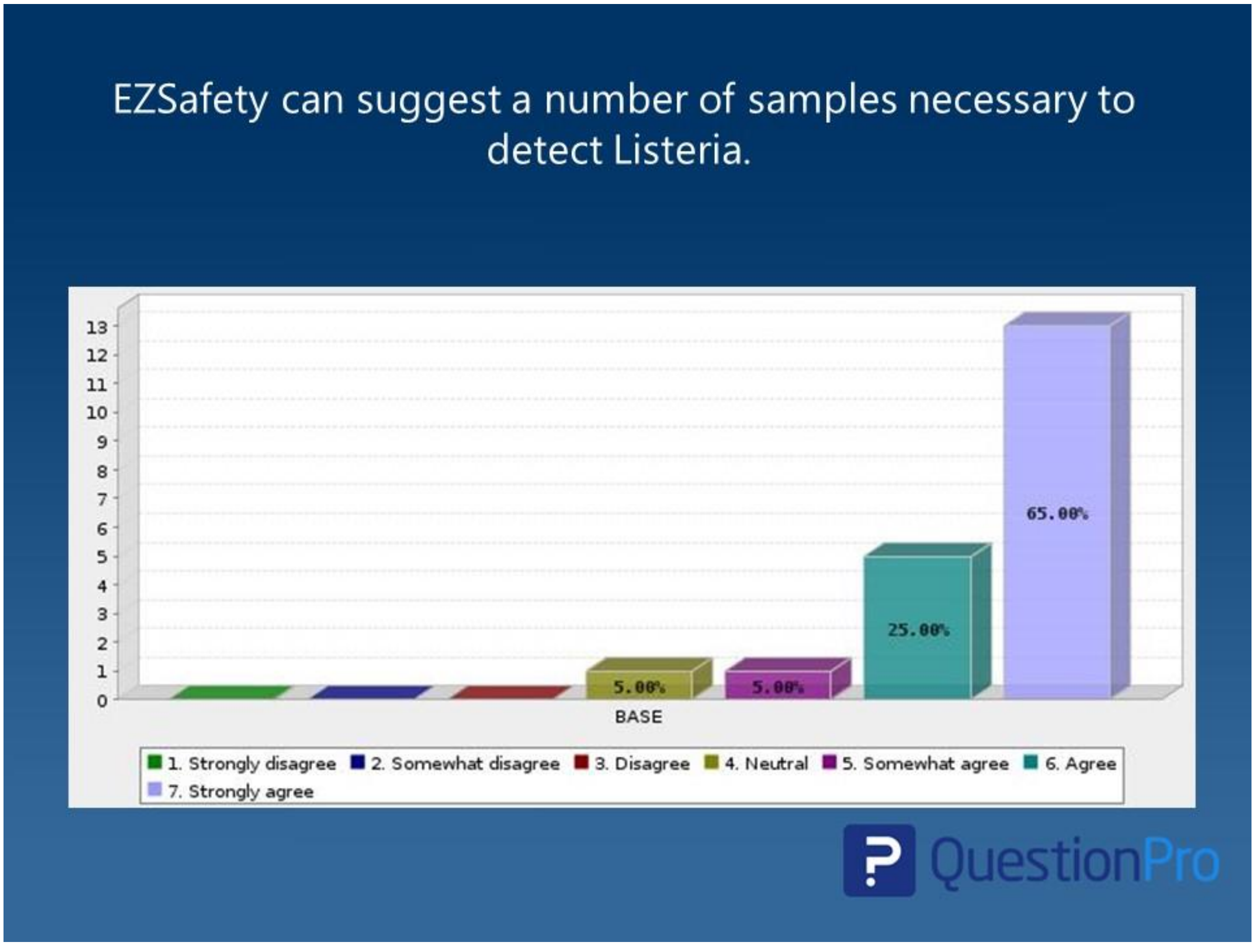

EZSafety has more effective tools to conduct environmental monitoring plans than programs like Excel, Word, etc.
10
9
8
7
6
5
4
3
2
1
0
10.00%
20.00%
20.00%
50.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

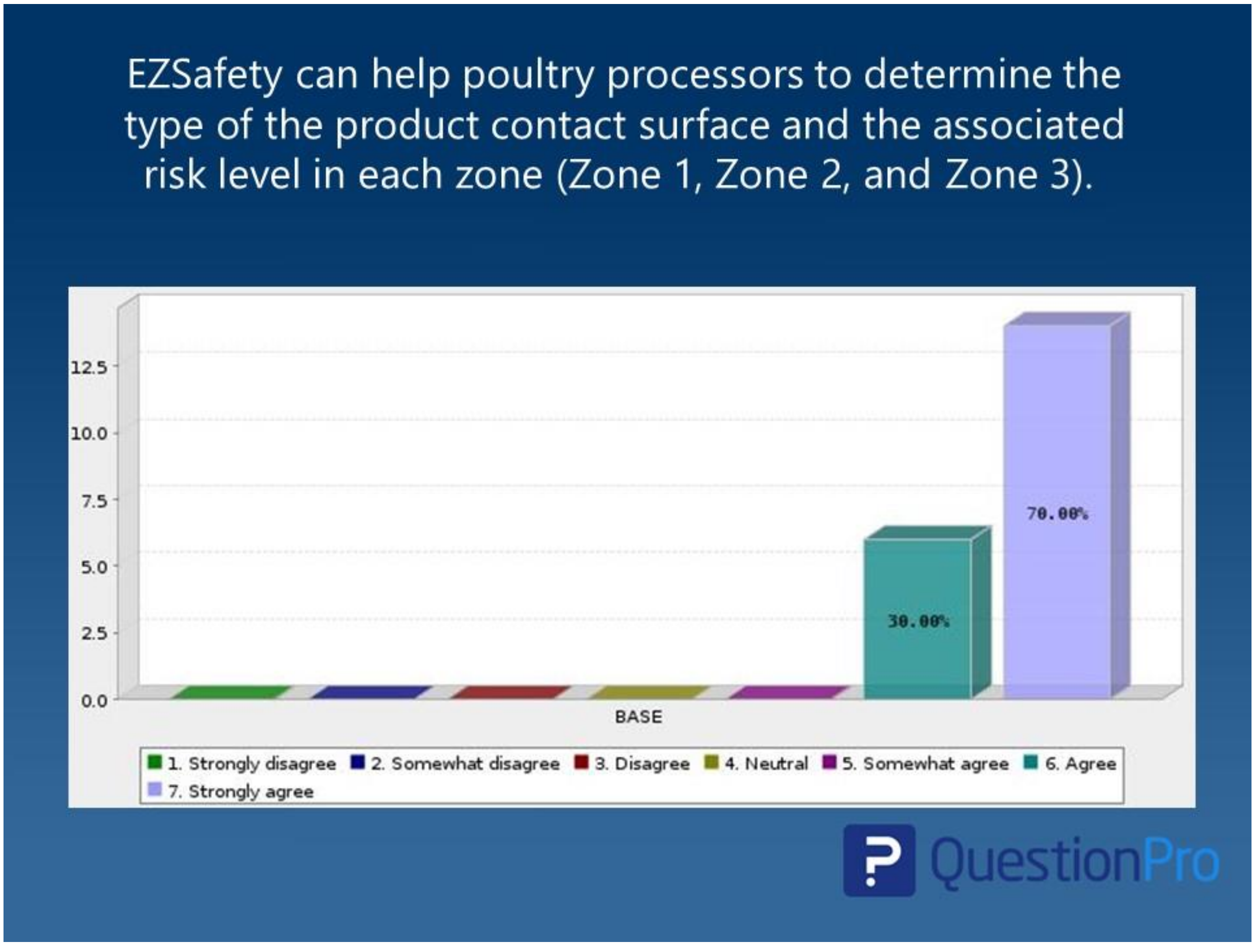

EZSafety can help poultry processors to determine the size of their processing facility (e.g., large, small, very small) and the recommended number of samples for each size.
12.5
10.0
7.5
5.0
2.5
0.0
70.00%
25.00%
5.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

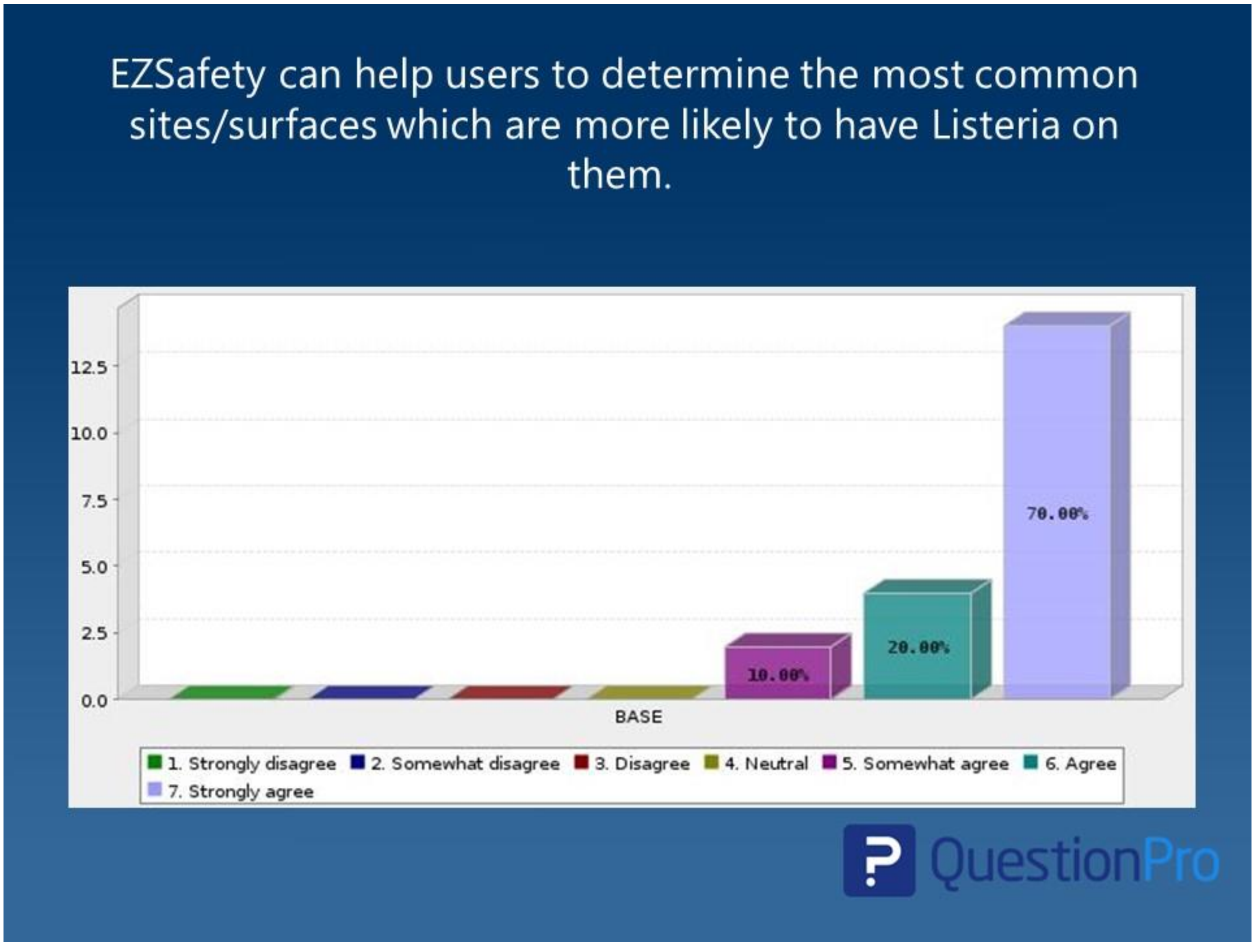

EZSafety can suggest corrective actions to troubleshoot Listeria contamination events in poultry processing plants.
11
10
9
8
7
6
5
4
3
2
1
0
20.00%
25.00%
55.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

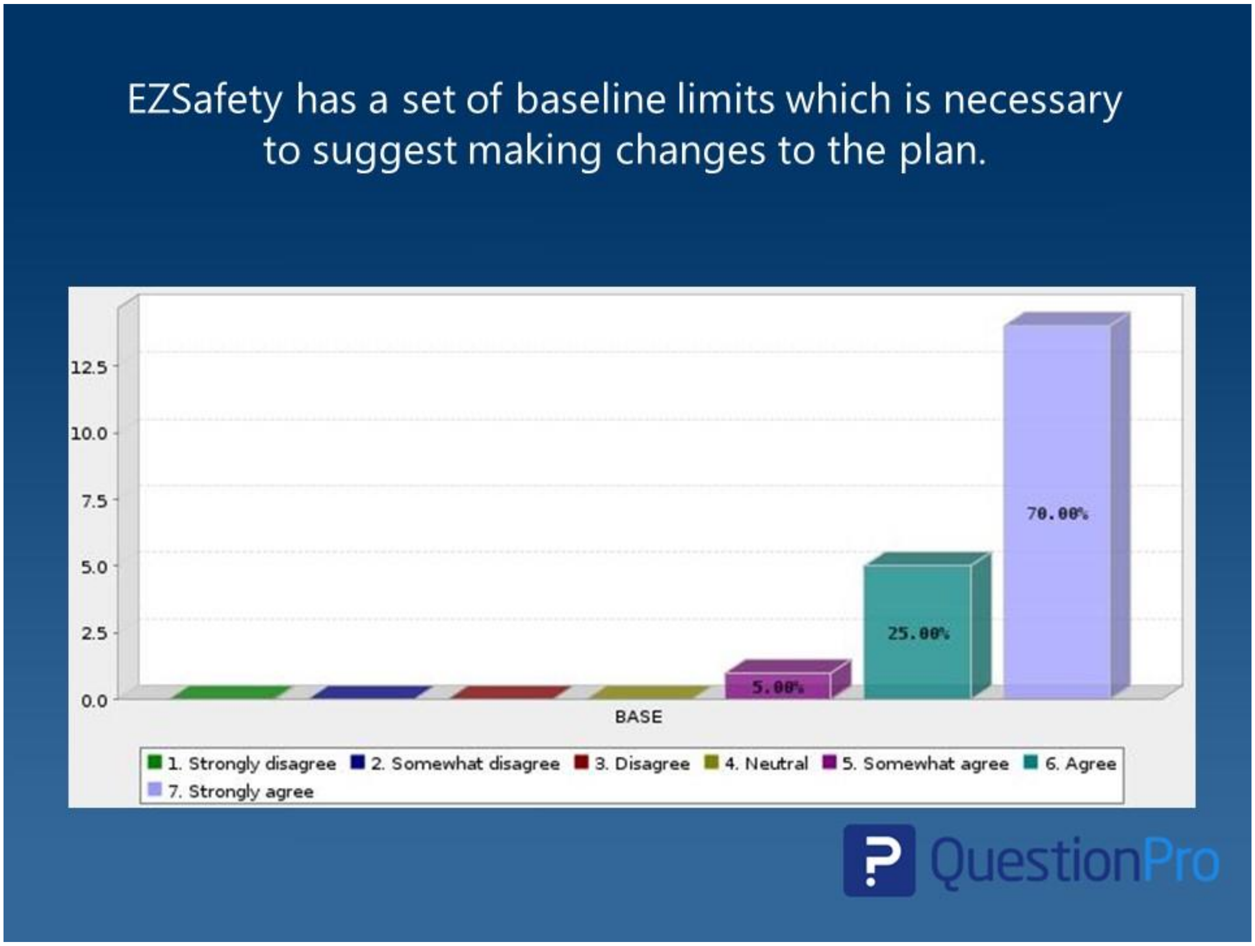

EZSafety can detect unacceptable test results related to Listeria, Salmonella, APC, EB, and ATP in poultry processing plants.
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
10.00%
85.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro


EZSafety can suggest the optimal time (before production or during production) necessary to conduct Listeria environmental swabbing.
13
12
11
10
9
8
7
6
5
4
3
2
1
0
5.00%
30.00%
65.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

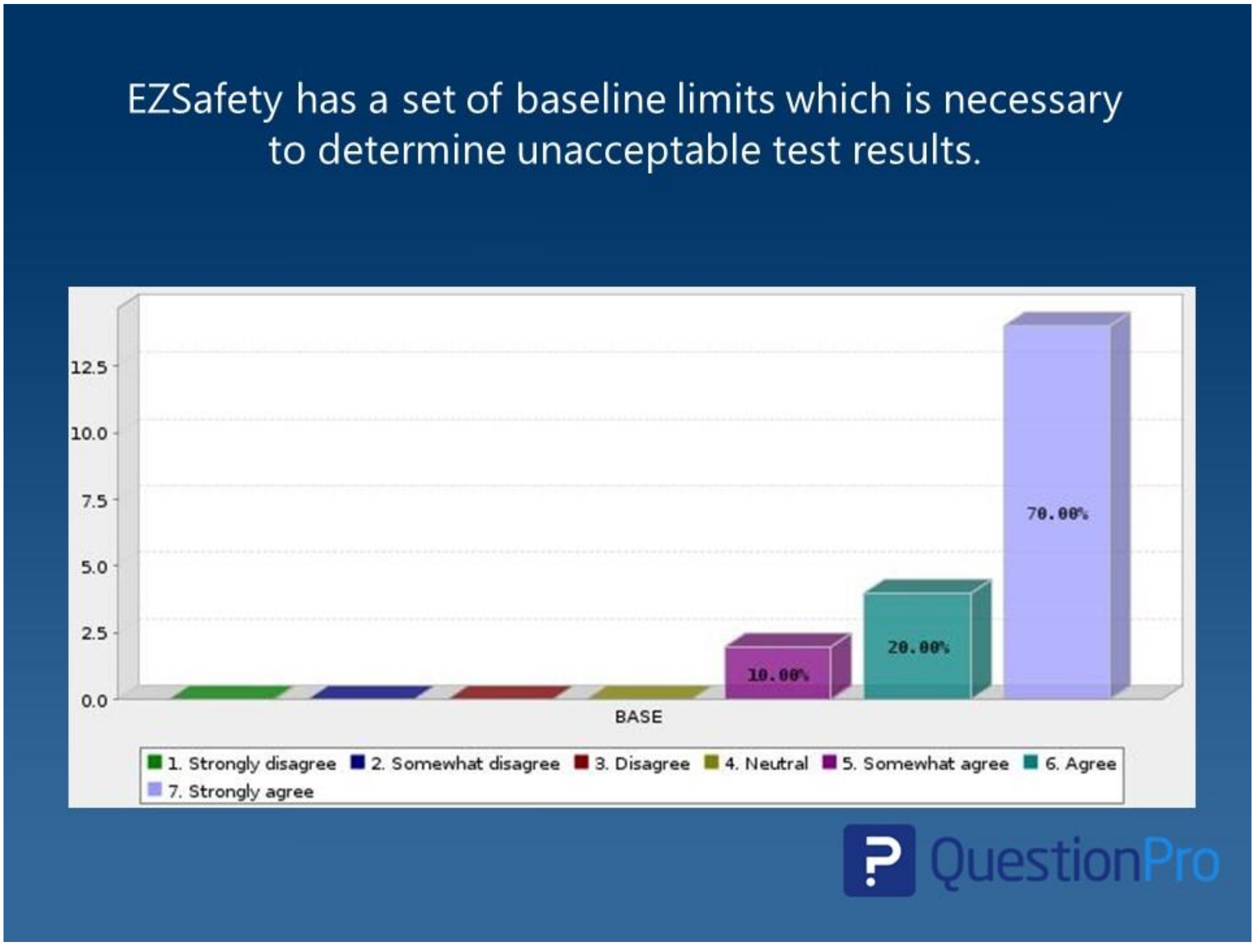

EZSafety can suggest a number of samples necessary to detect Listeria.
13
12
11
10
9
8
7
6
5
4
3
2
1
0
5.00%
5.00%
25.00%
65.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

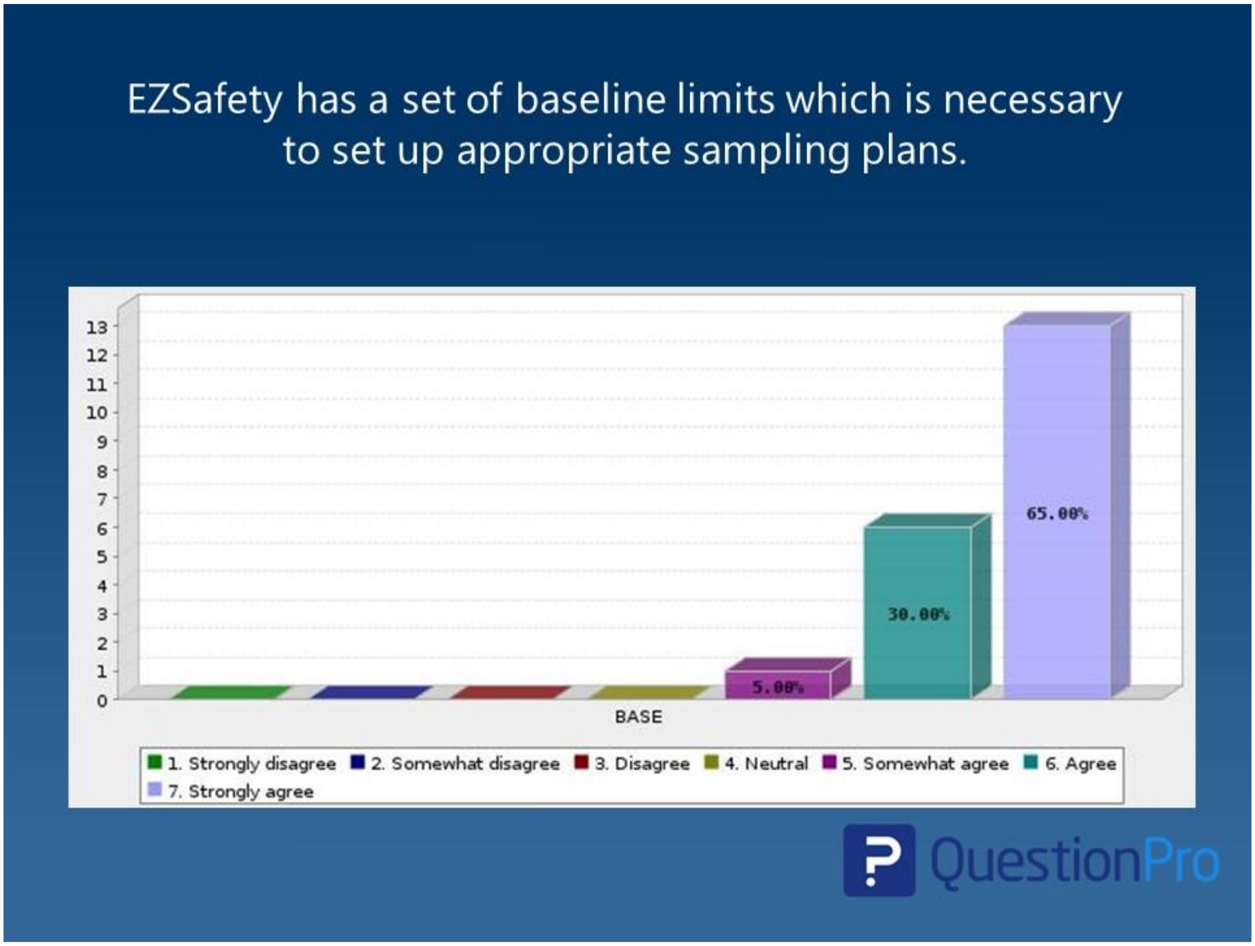

EZSafety can help poultry processors to determine the type of the product contact surface and the associated risk level in each zone (Zone 1, Zone 2, and Zone 3).
12.5
10.0
7.5
5.0
2.5
0.0
30.00%
70.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

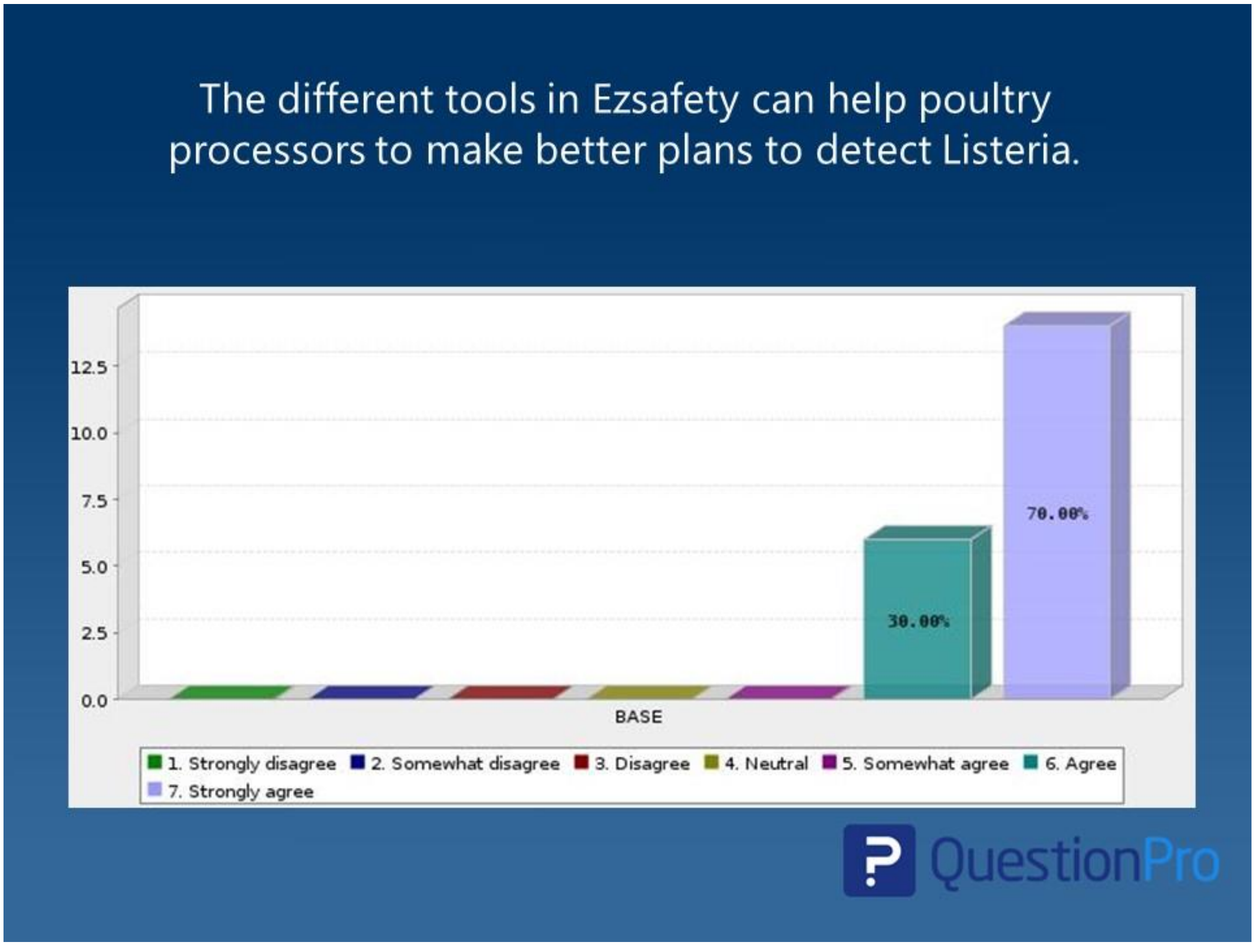

EZSafety can help users to determine the most common sites/surfaces which are more likely to have Listeria on them.
12.5
10.0
7.5
5.0
2.5
0.0
10.00%
20.00%
70.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro



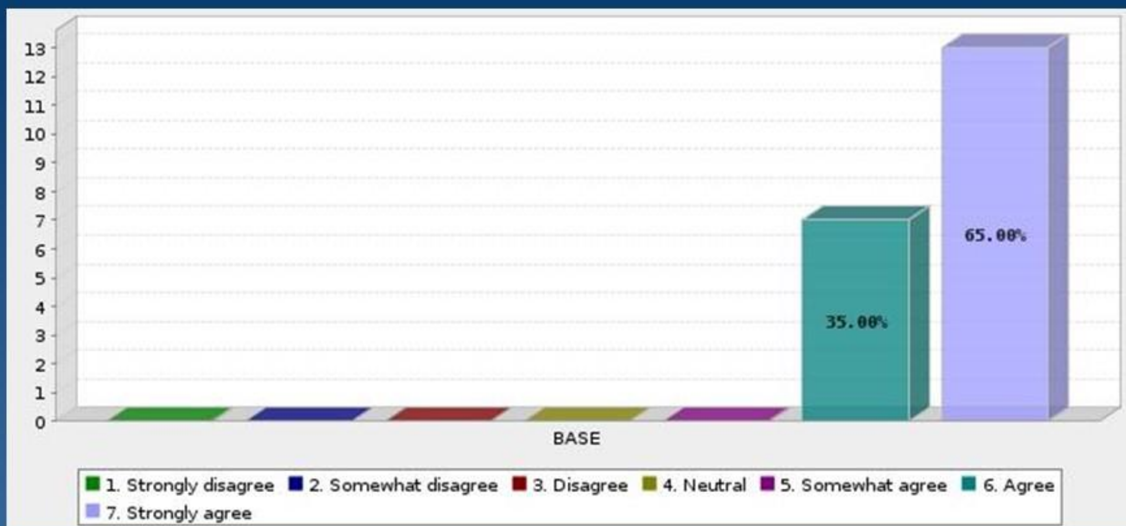
EZSafety has a set of baseline limits which is necessary to suggest making changes to the plan.
12.5
10.0
7.5
5.0
2.5
0.0
5.00%
25.00%
70.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

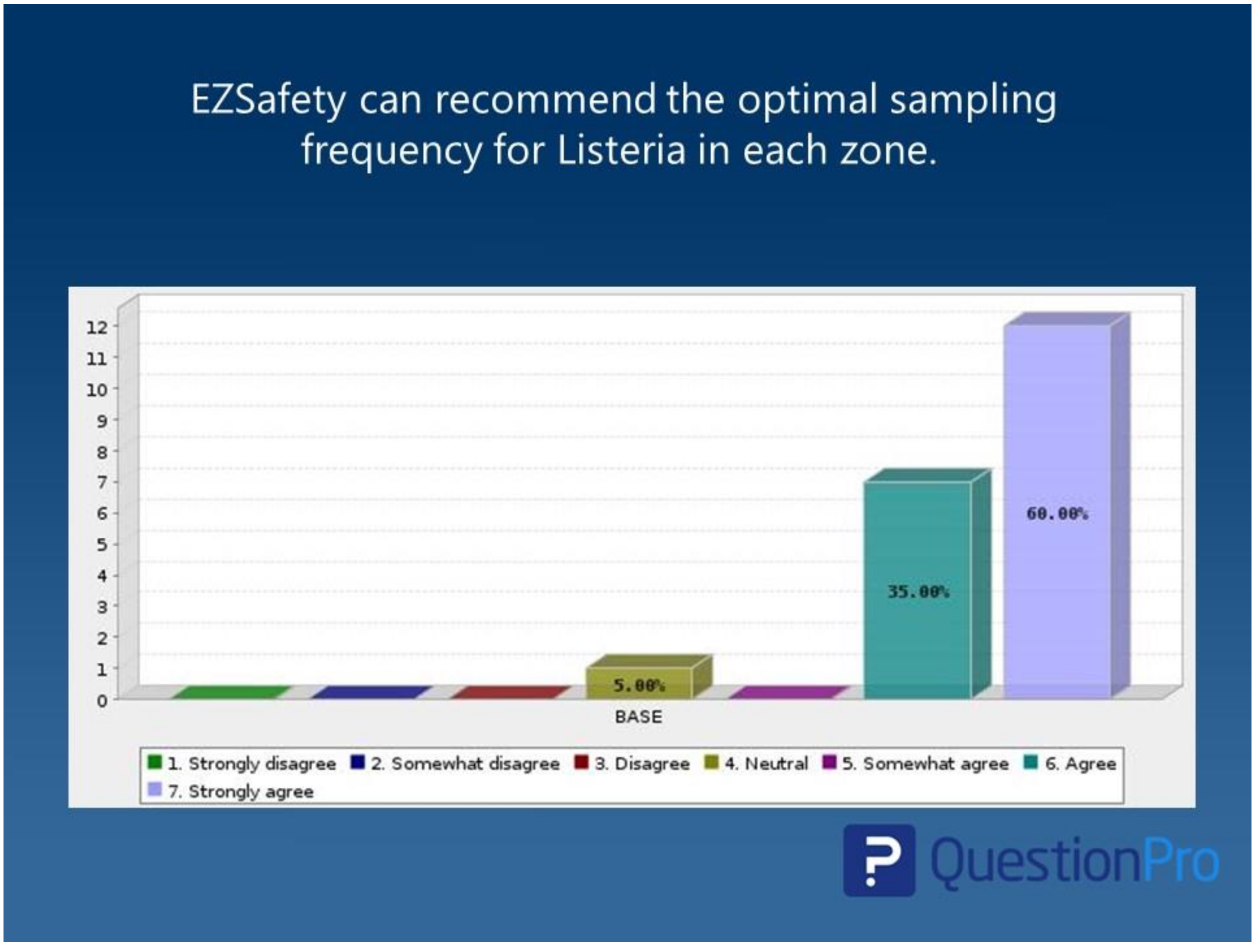

EZSafety has a set of baseline limits which is necessary to determine unacceptable test results.
12.5
10.0
7.5
5.0
2.5
0.0
70.00%
20.00%
10.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro


EZSafety has a set of baseline limits which is necessary to set up appropriate sampling plans.
13
12
11
10
9
8
7
6
5
4
3
2
1
0
5.00%
30.00%
65.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

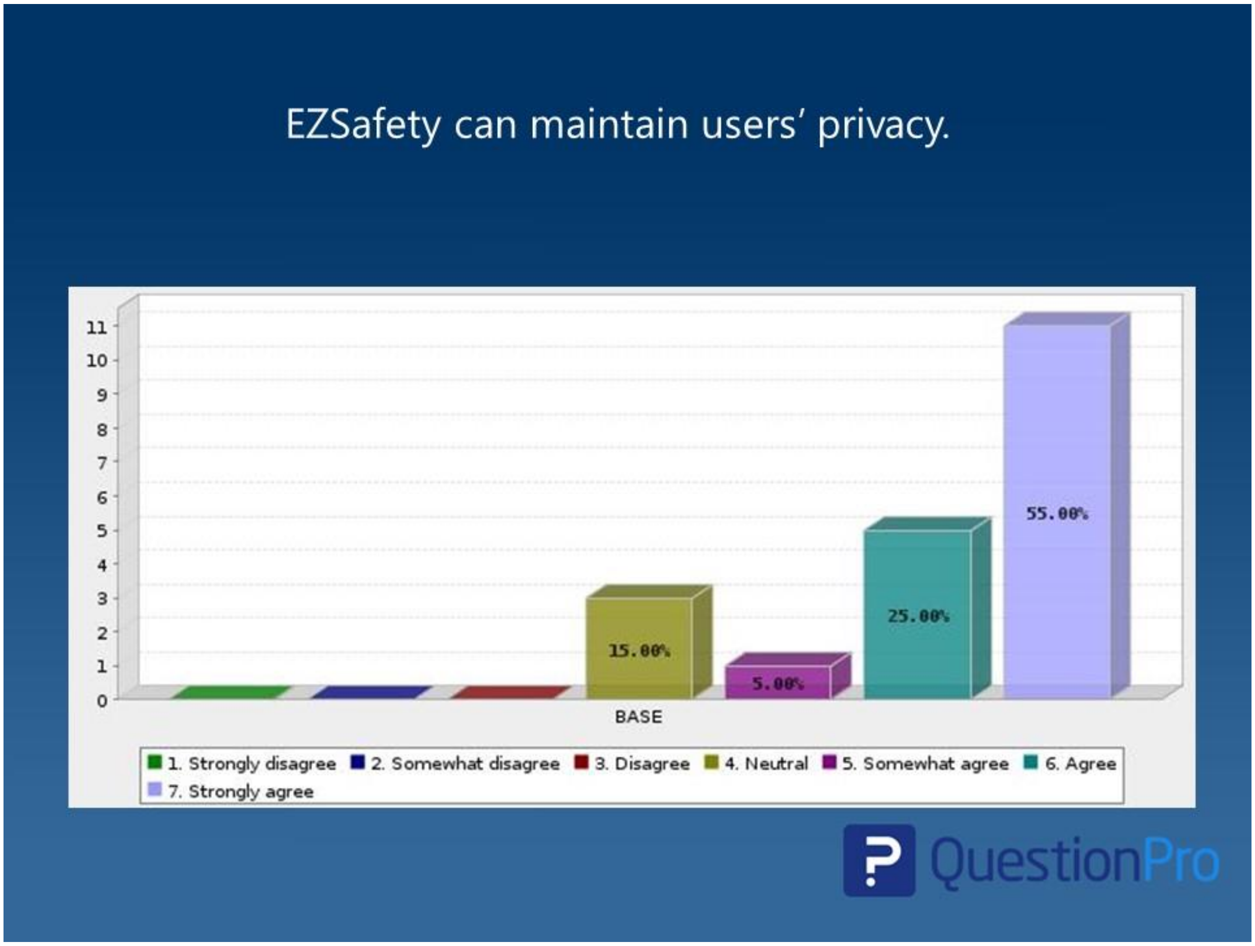

The different tools in Ezsafety can help poultry processors to make better plans to detect Listeria.
12.5
10.0
7.5
5.0
2.5
0.0
30.00%
70.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

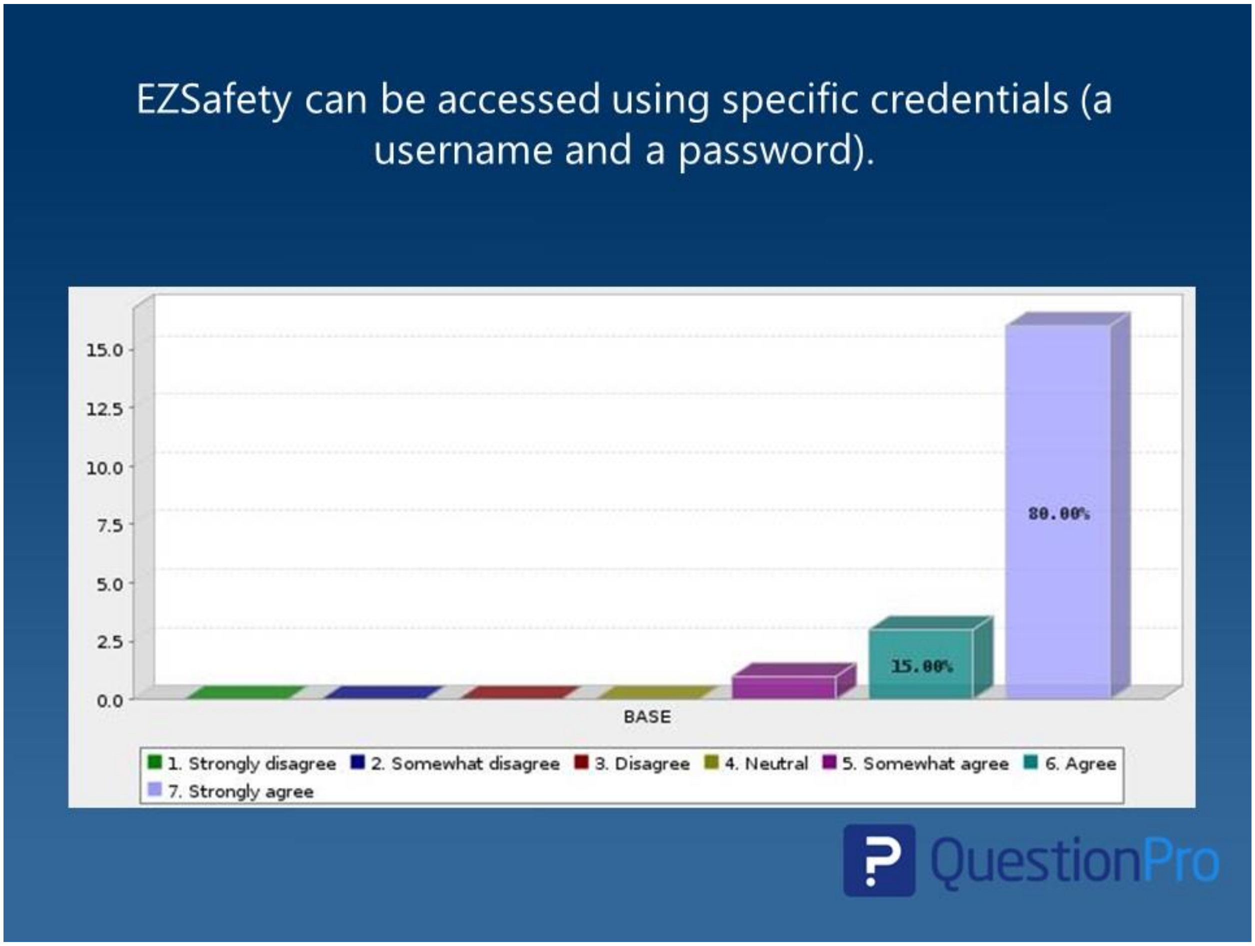
The information on the guidelines page can help poultry processors to have better plans to target Listeria and other contaminants.



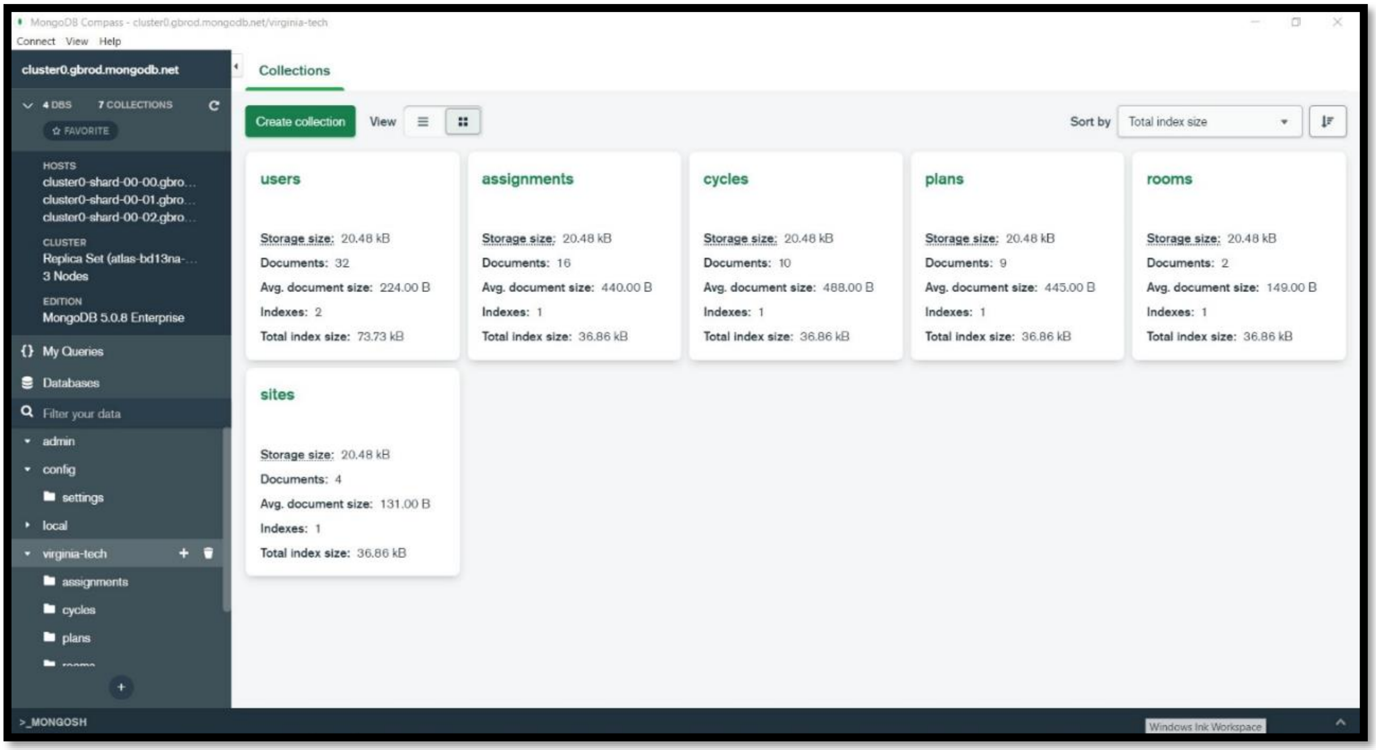


EZSafety can recommend the optimal sampling frequency for Listeria in each zone.
12
11
10
9
8
7
6
5
4
3
2
1
0
5.00%
35.00%
60.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

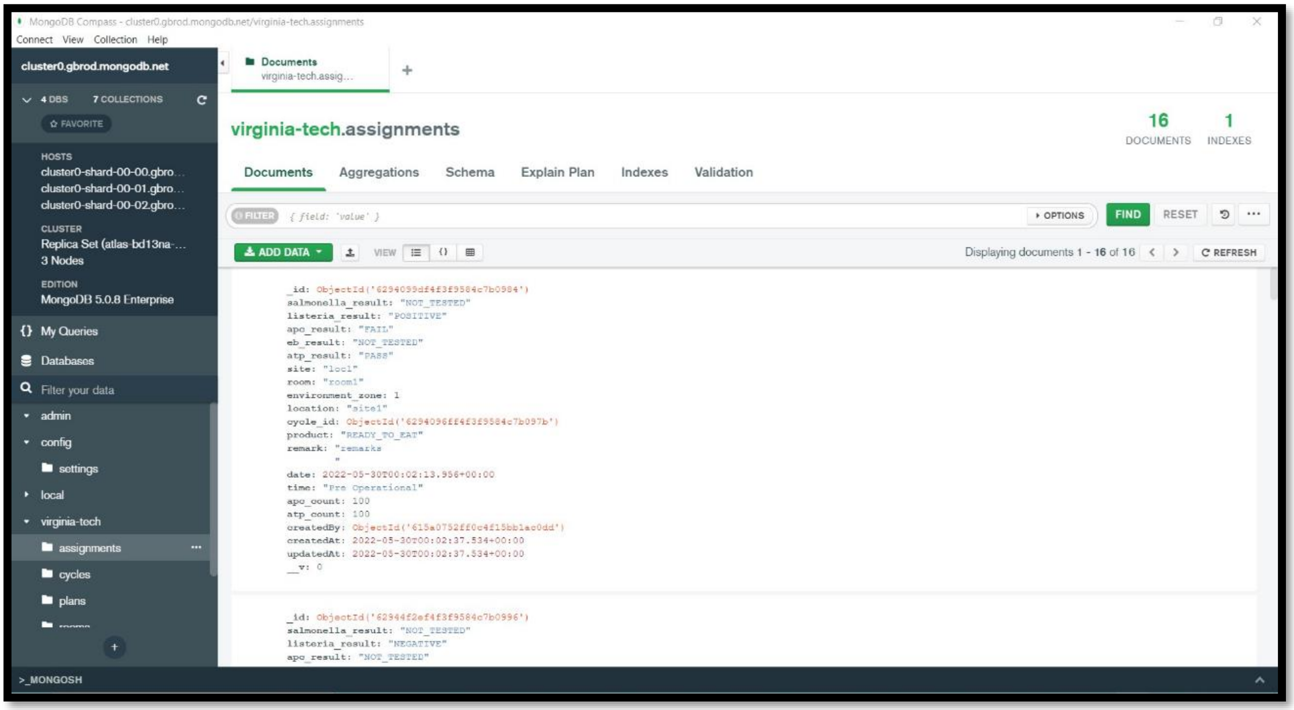

EZSafety can maintain users' privacy.
11
10
9
8
7
6
5
4
3
2
1
0
15.00%
5.00%
25.00%
55.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro


EZSafety can be accessed using specific credentials (a username and a password).
15.0
12.5
10.0
7.5
5.0
2.5
0.0
80.00%
15.00%
BASE
1. Strongly disagree
2. Somewhat disagree
3. Disagree
4. Neutral
5. Somewhat agree
6. Agree
7. Strongly agree
QuestionPro

Appendix B: Screenshots of displays of file structure for data storage and program files.



The above figure shows the architecture of the database. It can be used to refer to the activities seen on the website.



The above figure displays how each sample entered by the user is stored in the database.

Appendix C: An example of the codes used in EZSafety.

```
  >
    &#8203;
  </span>
  <Transition.Child
    as={Fragment}
    enter="ease-out duration-300"
    enterFrom="opacity-0 scale-95"
    enterTo="opacity-100 scale-100"
    leave="ease-in duration-200"
    leaveFrom="opacity-100 scale-100"
    leaveTo="opacity-0 scale-95"
  >
    <div className="inline-block w-full max-w-md p-6 my-8 overflow-hidden text-left align-middle transition-all transform bg-gray-100 shadow-xl rounded-2xl">
    <Dialog.Title
        as="h3"
        className="text-lg font-medium leading-6 text-black-900"
      >
        Action Needed! You are getting bad results.
        <br></br>
        Check <a href = "http://ezsafety.net/correctiveactions" target='_blank' style={{color: '#ff0000'}}><u>Corrective Actions</u></a> and consider the following:
        <br></br>
        1. Review your cleaning and sanitation practices.
        <br></br>
        2. Increase your sampling frequency and no. of samples.
        <br></br>
        3. Three consecutive negative samples are needed in order to return to routine monitoring.
        <br></br>
      </Dialog.Title>
      <div className="mt-4">
        <button
          type="button"
          className="inline-flex justify-center px-4 py-2 text-sm font-medium text-blue-900 bg-blue-100 border border-transparent rounded-md hover:bg-blue-200 focus:outline-none focus-visible:ring-2 focus-visi
          onClick={closeModal}
        >
          Got it, thanks!
        </button>
      </div>
    </div>
  </Transition.Child>
/div>
ialog>
nsition>
className="min-h-screen flex flex-col bg-gray-100 p-8">
v className="flex flex-col bg-white border border-gray-300 rounded-md shadow-2xl">
div className="font-sans flex font-large bold text-2xl pt-8 px-11">
 Add New Data
```

The codes in the above figure are responsible for checking what users are entering in the APC, ATP, and EB counts, and based on what number they have entered, the code will automatically set the result to either PASS, FAIL, or NOT SATISFACTORY using “If Else” statements. A list of codes will be executed if the result is FAIL, and the program will alert the user.