NEOGEN®

Cornell University

Environmental Monitoring Handbook

for the Food and Beverage Industries

2nd Edition

The Neogen® Environmental Monitoring Handbook is a helpful guide for food and beverage processors to use and is intended to provide general guidance only.

This second edition builds on information provided in the first edition and expands to include new chapters with more information. The technical information, recommendations and other statements contained in this document are based on experience and information that Neogen believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such information is intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information, taking into consideration the nature of their business, existing policies and particular laws and regulations that might apply.

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Key terms and definitions

Term	Definition
Adenosine triphosphate (ATP)	Energy molecule present in every cell, alive or dead.
Aggressive sampling[1]	Increased frequency and/or scope of sampling in response to a positive sample result. May also include addition of post-rinse sampling and other advanced sampling approaches.
Biofilm	Thin, slimy film of densely-packed bacteria that adheres to a surface. Biofilms may form on rough or scratched surfaces and in hard-to-reach areas, making them difficult to eliminate. Biofilms can represent a persistent harborage for microorganisms and a source of contamination of food products, as they can contain spoilage organisms or pathogens.
Clean out-of-place (COP)	Method of cleaning equipment items by removing them from their operational area and taking them to a designated station for disassembly and cleaning.
Clean-in-place (CIP)	Method of cleaning interior surfaces of process equipment, pipes, vessels, filter and associated fittings without disassembly.
Correction[2,3]	An action to eliminate a detected nonconformity. These can be immediate activities to identify and correct a problem that occurred during the production of food, such as re-cleaning and sanitizing a line before start-up of production when food residue remains after cleaning. This should not be confused with corrective action, as it may not address the cause of the problem.
Corrective action[2,3]	An action to eliminate the cause of a detected nonconformity or other undesirable situation, to prevent recurrence. This should not be confused with correction, which may not address the cause, or preventive action, which is taken to prevent occurrence of a potential problem.
Corrective and preventive action (CAPA)[4]	A quality management concept found within GMP, HACCP and ISO standards that aims to rectify a task, process, product or behavior that has resulted in errors or deviations from the intended plan. CAPA is split between two distinct functions — corrective actions and preventive actions — to systematically investigate the cause of the identified problems and prevent their recurrence or occurrence, respectively.

Term	Definition
Critical control point (CCP)[2,5]	A point, step, or procedure in a food process at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce the hazard to an acceptable level.
Critical limit[5]	A maximum and/or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of a food-safety hazard.
Environmental monitoring program (EMP)	Defined program for monitoring the environment of a food manufacturing facility to prevent cross contamination of the finished product from the environment. The term EMP typically is used to describe a program that verifies cleaning, sanitation, and other environmental pathogen control programs, and an EMP typically include sampling sites, frequency, testing methodology, acceptable criteria and corrective actions. More broadly, environmental monitoring programs often encompass a range of tests — from ATP and indicator organisms to pathogens, spoilage organisms, and allergens — and may serve to perform either validation or verification of specific pre-requisite programs (e.g., sanitation and sanitary equipment design) or may be more generally seen as a strategy to monitor the environment for unhygienic conditions that may lead to food safety and/or quality issues.
Environmental monitoring sampling zones[1,6,7,8,9]	Environmental sampling programs use a zone classification to identify the risk level of areas or sites where product may be exposed to post-lethality environmental contamination. In most countries and regions, sampling sites in processing facilities are assigned to one of four zones: (i) Zone 1 is the highest-risk area consisting of exposed food contact surfaces; (ii) Zone 2 contains non-food contact surfaces in close proximity to food and food contact surfaces, (iii) Zone 3 contains more remote non-food contact surfaces located in or near the processing area; (iv) Zone 4 includes non-food contact surfaces outside of the processing areas. In some countries, sampling sites may be classified into three zones, typically combining Zones 2 and 3 into one zone.
Firefighting	The (often-unsuccessful) approach of repeatedly attempting the same solution on a recurring problem in effort to obtain microbiological control.
For-cause[1]	Investigative sampling that follows a positive sample from a product, contact surface or other verification site.
Good manufacturing practices (GMP)	The conditions and practices for processing safe food under sanitary conditions, including personnel, plant and grounds, sanitary operations, sanitary facilities and controls, equipment and utensils, processes and controls, warehousing and distribution, and defect action levels considerations.

Term	Definition
Growth niche[1]	Location that supports microbiological growth and is protected from the sanitation process; characterized by high microbial counts after cleaning and sanitation.
Harborage site[1]	Growth niche that contains the pathogen or its indicator.
Hazard[2,5]	Any biological, chemical (including radiological), or physical agent that has the potential to cause illness or injury. Hazards may be introduced to or naturally present in the food.
Hazard analysis and critical control points (HACCP)[5]	A preventive food safety strategy that is a systematic approach to the identification and assessment of the risk of hazards from a particular food or food production process or practice and the control of those hazards that are reasonably likely to occur.
Hurdle	Methods, processes, preservations and technologies used in combination to ensure that pathogens in food products are appropriately eliminated or controlled.
Hygienic zoning	Division of a food manufacturing facility into different areas to avoid food contamination risks. Areas are designated based on risk and can include non-production areas (e.g., offices), basic GMP areas (e.g., raw material storage), and the primary pathogen control area (PPCA) where processed RTE product is exposed to the environment prior to packaging. Hygienic zones should not be confused with environmental monitoring sampling zones, which are used to designate target areas for environmental sampling (i.e., Zones 1–4).
Index organism	An organism or group of organisms whose presence relates to the possible occurrence of ecologically similar pathogen(s) (e.g., *Listeria* spp.).
Indicator organism	An organism or group of organisms whose presence reflects the general microbiological condition of the food or environment (e.g., coliforms, *Enterobacteriaceae*).
Intervention[1]	Procedure capable of eliminating the pathogen from the affected area (e.g., heat treatment, complete disassembly followed by cleaning and sanitation).

Term	Definition
***Listeria* intervention and control program**[1]	Documented regulatory compliance program designed to meet the regulatory needs of the establishment. The *Listeria* intervention and control program clearly defines (i) actions taken to verify the effectiveness of the establishment's control of the environment and (ii) actions taken when a sample from product, contact surface or verification site is positive for *Listeria monocytogenes* or *Listeria* spp.
Mesophilic[10]	Temperatures ideal for the growth of mesophiles, which are organisms in which optimal growth occurs between 25° and 40°C.
Microbiota	The population of microorganisms found in a specific environment.
Non-process preventive control[11]	Activities or systems that are not directly involved in the production or transformation of food, but play a crucial role in ensuring food safety, quality, and regulatory compliance.
Pathogen environmental monitoring (PEM) program	A defined program for monitoring the environment of a food manufacturing facility for pathogenic microorganisms. The goal of a PEM program is to find and eliminate pathogen contamination in the processing environment. They are typically used to (i) verify an overall food safety system (or specific components of a food safety system) and to (ii) provide early indication of potential food safety hazards.
Periodic deep cleaning and sanitation[1]	Disassembly of equipment or other components of a processing plant beyond the normal level, followed by cleaning and sanitization.
Post-rinse sampling[1]	Samples taken after production, disassembly and the initial rinse but before the application of soap or sanitizer. Typical sites are below the product line and in areas that tend to collect spatter from the rinsing process (e.g., machine sides, legs, support structure, floor wall juncture). Post-rinse samples are good broad indicators of the presence of the organism in the post-lethality exposed product area. Detection of the organism does not mean there is a harborage site within the scope of the sampled area. Positive post-rinse samples will typically trigger aggressive sampling.
Pre-requisite program[12]	A set of basic practices and procedures that are necessary to maintain a hygienic environment throughout food production.

Term	Definition
Preoperative sampling	Samples taken after sanitation but before starting production, typically during or after assembly and setup.
Preventive action[3]	An action to eliminate the cause of a potential non-conformity or other undesirable situation to prevent occurrence.
Preventive control (PC)[2]	Proactive control measures designed and undertaken to reduce or eliminate food safety hazards. These include risk-based, reasonably appropriate procedures, practices, and processes that a person knowledgeable about the safe manufacturing, processing, packing, or holding of food would employ to significantly minimize or prevent the hazards identified under the hazard analysis that are consistent with the current scientific understanding of safe food manufacturing, processing, packaging, or holding at the time of the analysis.
Primary pathogen control area (PPCA)	A designated hygiene zone. The PPCA is an area where product is exposed to the environment post-lethal processing. Also known as the ready-to-eat (RTE) area, high-risk area or high-hygiene area.
Qualitative test	A test that determines presence or absence of an analyte(s) in a sample.
Quantitative test	A test that measures the level or concentration of an analyte(s) in a sample.
Relative light unit (RLU)	The reading of the amount of light as determined by an individual ATP-based hygiene monitoring system. ATP system manufacturers may have different values for 1 light unit and all measurements are made relative to that value.
Sanitation process control program[1]	Overall process used to manage environmental control; includes both food safety components and non-regulatory quality components. Regulatory components include HACCP, SSOPs, pre-requisite programs and pathogen control program. “For-cause” investigative sampling is part of the pathogen control program. “Not-for-cause” sampling is a part of the sanitation process control program but is not necessarily a component of the regulatory compliance program.
Sanitation standard operating procedures (SSOPs)	Written procedures that a food manufacturing facility develops and implements to ensure sanitary conditions and prevent direct contamination or adulteration of food product. These include written steps for cleaning and sanitation, and are considered as one of the pre-requisite programs of HACCP.

Term	Definition
"*Seek and Destroy*" process[1]	A multi-faceted systematic approach to finding sites of persistent strains (niches) in food processing plants, with the goal of either eradicating or mitigating effects of these strains. This process has been used effectively to address persistent *Listeria monocytogenes* contamination in food processing plants. The continued use of this science-based strategy can not only control environmental pathogens, but it can also be deployed for controlling microbial spoilage in ready-to-eat (RTE) foods. The "*Seek and Destroy*" process can help to: • Finds pathogenic growth niches • Finds potential growth niches requiring monitoring and control • Defines normal level of disassembly • Defines periodic deep level of disassembly • Defines frequency of periodic deep level of disassembly • Qualify a new piece of equipment (e.g., run for 90 days then conduct *Seek and Destroy* investigation) • Validate effectiveness of equipment cleaning protocol • Validate effectiveness of intervention applied to a piece of equipment (e.g., heat treatment or other method)
Time-Action-Concentration-Temperature (TACT)	An approach to evaluate a root-cause failure of a cleaning process by examining the time, mechanical action, concentration of chemicals and/or the temperature of the intervention process.
Transfer pathway[1]	Path of travel an organism takes to move from transfer point to transfer point (e.g., the pathway between the harborage site and a contact surface or product); this typically reflects transfer of a pathogen by objects or people. Water, employees, equipment, product, materials and aerosols are common transfer vectors.
Transfer point[1]	Surfaces that are exposed to cleaning and sanitation and can serve as points of contact facilitating the transfer of an organism from one surface to another, e.g., gloved hands. Transfer points should not be growth niches when effective cleaning and sanitizing procedures are used.
Validation[5]	Providing scientific evidence that a strategy controls a given hazard. Environmental monitoring is a key strategy that can be used to validate cleaning and sanitation procedures. This typically involves testing of equipment, using a "*Seek and Destroy*" approach after cleaning and sanitation have been performed, including complete disassembly of the equipment and collection of samples on the disassembled equipment to validate that the procedures used completely clean and sanitize a piece of equipment.

Term	Definition
Vector swabbing	Additional investigative swabbing conducted in all directions, including up and down where possible, from the site of an initial positive detection.
Verification monitoring program[1]	Routine program to verify the consistent application of the sanitation process control program; includes sampling of Zone 1, 2, and 3 environmental sites in the ready-to-eat (RTE) area. This program is used for regulatory compliance and is a part of an establishment's HACCP or SSOP program.
Verification sites, contact surface (Zone 1)[1]	Testing of Zone 1 (food contact surface) sites is typically the primary verification measure for the consistent application of the environmental pathogen control program to prevent product contamination. In high-risk product manufacturing, these sites should be evaluated weekly; lower risk lines may be evaluated less frequently as long as the process is under control.
Verification sites (Zones 2 and 3)[1]	Locations sampled during operations to detect the presence of the organism in the normal operating environment. Verification sites are surfaces that are exposed during the normal operating conditions and are likely to serve as transfer points (i.e., they are located in transfer pathways). Monitoring of verification sites detects the organism as it is being moved from its harborage location to a contact surface or the product.
Zone 1[1,6,7,8,9]	Direct food contact surfaces post lethal processing, e.g., slicers, peelers, fillers, hoppers, screens, conveyor belts, air blowers, employee hands, knives, racks, work tables.
Zone 2[1,6,7,8,9]	Non-food contact surfaces in close proximity to food and food contact surfaces, e.g., processing equipment exterior and framework, refrigeration units, equipment control panels, switches.
Zone 3[1,6,7,8,9]	More remote non-food contact surfaces located in or near the processing area, e.g., forklifts, hand trucks, carts, wheels, air return covers, hoses, walls, floors, drains.
Zone 4[1,6,7,8,9]	Non-food contact surfaces outside of the processing areas, e.g., locker rooms, cafeterias, entry/access ways, loading bays, finished product storage areas, maintenance areas.

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CHAPTER 1

The importance of environmental sampling in food safety and quality programs

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1.1 Increasing recognition of the food processing environment as a contamination source

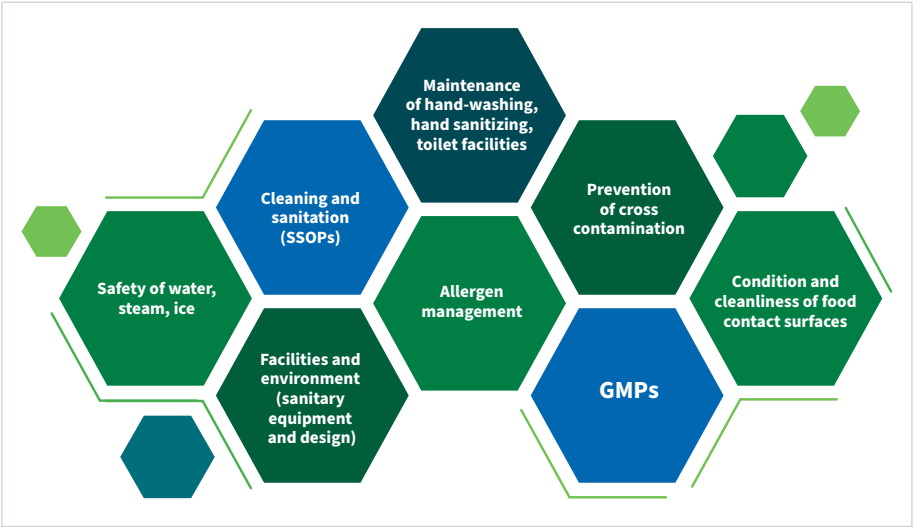
There is increasing recognition that environments in food processing facilities, as well as other built environments used in food production and distribution (e.g., retail food handling spaces, restaurants or packing houses for produce) can be important sources of biological agents, chemical compounds and physical hazards that may negatively affect food safety and quality.

Classical food safety and quality systems strongly relied on the concept of Hazard Analysis and Critical Control Points (HACCP) to ensure food safety and food quality, with an emphasis on identifying a specific, targeted critical control point (CCP) for each hazard identified, as reasonably likely to occur. Specific parameters that would allow for effective control of the target hazard at the CCP would have to be established (by "validation") then continuously monitored (by "verification"). A typical example of a CCP would be a heat treatment meeting a certain minimum temperature and time requirement for pasteurization of milk.

However, HACCP and quality management systems that utilize similar concepts, require "pre-requisite programs" to be in place to ensure that HACCP-based food safety programs and similar food quality programs work effectively.

Examples of classical pre-requisite programs include pest control, sanitation and sanitation standard operating procedures (SSOPs), personal hygiene and Good Manufacturing Practices (GMPs) (Figure 1).

Figure 1. HACCP and selected pre-requisite programs that can be validated and verified by environmental monitoring



Despite the value of HACCP-based food safety systems and similarly structured food quality systems, it has become clear that many food safety and quality issues experienced around the world are due to failures and problems with pre-requisite programs.

Common shortcomings with pre-requisite programs, such as sanitation programs (including sanitary equipment and facility design) and GMPs (including hygienic zoning), include a lack of validation and verification.

Examples of food safety and quality issues caused by failures with pre-requisite programs include listeriosis outbreaks linked to ready-to-eat (RTE) foods where contamination could be traced back to locations in the processing plant environment. These are often linked to growth niches where *Listeria monocytogenes* could survive over time and contaminate finished product.[1] Similar issues have also been observed for *Salmonella*.[2]

Microbial spoilage issues in RTE food and beverages can also often be traced back to sources in processing plant environments that were not effectively controlled through sanitation and GMPs. Examples of spoilage organisms typically traced back to sources in processing plant environments include *Pseudomonas* spp. and lactic acid bacteria, as well as yeast and mold.

Similarly, allergen contamination issues and recalls can sometimes be traced to failures in pre-requisite programs.

Due to the recognition of the association between processing facilities and sources of food safety and quality issues, the food industry and its regulators are heightening their emphasis on environmental monitoring programs. Conceptually, environmental monitoring may serve as either validation or verification of specific pre-requisite programs (e.g., sanitation and sanitary equipment design) or may be more generally seen as a strategy to monitor the environment for unhygienic conditions.

The increasing importance of environmental monitoring programs is particularly well-illustrated by changes to food safety regulations and standards around the world (Tables 1–3). The U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) and similar regulations in other countries have elevated the importance of pre-requisite programs. For example, in the FSMA Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food Rule (PC Rule), many of the specified “preventive controls” represent programs that would have previously been classified as pre-requisite programs. However, FSMA preventive controls include a requirement for verification of the preventive controls, which was not in place for pre-requisite programs.

Additionally, the FSMA PC Rule includes specific recognition of environmental monitoring as a key verification strategy for certain non-process preventive controls such as sanitation: “Environmental monitoring, for an environmental pathogen or for an appropriate indicator organism, if contamination of a ready-to-eat food with an environmental pathogen is a hazard requiring a preventive control, by collecting and testing environmental samples.”[3]

This provision demonstrates the growing consensus on the importance of environmental monitoring programs as an essential part of food safety and quality systems.

Table 1. Regulations and standards for environmental monitoring (1)

United States Food and Drug Administration (U.S. FDA)[3]	
Regulation/ Policy	Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food (PC Human Food Regulation) **21 CFR §117.165 Verification of implementation and effectiveness**
Scope	Human foods, including ready-to-eat (RTE) foods
Requirement	Environmental monitoring, for an environmental pathogen or for an appropriate indicator organism, if contamination of a ready-to-eat food with an environmental pathogen is a hazard requiring a preventive control, by collecting and testing environmental samples.
United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS)[4]	
Regulation/ Policy	**9 CFR § 430.4 — Control of Listeria monocytogenes in postlethality exposed ready-to-eat products**
Scope	Ready-to-eat (RTE), meat and poultry products *(L. monocytogenes)*
Requirement	(iii) If an establishment chooses ... to use only an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes*, its sanitation program must: a) Provide for testing of food contact surfaces in the postlethality processing environment to ensure that the surfaces are sanitary and free of *L. monocytogenes* or of an indicator organism; b) Identify the conditions under which the establishment will implement hold-and-test procedures following a positive test of a food-contact surface for *L. monocytogenes* or an indicator organism.
Health Canada[5]	
Regulation/ Policy	**Policy on *Listeria* monocytogenes in ready-to-eat foods** 7.1 Control of ready-to-eat food manufacturing
Scope	Ready-to-eat (RTE) foods *(L. monocytogenes)*
Requirement	An effective environmental monitoring program, supported by investigative sampling to detect sources of *Listeria* spp., should be used to identify additional steps the manufacturer should take to continuously improve its food safety system. Experience indicates that environmental sampling is the most sensitive tool to verify the effectiveness of control measures to prevent the introduction of *L. monocytogenes* into RTE foods (Tompkin et al., 1992; Tompkin 2002; Farber et al., 2021).

Table 2. Regulations and standards for environmental monitoring (2)

European Union[6]	
Regulation/ Policy	**COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs** Article 5
Scope	Foodstuffs
Requirement	Specific rules for testing and sampling 2. Samples shall be taken from processing areas and equipment used in food production, when such sampling is necessary for ensuring that the criteria are met. In that sampling the ISO standard 18593 shall be used as a reference method. Food business operators manufacturing ready to eat foods, which may pose a *Listeria monocytogenes* risk for public health, shall sample the processing areas and equipment for *Listeria monocytogenes* as part of their sampling scheme. Food business operators manufacturing dried infant formula or dried foods for special medical purposes intended for infants below six months, which pose a *Cronobacter* spp. risk shall monitor the processing areas and equipment for *Enterobacteriaceae* as part of their sampling scheme.
Codex Alimentarius[7]	
Standard	**CXG 61 2007 (Guidelines on the Application of General Principles of Food Hygiene to the Control of *Listeria* monocytogenes in Foods)** Annex I: Recommendations for an Environmental Monitoring Program for *Listeria monocytogenes* in Processing Areas
Scope	Ready-to-eat (RTE) foods *(L. monocytogenes)*
Requirement	The necessity for an environmental monitoring program is highest for ready to eat foods that support *L. monocytogenes* growth and that are not given a post packaging listericidal treatment. Recontamination has led to many of the recognized outbreaks of listeriosis. One effective element of managing this risk is to implement a monitoring program to assess control of the environment in which ready to eat foods are exposed prior to final packaging.

Table 3. GFSI-benchmarked certification program and standards related to environmental monitoring

Food Safety System Certification 22000 (FSSC)[8]	
Certification Program/ Standard	**FSSC 22000: Version 6** Requirements for Organizations to be Audited 2.5.7 Environmental Monitoring (Food Chain Categories BIII, C, I & K)
Scope	Food manufacturing
Requirement	The organization shall have in place: a) A risk-based environmental monitoring program for the relevant pathogens, spoilage, and indicator organisms; b) A documented procedure for the evaluation of the effectiveness of all controls on preventing contamination from the manufacturing environment and this shall include, at a minimum, the evaluation of microbiological controls present; and shall comply with legal and customer requirements. c) Data of the environmental monitoring activities, including regular trend analysis; and d) The environmental monitoring program shall be reviewed for continued effectiveness and suitability, at least annually, and more often if required, including when the following triggers occur: i. Significant changes related to products, processes, or legislation; ii. When no positive testing results have been obtained over an extended period of time; iii. Trend in out of specification microbiological results, related to both intermediate and finished products, linked to environmental monitoring; iv. A repeat detection of pathogens during routine environmental monitoring; and v. When there are alerts, recalls, or withdrawals relating to product/s produced by the organization.

Safe Quality Food (SQF) Institute[9]	
Certification Program/ Standard	The SQF Food Safety Code: Food Manufacturing (Edition 9) **PART B: System Elements** **2.4.8 Environmental Monitoring**
Scope	Food manufacturing
Requirement	2.4.8.1 A risk based environmental monitoring program shall be in place for all food manufacturing processes and immediate surrounding areas, which impact manufacturing processes. The responsibility and methods for the environmental monitoring program shall be documented and implemented. 2.4.8.2 An environmental sampling and testing schedule shall be prepared. It shall at a minimum: i. Detail the applicable pathogens or indicator organisms to test for in that industry; ii. List the number of samples to be taken and the frequency of sampling; iii. Outline the locations in which samples are to be taken and the rotation of locations as needed; and iv. Describe the methods to handle elevated or undesirable results. 2.4.8.3 Environmental testing results shall be monitored, tracked, and trended, and preventative actions shall be implemented where unsatisfactory results or trends are observed.

BRCGS[10]	
Certification Program/ Standard	**BRCGS** **Global Standard Food Safety (Issue 9)**
Scope	Food manufacturing
Requirement	The design of the environmental monitoring program shall be based on risk and, at a minimum, include: • sampling procedures • identification of sample locations • frequency of tests • target organism(s) (e.g. pathogens, spoilage organisms and/or indicator organisms) • test methods (e.g. settle plates, rapid testing and swabs) • recording and evaluation of results. The program and its associated procedures shall be documented.

Examples of *Listeria monocytogenes* and *Salmonella* persistence events responsible for outbreaks

In the United States, the Centers for Disease Control and Prevention (CDC) and state-level health departments continually monitor the number of cases of foodborne illness. When there is a spike in the number of cases caused by a given pathogen, this may be an indication that an outbreak is occurring.

For example, in October of 1998, there was a spike in the number of listeriosis cases in New York, indicating a potential outbreak. In response, the *Listeria monocytogenes* isolates collected from these clinical cases, as well as cases in other states, were characterized by subtyping to determine if their "fingerprints" matched. A single subtype was common among several cases from that October, as well as some isolates from previous months initially deemed to be sporadic cases. Interviews with the patients were then conducted to determine if there were any common foods consumed among them.

The results showed that 89 percent of the patients infected with the outbreak strain had consumed cooked frankfurters, and only 32 percent of patients not infected with the outbreak strain had consumed cooked frankfurters. Of those patients infected with the outbreak strain, 78 percent reported eating a single brand of frankfurters.[1]

From there, finished product *Listeria monocytogenes* testing was performed on the frankfurters of the identified brand. Subtypes of isolates from the finished product matched those isolated from clinical cases, implicating this company in the outbreak.

By the end of the outbreak, there were 108 cases of listeriosis and 14 associated deaths. Even though the company had an appropriate HACCP plan, they were still producing unsafe product. It was later determined that the *Listeria monocytogenes* contamination originated from the processing plant environment. This case illustrates the need for effective environmental monitoring programs (including appropriate corrective and preventive actions) even in facilities that have HACCP plans.

Similarly, a *Salmonella* Agona outbreak was traced back to toasted oat cereal in 1998, which caused 209 cases of salmonellosis.[2] The *Salmonella* was determined to be coming from the processing plant environment. Then, 10 years later in 2008, another *Salmonella* Agona outbreak was traced back to puffed rice cereal, which caused 28 salmonellosis cases. It was determined that the strains implicated in both outbreaks were of the same subtype, indicating that the *Salmonella* had survived in the plant for a decade. This case illustrates that effective environmental monitoring programs are not only necessary for *Listeria monocytogenes*, but are also essential for *Salmonella*, particularly in facilities that produce low-water activity RTE products.

1.2 Importance of identifying specific purposes and goals for environmental monitoring programs

Environmental monitoring programs and environmental sampling activities can serve multiple, and sometimes complementary, purposes. In practice, environmental monitoring programs often encompass a range of tests — from ATP and indicator organisms to pathogens, spoilage organisms, and allergens — conducted on a variety of samples collected throughout a facility, at various time points, with varying frequencies. Often, these programs have been used for years and modified over time to address specific customer and regulatory requirements or particular issues or concerns. This can lead to programs that represent an uncoordinated, non-unified approach that may not use resources effectively, particularly if new requirements for environmental monitoring are frequently added. Therefore, it is often essential for the food industry and specific processing plants to specifically define the purpose of current and planned environmental monitoring programs.

While there does not appear to be a universally recognized framework for this, some potential approaches would seem logical and consistent with other aspects of food safety and quality management, such as HACCP.

A HACCP-informed approach to develop purpose-driven environmental monitoring programs could, for example, start with an identification of food safety hazards and quality threats. A food manufacturer might then determine which specific hazards could potentially be transmitted through the processing plant environment, recognizing that the processing plant itself could be a source or a vehicle for cross contamination, or both.

Control strategies (e.g., sanitation, GMPs, sanitary equipment design) would then be prescribed to control each hazard or threat. Subsequently, a facility could identify environmental monitoring activities needed to validate that a given, non-process preventive control addresses the target hazard (which often would be non-trivial). A facility would then implement a routine environmental monitoring program that would verify the effectiveness of the validated control and ensure it would be consistently implemented (Figure 2).

Importantly, verification may include obtaining measurements and establishing records other than those for classical environmental monitoring tests. For example, ATP testing (used to verify cleaning), combined with visual inspection, sanitizer concentration measurement records, and check sheets documenting the duration of sanitizer application, could be sufficient to verify sanitation. Furthermore, corrective actions should be developed in case the verification critical limits are not met.

Environmental monitoring programs could be developed for specific purposes and implemented by identifying key preventive controls (without necessarily assigning specific hazards to be controlled by each preventive control). Subsequent identification of environmental monitoring activities would be needed to validate and verify each control. These approaches may also facilitate realignment of existing environmental monitoring activities, including elimination or revision of specific tests that no longer have clearly defined goals and purposes.

Figure 2. A HACCP-informed approach to environmental monitoring

- Identify food safety hazards and quality threats that can be introduced into the product from the processing environment
- Conduct a risk assessment to evaluate the likelihood and severity of identified hazards/threats, prioritizing control measures based on the level of risk

- Identify non-process preventive controls/pre-requisite programs needed to control each hazard/threat
- Examples: Sanitation, GMPs, and sanitary equipment design

- Determine the acceptance criteria required to control the hazard/threat
- Examples: Concentration/temperature/flow of cleaning solution, visual inspection pass or fail, ATP pass/fail values

- Develop a monitoring plan with the established acceptance criteria and set a frequency
- Examples: Measuring the concentration/temperature/flow of cleaning solution, daily visual inspection, daily ATP testing

- Determine how to respond if acceptance criteria is exceeded
- Example: Re-cleaning and re-sanitation if ATP is above threshold

- Maintain records on the validation, monitoring, verification, corrections, and corrective actions

- Use environmental monitoring to verify that controls are working
- Examples: Weekly, at least 4 hours after processing starts; possible criteria may include *Enterobacteriaceae* (EB) counts below a certain threshold or "negative for *Listeria* spp."

- Regularly analyze collected environmental monitoring data to identify trends, deviations, or areas requiring further action
- Communicate findings to relevant stakeholders, including quality assurance teams and management, to drive continuous improvement in food safety and quality strategies

1.3 Target analytes for environmental monitoring programs

When designing and implementing environmental monitoring programs, it is essential to identify the proper chemical and biological target analytes for testing various samples and achieving different goals (such as verification and validation). Typical target analytes used in environmental monitoring programs include compounds that can assess cleaning efficacy (e.g., ATP or protein), allergens, indicator organisms, pathogens, and spoilage organisms.

Understanding these target analytes, as well as the sensitivity and specificity of the tests used, is essential for the design and implementation of appropriate environmental monitoring programs. A summary of analytes is shown Table 4, and more detail is provided in subsequent chapters.

Table 4: Target analytes and their role in environmental monitoring programs

ATP	• Verification of effective cleaning procedures • Real-time testing results can lead to immediate corrections, and data trending enables identification and correction of cleaning failures before they impact food safety and/or quality
Allergens	• Validation and verification of allergen cleaning procedures to minimize risk of cross-contact contamination in shared equipment of production lines • Identification of food allergen harborage sites
Indicator Organisms	• Validation and verification of sanitation procedures and help to determine hygienic status of the processing equipment and environment • Quantitative results allow for baseline determination, acceptable limits and analysis of trends
Spoilage Organisms	• Reduction of post-processing contamination that can result in food and beverage spoilage • Quantitative results allow for baseline determination, acceptable limits and analysis of trends
Pathogens	• Verification of effective sanitation procedures and other strategies for pathogen control • Identification of environmental sources and transmission pathways for pathogens

1.4 Importance of coordination and integration of environmental monitoring programs

Coordinating and integrating aspects of an environmental monitoring program can increase the program's effectiveness and efficiency. For example, in some facilities, ATP testing, visual inspection, environmental allergen tests, and environmental microbiological tests may not always be coordinated or analyzed together, despite that all of them typically help validate or verify sanitation practices. Coordinated analyses of the different tests therefore may allow rapid and sensitive detection of sanitation issues.

For example, coordinated environmental sampling programs should include record-keeping and data analyses of all environmental monitoring data (ATP, indicator organisms, allergen monitoring, and pathogen monitoring) and should include a standardized sampling site list that encompasses all sites tested. Best practices for environmental monitoring programs may include (but are not limited to) electronic record-keeping, consistent designation of sampling sites (some facilities may have thousands of sampling sites, each with a unique identifier), coordinated and integrated analysis of different environmental monitoring data, and regular, in-person reviews of all environmental monitoring data (typically, at least every six to 12 months) as well as other approaches to coordinate and integrate multiple environmental sampling programs.

Additional strategies and activities that facilitate coordination and integration of environmental monitoring programs include use of floor plans and trending charts that allow for integrated temporal and spatial analysis of different environmental monitoring data as well as SOPs for sample collection and follow-up of results that are out-of-specification. Examples of these strategies can be found in Chapter 10, Data Management and Utilization in Environmental Monitoring.

Control of environmental sources of microbial contaminants is important for proactively addressing food spoilage issues

Using social media, a single consumer was capable of reaching close to a half million people regarding her dissatisfaction with the premature spoilage of a juice pouch. In this case, the effects were so powerful that the company was forced to conduct a costly redesign of their package so that consumers could see the juice in the pouch to ensure it had not spoiled.[11] Since a processing plant environment is the likely source for a number of spoilage organisms, environmental monitoring programs can play a key role not only in improving the safety of food products, but helping identify and eliminate or manage niches of spoilage-causing organisms.

With social media allowing consumers to easily communicate spoilage issues to large audiences, proactive approaches to preventing even rare spoilage issues are becoming increasingly more important. Well-designed environmental programs therefore provide multiple benefits for food companies and a larger return-on-investment than many may realize.

1.5 Business needs for environmental monitoring programs

While the primary goal for environmental monitoring programs typically is to control and reduce food safety hazards (e.g., allergens, microbial pathogens), environmental monitoring programs also play an important role in protecting businesses from potentially expensive recalls. For example, recalls of RTE food products due to contamination with pathogens such as *Listeria monocytogenes* and *Salmonella* can often be attributed to environmental sources.

Effective environmental monitoring programs, particularly those linked to specific goals such as sanitation validation and verification, can significantly reduce the risk of these recalls. For instance, good environmental monitoring data are often essential to allow companies to limit recalls to a single lot, production day, or production week. This is because without appropriate validation and verification data, it is challenging to sufficiently prove that finished product contamination on a given day could not have been transferred to subsequent lots (often referred to as a "clean break").

In addition to food safety hazards, spoilage issues (including problems caused by organisms introduced from the environment in processing plants) represent an increasing business and brand risk for food companies. Consumers often use social media platforms to communicate food spoilage issues and pressure companies into action, as described above.

Reduced risks of spoilage issues and associated recalls due to effective environmental monitoring programs, thereby represent another benefit to food companies.

Although it is widely recognized that recalls are extremely costly for companies, quantification of the benefits of environmental monitoring programs is often still considered challenging. While recalls tend to occur rarely, improved foodborne disease surveillance systems place companies at an increased risk of being identified as the source of an outbreak.

However, food companies have also realized that effective environmental monitoring programs can facilitate extended run-times, thereby improving production efficiency. For example, environmental monitoring may identify difficult-to-clean areas that can be eliminated through equipment redesign, which will subsequently allow for longer production runs.

Learn more about environmental monitoring

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CHAPTER 2

Using environmental sampling to validate the effectiveness of non-process preventive controls and pre-requisite programs

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2.1 Introduction

There is emerging recognition that environmental sampling can be a key tool for the validation of food safety controls. Specifically, it can be argued that **validation** of key non-process preventive controls (non-PPC) and pre-requisite programs (PRP) (e.g., cleaning and sanitation) is an important step that assures routine environmental monitoring activities (which, as detailed in subsequent chapters, would typically be considered as verification activities) are indeed verifying valid control strategies. This role for environmental sampling was originally incorporated into routine environmental monitoring programs (often under the concept of "seek and destroy activities"; see Chapter 8). However, future food safety efforts and best-in-class programs will likely continue to develop more formal and separate processes for validation of non-process preventative controls and pre-requisite programs as detailed in this chapter.

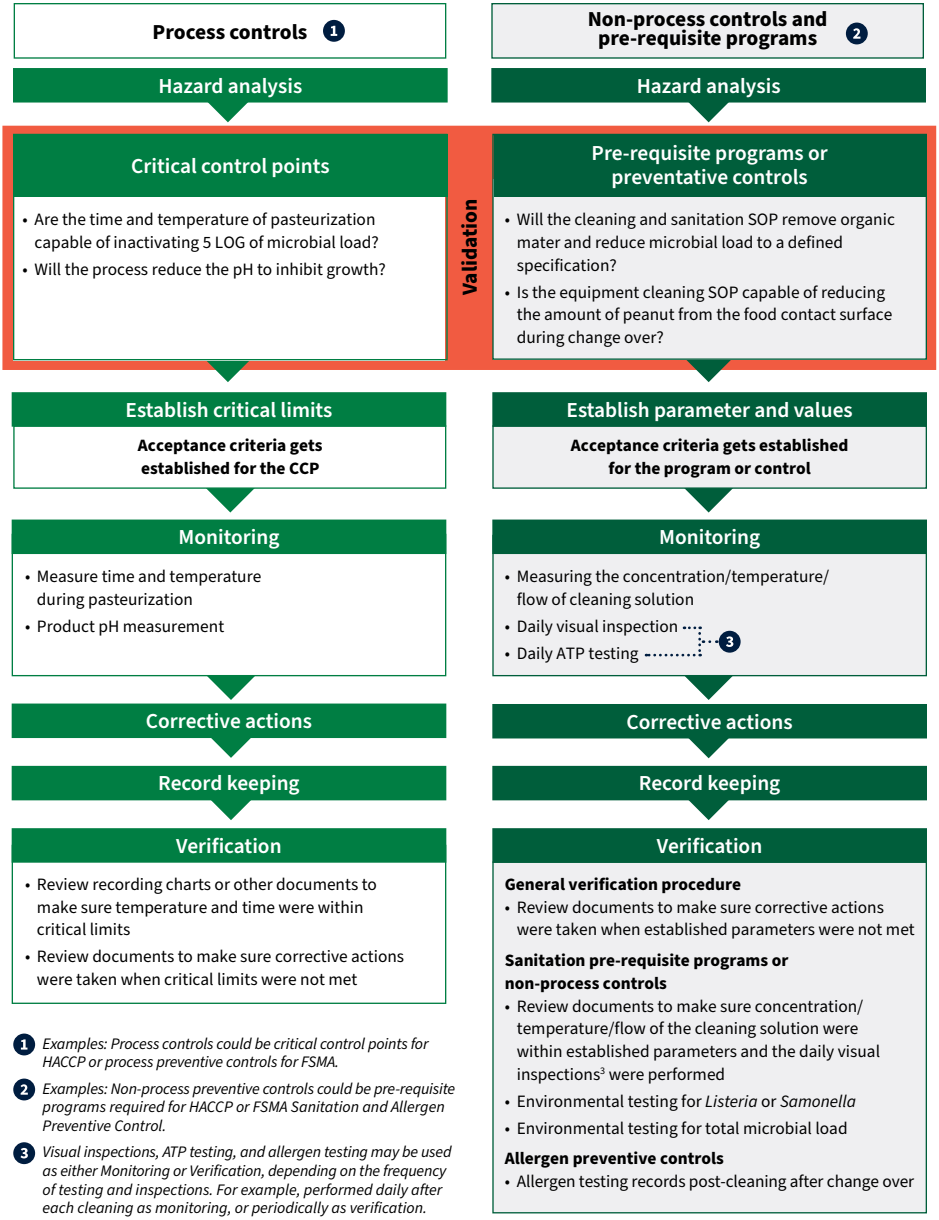
2.2 What is validation and why is validation of non-process controls and preventive controls important?

Modern food safety management programs take a risk-based approach by performing a hazard analysis to identify biological, chemical, and physical hazards that require Preventive Control (PC). Process Preventive Controls (PPCs) or Critical Control Points (CCPs) are considered the last line of defense against some of these hazards; for example, reducing the risk of *Clostridium botulinum* by using a canning process, or reducing the risk of physical hazards by using a metal detector.

Due to this reliance for assuring the safety of food products, regulations require validation of PPCs and CCPs. **Validation** is a scientific process that demonstrates specific procedures established as PPCs or CCPs are truly effective at reducing risk to an acceptable level (Figure 1). Once these procedures are established and validated, specific steps and parameters are **monitored** and **verified** every time these procedures are performed to confirm they are aligned with validated procedures. **Monitoring** is the measurement of key parameters that show critical limits were met (e.g., time/temperature), and **verification** is the process of making sure procedures were followed (e.g., time/temperature were measured, and corrective actions were taken if critical limits were not met).

Similar validation, monitoring, and verification procedures can also be established for non-PPC and PRP. Although non-PPC and PRP may be seen by some as less critical than PPCs and CCPs, these food safety management components are essential to the success of the entire food safety system and control of identified hazards. In a production environment, these hazards can be introduced into food after the "kill step;" for example, *Listeria monocytogenes* in ready-to-eat foods.

Figure 1. Flow of food safety management principles with examples of activities performed as part of monitoring and verification procedures. Key Non-Process Preventive Controls and Pre-requisite Programs (orange box) should be validated to assure routine environmental monitoring activities used for verification are verifying valid control strategies.



1 *Examples: Process controls could be critical control points for HACCP or process preventive controls for FSMA.*

2 *Examples: Non-process preventive controls could be pre-requisite programs required for HACCP or FSMA Sanitation and Allergen Preventive Control.*

3 *Visual inspections, ATP testing, and allergen testing may be used as either Monitoring or Verification, depending on the frequency of testing and inspections. For example, performed daily after each cleaning as monitoring, or periodically as verification.*

Environmental sampling can be used to validate, monitor, and verify cleaning and sanitization procedures that are part of Allergen PCs, Sanitation PCs, or Sanitation PRP (Figure 1 [ii]). For example, environmental testing procedures for total microbial load, indicator organisms (e.g., *Enterobacteriaceae*), or pathogens (e.g., *Salmonella*) are used for verification, while ATP and allergen testing are (depending on the frequency of testing) used for either monitoring (daily after each cleaning procedure) or verification (periodic occasional testing). Allergen PCs, Sanitation PCs, and Sanitation PRP, that rely on environmental testing procedures for monitoring and verification, should be validated to ensure monitoring and verification procedures indicate true (not simply perceived) control over environmental food safety and quality risks.

Neglecting to validate the effectiveness of non-PPC, as well as their continuous monitoring and verification, can lead to significant food safety issues. Historical data indicates that failures in implementing pre-requisite programs, such as inadequate sanitation, have frequently been implicated in food safety breaches (Table 1). Environmental monitoring serves a dual purpose in this context, acting as both a validation and a verification tool for specific pre-requisite programs and non-process controls. By collecting and analyzing environmental samples, organizations can assess the effectiveness of these programs and determine if modifications are necessary to enhance food safety outcomes.

Table 1. Examples of foodborne outbreaks in which the root cause was related to issues in non-process controls and pre-requisite programs.[1–4]

Food item	Microorganism	Year	Potential root cause
Peanut butter[6] 	*Salmonella*	2008–2009	• Contaminated raw materials • Improper sanitation practices and inadequate cleaning • Failure to follow safety protocols
Ice cream[7] 	*Listeria*	2015	• Improper sanitation of equipment and production areas • Difficult-to-clean equipment • Improper or inadequate testing and monitoring practices
Cheese[8] 	*Listeria*	2020	• Improper sanitation • Environmental contamination • Improper testing • Improper temperature controls
Deli meat[9] 	*Listeria*	2024	• Food accumulation in equipment and walls • Mold and mildew around handwashing sinks • Insect infestation in processing area

Benefits of validating non-PPC and PRP can include:

- Demonstrating a cleaning and sanitation standard operating procedure (SSOP), developed as part of pre-requisite programs, can effectively remove organic residues, potential pathogens, and spoilage organisms from equipment introduced during a production run and prevent from contaminating the next production run.
- Demonstrating the frequency of preventive maintenance is appropriate for control of food safety and quality risks associated with a company's products.
- Demonstrating the frequency of a master sanitation schedule is appropriate for control of food safety and quality risks associated with a company's products.
- Demonstrating a new piece of equipment brought into a food production facility has an appropriate hygienic design and can be cleaned following an established SSOP.
- Demonstrating that extending production run time does not increase the presence of organic residues, potential pathogens, or spoilage organisms above limits that indicate unexcitable risks to the safety and quality of a company's products.

While regulatory requirements for **validation** are often associated with **process controls**, they are equally applicable to **non-PPCs and PRPs**. Today, a number of globally recognized certification programs require the validation of non-PPCs and PRPs.

Examples of certification programs requiring validation of non-PPC or PRP:

Safe Quality Food (SQF) Program, SQF Code, Edition 9, Section 2.5.1

Emphasizes the need for validating all applicable elements of an SQF Program to ensure their effectiveness, including Good Manufacturing Practices (GMPs).[5]

BRCGS Food Safety Standard, Issue 9, Section 2.12.1

Requires the validation of control measures, including those managed through pre-requisite programs, to confirm their efficacy in controlling identified hazards.[6]

Food Safety System Certification (FSSC) 22000, Clauses 8.5.2 and 8.8

Outlines the requirements for validating control measures and emphasizes the importance of verification activities to ensure the effectiveness of pre-requisite programs.[7]

PrimusGFS Standard, Version 3.2, Module 3, Sections 3.01.01 and 3.01.02

Highlight the need for documented validation and verification procedures for all preventive measures, including sanitation and other non-process controls.[8]

Canadian Food Inspection Agency (CFIA) Preventive Control Plan (PCP) under the Safe Food for Canadians Regulations (SFCR)

Mandates that food businesses develop, implement, and maintain a written PCP that includes validation and verification procedures to ensure the effectiveness of control measures in preventing, eliminating, or reducing hazards to acceptable levels.[9]

2.3 Key elements needed to perform validation of non-process controls and pre-requisite programs

Key elements are necessary to successfully execute a validation process. Examples are:

Create a sample plan

Sample plans for validation should consider a larger number of sites, which have been selected using risk-based analysis (Figure 2). All selected sites should also include sites expected to be included during routine verification and monitoring.

- Include sites in more than one hygienic zone (including food contact surfaces).
- Include all critical sites (areas of equipment that are difficult-to-reach and difficult-to-clean).

Use a defined and documented standard operating procedure for cleaning and sanitation, which will be validated

As part of any Food Safety Management System (FSMS), a well-documented and executed cleaning and sanitation program should already be in place to ensure effective hazard control. Once these foundational procedures are established, the next step is to validate their effectiveness through a structured approach.

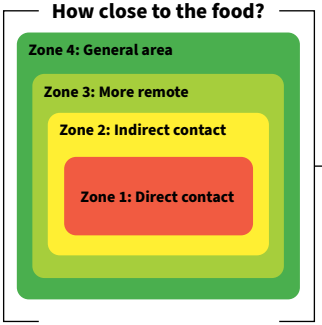
- The sanitation team should be familiar with the SOP that will be validated.
- If feasible, provide diagrams about the extent of dismantling required for equipment before it is cleaned and sanitized.
- Clear understanding of the sample plans, and descriptions and locations of the sample sites.

Assemble a validation team

Validation confirms that cleaning and sanitation processes consistently achieve the desired microbial, chemical, and physical hazard reduction. This requires a well-defined validation strategy, appropriate sampling plans, and collaboration among key functions.

Figure 2. Example of a risk-based matrix to select testing sites

Sites that are in direct contact to food (Zone 1) that are harder to clean could be considered as critical sites where the frequency of testing is higher. On the contrary, a site in Zone 2 that is easy to clean should be part of the sample plan but likely tested at a lower frequency.



Zone	How hard is it to clean?		
	Low	Medium	High
High (Typically Zone 1)	Mid-level priority	High priority	High priority
Medium (Typically Zone 2 and 3)	Low priority	Mid-level priority	High priority
Low (Typically Zone 4)	Low priority	Low priority	Mid-level priority

Establish a cross-functional team that will have the necessary expertise to design and execute a validation study. It is critical to involve members who understand equipment design, cleaning and sanitation chemicals, food composition, equipment maintenance, operations, food safety, etc.

- Assign roles and responsibilities to each member of the validation study team.
- If evaluation by observation is part of the validation procedure, team members should receive proper training before the validation study.
- Validation studies often require thorough disassembly of equipment, therefore, it is critical to have team members with the skills to perform this task.
- The team may also include third-party auditors, as well as subject-matter experts, who can provide objective feedback on the validation approach.

Coordinate with plant personnel and validation team members

Prior to and during validation of the non-PPC, it is critical to properly communicate with appropriate members of the company and plant. Justifying the relevance of validation activities may be needed to allocate necessary support and resources will be available.

- Validation studies may require extended downtime of equipment, as collection of samples may be required.
- Validation may require extensive dismantling of equipment.
- Validation may alter the plant's regular production schedule.

These activities will require full support and understanding of upper management and plant production personnel, as these activities will require financial investment and may alter the schedule of a food processing facility. **However, it is important to convey that validation of a PRP and non-PPC should be seen as a valuable investment**.

Non-PPCs or PRPs that are not properly validated can significantly impact the hygienic and safe manufacturing of food products. It is hoped these failures become evident during verification and monitoring. But by that time, valuable resources have already been spent on continuous corrective actions and will continue to be needed until new and appropriate preventive actions are established. Messages to justify investment in the validation of non-PPC and PRP could include:

- A properly validated non-PPC can **prevent repeated failures**, thereby saving time and resources.
- The need for **frequent corrective actions** may be reduced while operational efficiency may improve.
- These activities can support compliance, reduces risk of **recalls**, and strengthen brand protection.

2.4 The process of validating non-process controls and pre-requisite programs

Establish methods for sample collection, required measurements, and acceptance criteria.

Define how samples will be collected and analyzed. A standard operating procedure should already be implemented describing sample collection techniques, sample handling, and sample testing. It is important to select adequate measurement methods capable of detecting the specific hazard or an associated marker or indicator to generate scientific evidence that the non-PPC will be effective.

Defining acceptance criteria for tests that will be utilized is crucial during a validation study to determine if the SOP is yielding satisfactory results. Acceptance criteria should be based on measurable standards (when possible), with clear benchmarks or threshold that must be met. When such standards are not readily available or prescribed, historical data, subject-matter expertise, guidelines offered by method manufacturers and chemical suppliers, or third-party consultation can be valuable resources to establish these criteria.

Some of the following tests are often included in a validation study:

- **Cleaning:** Collection of samples for ATP measurements. Most ATP reader manufacturers offer a default ATP threshold level, which is often a useful value when evaluation begins. After the validation study has been completed, the threshold value could be adjusted to a level that is appropriate for the plant's equipment (See Chapter 4).
- **Allergen cleaning:** Collection of swabs and clean in place water. The use of specific allergen tests (immunoassays or PCR) is necessary. Allergen threshold results should show absence or no detection below a defined limit of detection for the method used (See Chapter 5).
- **Sanitation:** Collection of samples (swabs and sponges) to determine concentrations of microorganisms post-sanitation. Total aerobic count and *Enterobacteriaceae* are common targets but these may vary depending on an operation, product, etc. (See Chapters 6 and 7).
- **Physicochemical parameters:** Time, temperatures, concentration, action time, pressure, pH, etc. may be measured and recorded to demonstrate that the physicochemical conditions within the SOP are working correctly.

Each measurement method should be carefully considered before it is used for validation and verification of process and non-process controls. Therefore, it is critical to determine whether a method is fit-for-purpose and demonstrate proficiency with the method before its implementation.

If the evaluation method will be observational, it is first necessary to train team members and make sure they will use the same acceptance criteria. During these preparations, clearly stating and exemplifying concepts is important.

Determine how often samples should be collected during a validation study

Frequency of sample collection can vary from daily or multiple times per day to weekly, or even monthly, especially in smaller operations.

- Sampling frequency and the number of samples should be determined by considering a risk-based approach (Figure 2).
- As previously noted, sampling sites should already be selected, considering the difficulty of cleaning and closeness to the food product. Although sites have been pre-determined, they can be rotated randomly so not all sites are sampled in every instance.
- Determine sites to be sampled based on the plant, history, personnel activities, equipment design, and age.
- On a given day, sampled sites should include a larger percentage of critical sites which risk-analysis has shown to be difficult-to-reach, difficult-to-clean, and/or in close proximity to food.

Determine when samples should be collected

- Depending on the non-PPC under validation, equipment may be re-assembled and allowed to run without food for approximately one hour, then environmental samples can be collected. In other cases, samples may be collected during processing, at least 3 to 4 hours after the process begins.
- A randomly selected day should be chosen for validation, using a random number generator rather than relying on a convenient day, which might result in sampling on a less busy day. Validating under typical conditions or under a "worst case scenario" may result in a more robust validation study.
- If feasible, conduct the evaluation on different operational shifts.

Additional considerations in designing a validation study

- Observational sampling is critical to assess certain elements of a validation (i.e., execution of cleaning and sanitation procedures, disassembly and assembly of equipment). Therefore, it is critical to standardize sampling through training and if feasible by using diagrams to qualify those activities.
- Sampling should be dynamic to enable modifications based on circumstances (i.e., new equipment, construction, maintenance, and repair).

2.5 Executing the validation study

A validation study will vary depending on the non-process control or program under validation. The diagram depicted in Figure 3 shows an example of a validation study for equipment including processes for allergen cleaning, cleaning and sanitation and hygienic design. Notice that the diagram can be adapted to validate new equipment or equipment already in operation. Also, when validating a hygienic design, the validation may be incorporated within the validation study of a cleaning and sanitation SOP.

Considerations for validating sanitation processes

The process of sanitation often encompasses both cleaning and sanitation, defined here as:

1. **Cleaning**: removing organic matter and other residues from a finished product run
2. **Sanitization:** application of sanitizers to inactivate potential pathogens and spoilage organisms

Validation of standard operating procedures for cleaning and sanitation is critical to provide science-based evidence those operations can effectively remove organic matter, thereby preventing potential niches; and can effectively remove harmful microorganisms and thereby preventing cross-contamination of foods during processing.

Considerations for validating hygienic design

An essential non-PPC to reduce contamination is proper hygienic design of equipment. In manufacturing facilities, equipment of poor hygienic design can be difficult to clean and residues can be retained in crevices and dead areas. This can affect the efficacy of sanitizers, and such residues can become a niche or allergen reservoir. In FSSC 22000, requirement 2.5.15 states that companies should have an equipment management policy that considers sanitary design, compliance with quality standards (for new and secondhand acquisition), maintenance and repair, installation validation, and the Seek and Destroy process (See Chapter 8 or 11).[7]

Validating the hygienic design of equipment requires close collaboration with equipment suppliers and an internal team. This can ensure a thorough understanding of the process, the types of products being manufactured, plant layout and equipment placement, as well as cleaning and sanitation SOPs.

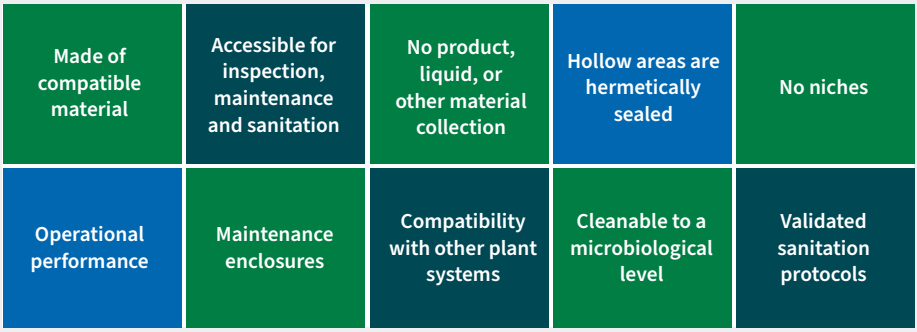
Guidance documents, such as those provided by the Meat Foundation, can serve as valuable resources for validation to ensure that equipment meets hygienic design standards. By following the principles outlined in Figure 3, similar approaches can be applied to validation processes, including those used for cleaning and sanitation, as well as the validation of new equipment (Figure 4).

Considerations for validating of a master sanitation schedule

A master sanitation schedule (MSS) refers to cleaning and sanitation programs whose activities occur periodically and involve both cleaning and sanitation of equipment and infrastructure. The objective is to reach those surfaces that are inaccessible during routine or daily cleaning activities.

Figure 3. General workflow for validation of non-process controls and pre-requisite programs aligned within an environmental monitoring program; interpretation of results and next steps

Figure 4. Principles of food safety equipment design (Meat Foundation)[10,11]



The objective of validating an MSS is to demonstrate that the cleaning and periodicity are appropriate and can effectively achieve the desired food safety outcomes.[10,11]

Defining frequency of validation studies for non-process controls and pre-requisite programs

Generally, validation will require sampling, testing, observations, and/or evaluation of processes and procedures within a defined process. Acceptable results should be within two to three production runs.

A process should be revalidated if a critical element has changed; examples include:

- Major changes in product formulation
- Changes in detergents or sanitizers
- Major maintenance and repair events
- Changes in process schedules
- Extended product process runs

If no changes have occured, periodic revalidation is appropriate. The frequency and extent of the revalidation should be determined using a risk-based approach, considering historical data that will be gathered during monitoring and verification.

Continuous revision of monitoring and verification records and re-validation studies, allow plants to continuously improve their sanitation practices.

Documenting the validation study

Documentation of the validation study serves as evidence that the validated non-PPC or PRP is effective and meet the delineated acceptance criteria. A final document should summarize how the validation study was designed, the established acceptance criteria, and results of the validation study. This documentation is valuable for audit and inspection support and it can become a tool for training and knowledge transfer. It also may serve as a troubleshooting tool when deviations are found, as it can help in tracking potential sources or sites of contamination identified during a risk analysis. If validation is repeated, documentation serves to demonstrate consistency in the process and provides a baseline at which the non-PPC or PRP are effective.

A note on other non-PPC and PRP

This manual has focused on the importance of validation within programs that can be assessed using tools from environmental sampling, particularly about validation of equipment. However, other non-PPC and PRP share the same goal: minimizing food safety risks. These programs, too, can be validated. For instance, supplier programs can be validated through a comprehensive audit to provide evidence their food safety systems will provide raw materials that meet acceptance criteria. Similarly, the implementation of handwashing and personal hygiene procedures can be validated by direct visual observation or testing for allergens or microorganisms on items like aprons after washing. In essence, validation is simply about demonstrating that a proposed process or action will achieve the desired outcome.

2.6 Conclusions

Validating non-PPC and PRP is essential to demonstrate they will effectively manage food safety hazards when implemented. This process involves generating sufficient data to demonstrate that specific controls or programs meet their intended objectives.

Ultimate outcomes of the overall validation process for non-PPC or PRP include:

- Processes that prove effective when followed according to standardized procedures.
- Control points and parameters effectively verify that procedures consistently adhere to standardized practices each time they are performed.

Once **validated**, it is crucial to consistently record evidence during each execution of the SOP to confirm these controls continue to perform as intended through **verification and monitoring** activities.

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CHAPTER 3

Environmental sampling guidance

By

Scott Egan | Neogen

3.1 Sampling neutralizers

Sampling from food processing environments can present several challenges. Obtaining meaningful results that accurately reflect the level of microbial contamination on a surface is not an easy task. One such challenge is the presence of sanitizers, which may continue to have bactericidal or bacteriostatic activity after a sampling event. This continued activity can reduce the microbial population within a sample prior to detection, affect enumeration (e.g., during transportation) or inhibit an organism's growth in the culture medium used in a testing process. These effects can ultimately result in reduced counts for quantitative methods or negative results for qualitative methods and therefore will not accurately represent the risks present in the production environment. To overcome this challenge, sample collection devices such as swabs or sponges should incorporate components that can effectively neutralize any sanitizers that are present. Selection of a neutralizer (or combination of neutralizers) should be undertaken with knowledge of the types of sanitizers used within a facility, as not all neutralizers, alone or in combination, are equally effective against different types of sanitizers.

When selecting neutralizers, two other important factors must be considered. First is their compatibility with the test method to be used. Second is whether the test method is qualitative or quantitative. For quantitative tests, the selected neutralizers should not support organism growth but should merely maintain the population at the level found during sampling or a similar level.

These considerations can often be overlooked not only during initial selection of a neutralizer, but when sanitizers, test methods or sampling regimes change.

Most commercially available swabs and sponges will incorporate a combination of neutralizers as part of their standard or proprietary formulations. The most common neutralizing or sampling liquids and their various levels of effectiveness are summarized later in this chapter in Table 5.[2,5-8] When using a proprietary neutralizer, contact the manufacturer to obtain information about the components or sanitizers for which the neutralizer has been found effective (see Table 6 for an example).

Letheen Broth is commonly used as a neutralizer for environmental sampling in the food, nutraceutical, cosmetic, and pharmaceutical industries.[1,2] It has neutralizing capability for iodine, quaternary ammonium compounds and chlorine sanitizers. However, it has no ability to neutralize mercurials, formaldehyde, or glutaraldehyde, so again the sanitizer being used must be considered.

Additionally, Letheen Broth has some enrichment capabilities, so the surface should be re-sanitized after sample collection. (Table 1.)

Table 1. Composition of Letheen Broth

Composition: (typical formula g/L)	
Enzymatic Digest of Animal Tissues	10.0 g
Beef Extract	5.0 g
Polysorbate 80	5.0 g
Sodium Chloride	5.0 g
Lecithin	0.7 g

Dey/Engley (D/E) Neutralizing Buffer was developed by Dey and Engley to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals. However, it was designed for testing the efficacy of disinfectants rather than for environmental sampling. Although it counteracts the biocidal activity of all principal sanitizers, it also contains an indicator dye and has enrichment properties. Therefore, a surface must be resanitized after sample collection. (Table 2.)

Table 2. Composition of D/E Neutralizing Buffer

Composition: (typical formula g/L)	
Enzymatic Digest of Casein	5.0 g
Yeast Extract	2.5 g
Polysorbate 80	5.0 g
Dextrose	10.0 g
Lecithin	7.0 g
Sodium Thioglycollate	1.0 g
Sodium Thiosulfate	6.0 g
Sodium Bisulfite	2.5 g
Brom cresol Purple	0.02 g

Neutralizing Buffer, often thought to be a generic term, is a specified formulation commonly used in industry for *Listeria*, Total Plate Count, *Salmonella*, *E. coli,* and other types of testing.[2,3] It does not effectively neutralize phenolic, mercurial, formaldehyde, or glutaraldehyde sanitizers (although these are uncommon in the food industry due to their toxicity). This formulation contains aryl sulfonate complex and may require dilution of a sample prior to testing with a molecular-based method. (Table 3.)

Table 3. Composition of Neutralizing Buffer

Composition: (typical formula g/L)	
Aryl Sulfonate Complex	5.0 g
Sodium Thiosulfate	0.16 g
Potassium Phosphate, monobasic	0.0425 g

Buffered Peptone Water (BPW) is often used in abattoirs to collect samples from carcasses as directed by regulations. It is not recommended for use on sanitized surfaces as it has minimal neutralizing capability. Buffered Peptone Water is an enrichment broth, so if it is used for environmental sampling, the surface should be re-sanitized after sample collection.[4] (Table 4.)

Table 4. Composition of Buffered Peptone Water

Composition: (typical formula g/L)	
Peptone	5.0 g
Sodium Phosphate, dibasic	0.16 g
Sodium Chloride	0.0425 g
Potassium Phosphate, monobasic	1.5 g

The effectiveness of frequently used neutralizing media against common sanitizers can vary and specific neutralizing media may or may not neutralize certain sanitizers. (Table 5.)

EN 1650 Annex B[9] can be consulted to see examples of neutralizers for residual disinfectants. The effectiveness of any disinfectant neutralizer should be validated under real-use conditions. Any remaining enrichment broth or residue of neutralizing solution should be removed from a sampled surface after sample collection, according to user-established procedures.

Table 5. Effectiveness of common neutralizing media against common sanitizers[2,5-8]

Sanitizer	Letheen Broth	D/E Neutralizing Buffer	Neutralizing Buffer	Buffered Peptone Water
Quaternary ammonium compounds	Yes	Yes	Yes	No
Phenols	Yes	Yes	No	No
Iodine and chlorine	Yes[5,6]	Yes	Yes	No
Mercurials*	No	Yes	No	No
Formaldehyde*	No	Yes	No	No
Glutaraldehyde*	No	Yes	No	No
Peroxyacetic acid and hydrogen peroxide	Some[5,6]	Yes[7,8]	No	No
Acids	Yes[5,6]	Yes[7,8]	No	No

*Not commonly used in the food industry due to their toxicity

Table 6. Example of a proprietary neutralizing formulation against common sanitizers

	Quaternary ammonium compounds	Phenols	Iodine and chlorine	Peroxyacetic acid and hydrogen peroxide	Acids
Neogen Wide Spectrum Neutralizer	Yes	Yes	Yes	Yes	Yes

3.2 Selection of sampling devices

When selecting a sampling device, unless governed by specific regulations, the primary decision should be the type of device (sponge or swab) to be used. Key considerations are the size of the area being sampled, whether the area is readily accessible, and the type of testing to be conducted on the sample. An effective environmental monitoring program will use a combination of sponges and swabs.

Consideration should be given to the quality, strength, and materials used in sampling devices, as fragments of the device may separate, leading to foreign object contamination of a facility and resulting implications. Additional features, such as blue-colored design for enhanced visibility in the processing environment or metal detectability, may also be beneficial.

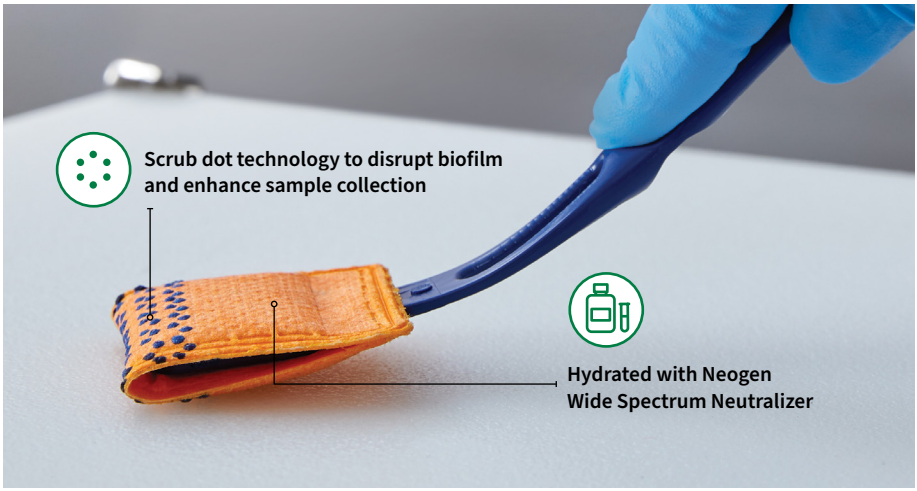
Sponges

Sponges are larger sampling devices, available in a variety of formats, from an individual sterilized sponge to a sponge attached to a handle to aid in aseptic handling.

Sponges are preferred if qualitative pathogen testing is to be conducted, as they can be used to sample a larger area, thereby increasing the likelihood of organism detection. The area sampled should be greater than 100 square centimeters (15.5 square inches) and preferably greater than or equal to 1,000 square centimeters (155 square inches).[4,10]

However, in many cases, particularly when swabbing for detection of pathogens or index organisms (e.g., *Listeria* spp.), sampling an

Figure 1. The Neogen Environmental Scrub Sampler uses a novel design to help improve the mechanical disruption of microorganisms from surfaces



area of a specific size is not appropriate or feasible as locations likely to harbor pathogens are not typically areas that can be easily accessed (e.g., long cracks in floors). In these cases, it is important to sample as large an area as possible (e.g., several meters or yards of a floor crack).

The materials used in the manufacture of sponges are most commonly cellulose or polyurethane.[10,11] Studies have evaluated the efficacies of these materials in collecting and facilitating improved detection rates. However, these studies generally have shown no significant differences.[12,13]

Sampling devices (Figure 1) may also have novel designs, construction, or materials to improve mechanical disruption of biofilms and dislodgement of entrapped micro-organisms.

Such devices may give a more accurate assessment of the microbial risk in the processing environment, as several spoilage organism and pathogens are known to be associated with biofilms.[14,15]

Sponges should be free of inhibitory substances. Typical household sponges are not recommended for environmental sampling, as they may contain biocides that could inhibit microbial growth.

Swabs

Swabs are smaller sampling devices consisting of a tip or bud for collecting the sample, attached to a long, flexible stem. Because of their smaller size, they are generally better-suited for sampling in difficult-to-reach places and are typically used for areas of 100 square centimeters or less.[4,10]

Due to their smaller size and ease-of-use in sampling defined areas, swabs can be particularly effective in quantitative environmental testing (e.g., for indicator organisms). This is important because the defined area will be used in the calculation of results.

Swab material is typically a synthetic such as alginate, Dacron or rayon. However, cotton is also used.[1,4] Evidence should be obtained, either through supplier documentation or product validation/verification, that the chosen device does not have any bacteriostatic or bactericidal activity.

3.3 Sampling methods

Sampling methods will vary, depending on the type of device being used and the intended subsequent testing.

Moisture is one of the most important factors for bacterial survival on surfaces. Therefore, regardless of the device or intended testing, sampling from a moistened surface or using a moistened collection device is recommended to improve recovery.[12]

A noteworthy exception can be sampling of dry environments where the introduction of moisture may be undesirable, as it enhances risk of microbial growth. In these cases, specialized tools (e.g., spatulas, spoons, scoops) may be used to collect dry materials and dust from the environment.

It is also important to sample only a single item or area with each sampling device. This can help prevent cross-contamination among items or areas in the facility.

Pathogen sampling should include as much surface area as possible to improve the likelihood of detection. Although regulations may specify the size of a sampling area, these typically can be considered minimum sizes. As discussed in Chapter 8, training materials often cite specific sizes of sampling areas (for example, 12 by 12 inches, or 30 by 30 centimeters), but many surface areas are not square or flat enough to accommodate these measurements. In most cases, the area to be sampled prescribed in a standard operating procedure (SOP) may be determined by assessing of the site to help ensure capture of an organism (if present) while meeting the sampling objective.

If sampling sites are not easily accessible, using a swab may be more suitable. Again, the intention should be to obtain as much surface contact as possible to maximize the likelihood of detection.

Quantitative sampling may require more care to be taken. For example, if a test result is to be expressed in CFU/cm^2, a sample area of a specific size is typically defined and tested. Sampling templates can assist in sampling a defined area, but caution should be taken as their use can lead to cross-contamination.

It would not be uncommon, even for quantitative sampling, to target an area of undefined size. For example, testing for Total Plate Count may be used to assess the efficacy of sanitation on difficult-to-reach areas, in which case it may be impossible to sample a defined area. In testing these unmeasured surface areas, results may be reported based on the entire sampling site instead of the measured surface area.

When environmental samples are taken, it is critical that proper aseptic technique be used to prevent inadvertent contamination of samples. It is recommended that before opening a sampling device, a technician should wash or sanitize their hands. Each type of sampling device also has particular techniques that should be followed, along with any additional guidance from manufacturers, for instance when using proprietary swabs.

Swabs (Figure 2) should be aseptically removed from their container. Caution should be taken not to touch the bud or any area of the stem that will be returned to the container. During removal, the swab tip should be pressed against the container to remove excess liquid.

Where possible, particularly with easily accessible areas sampled for quantitative analysis, multiple directions should be used when sampling and a swab should be rotated between the thumb and forefinger during sampling. After swabbing the first direction, the swab should be returned to the container and rinsed in the neutralizing solution to remove collected organisms and re-moisten the tip. The same procedure should then be repeated in two other directions. Then the swab should be sealed in its container for transportation.

Sponges (Figure 3) should be aseptically removed from their containers using sterile gloves or forceps, or by manipulating the container to access the handle of the device. Care should be taken not to contaminate the sponge or any other part of the device that will be inserted back into the container.

Figure 2. Example of sampling technique using Neogen Swab-Sampler

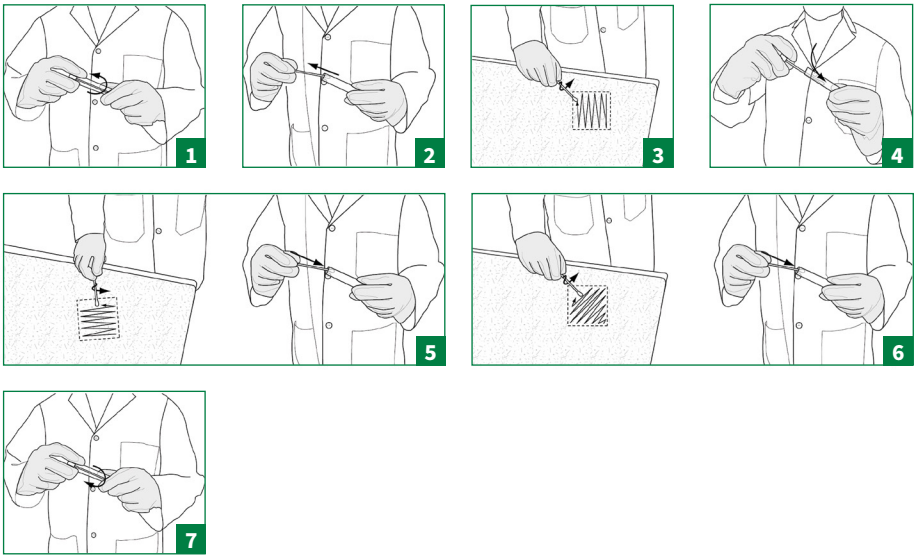
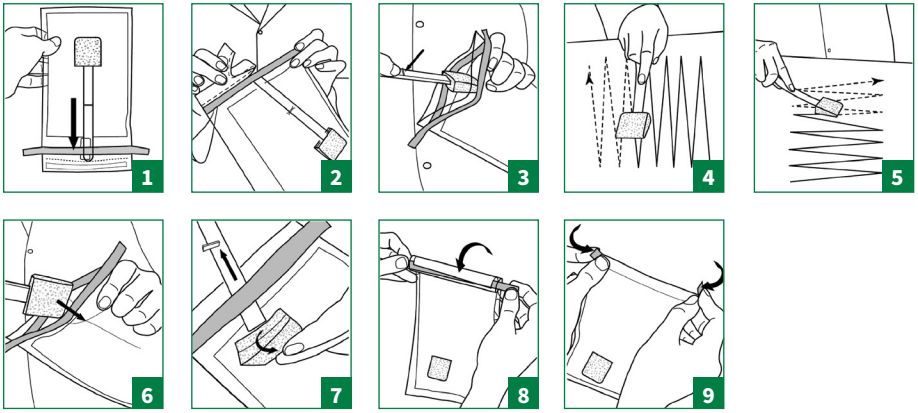


Figure 3. Example of sampling technique using Neogen Sponge-Stick



A sponge should be wiped over the sampling surface using firm and even pressure. This can help dislodge organisms that may be protected by a biofilm. After sampling in one direction, the sponge should be turned over and used to swab in a perpendicular direction. The sponge should then be placed in its container, aseptically taking care not to insert any portion that is not part of the sample (e.g., the handles of some devices). The container should then be sealed for transport.

After any sampling has taken place, surfaces should be cleaned of any neutralizing solution and re-sanitized.

Sample transport is the final step in the environmental sampling process and particular attention should be given to some aspects. Samples should be kept at refrigeration temperature and delivered for analysis as soon as possible, preferably within 24 hours, as described in ISO 18593:2018.

Containers used for transportation should be cleaned and sanitized. They should also include ice packs and be able to maintain refrigeration temperature for the duration of transportation.

Upon receipt at the laboratory, an internal temperature of the cooler should be verified using a thermometer.[2] Samples should not be allowed to freeze under any circumstances, as exposure to sub-zero temperatures may kill or injure microbes that are present.

If it is not possible to perform sample analysis within the recommended time frame or transport samples appropriately, alternatives should be developed and validated accordingly to ensure they do not undermine the sensitivity of the method.[3]

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CHAPTER 4

ATP and protein-based hygiene monitoring

By

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Taylor Lecy | Neogen

4.1 Purpose of ATP or protein-based hygiene monitoring

Each day, the high-risk decision to start food production must be made.

Hygiene monitoring is an important component of an environmental monitoring program that can help with this decision, by verifying the hygienic status and cleanliness of equipment and surfaces prior to food processing or preparation.

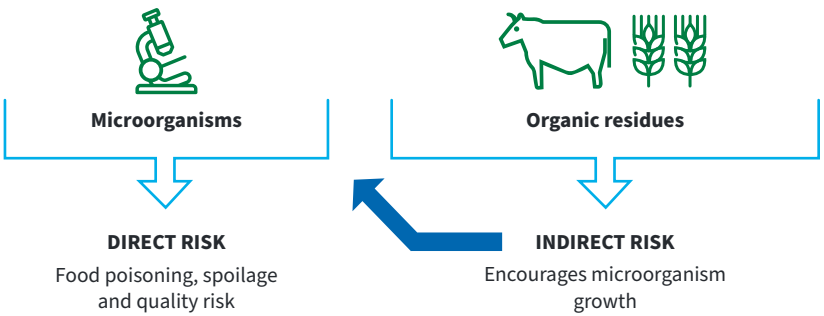
There are three established and recognized approaches that can be used for hygiene monitoring: visual inspection, microbiological testing, and Adenosine Triphosphate (ATP) testing. A robust hygiene monitoring program would utilize all three complimentary methods. Protein testing may be used as a substitute for ATP testing for cleaning verification if needed. This chapter will focus on the ATP and protein-based technologies, but with an emphasis on ATP testing due to the additional benefits it offers over protein tests such as quantitative results and data trending capabilities.

ATP and protein-based technologies are rapid, simple-to-use methods for hygiene monitoring that provide a measurable and objective assessment of the cleanliness of equipment and surfaces prior to food processing or preparation.

Inadequate cleaning can result in organic matter being left on a surface that can nourish microorganisms. Removing this organic matter reduces the opportunity for bacteria and mold to multiply or grow, thus reducing the microbial risk within the processing environment (Figure 1). Removal of organic matter can also enhance the efficacy of sanitizers, further improving the overall sanitary status of a facility and reducing risk.

Figure 1. How ATP indicates direct and indirect risks

- Where the cleaning regime is inadequate or fails, residue from organic sources may remain on the surface.
- Where this occurs, both direct and indirect food contamination risks exist.



4.2 Principle of the methods

4.2.1 Principle of ATP testing

ATP (adenosine triphosphate) is present in every living cell. It is the energy molecule for the cell and is broken down to ADP (adenosine diphosphate), releasing energy for the cell to utilize.

As well as being present in living cells such as bacteria, yeast and molds, ATP is also found in residues from organic sources such as:

- Food debris remaining on a surface after cleaning.
- Biofilms produced by bacteria.
- Surfaces touched by operators.

The amount of ATP in a cell will vary based on several factors, including whether the cell is bacterial (prokaryotic) or somatic (eukaryotic). It is easier to detect ATP from eukaryotic food cells than prokaryotic microbial cells, as the amount of ATP in a food cell can be 10^7 times greater than a microbial cell (Figure 2). The growth phase of the microorganism can also impact the amount of ATP in a cell.

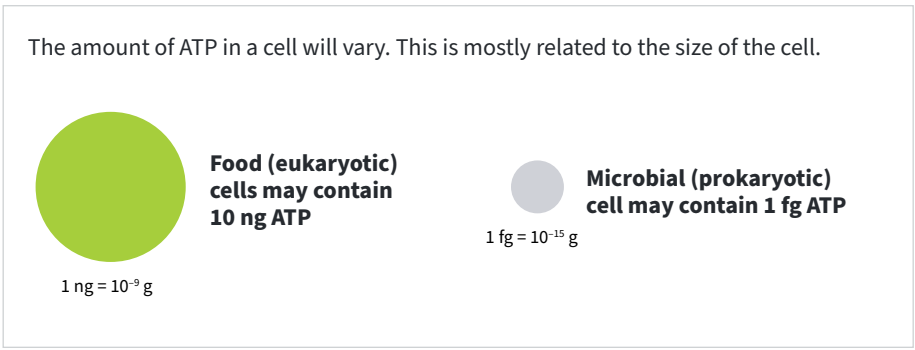
ATP hygiene monitoring utilizes the energy in the ATP molecule along with an enzyme complex known as Luciferin-Luciferase to produce light, the same chemical reaction used by fireflies.[1]

In the bioluminescence reaction, luciferase utilizes ATP to catalyze the oxidation of luciferin to oxyluciferin, yielding light (Figure 3). The light produced is proportional to the amount of ATP present. By measuring the light produced, a correlation can be formed with the amount of ATP present and therefore the amount of ATP-containing organic matter present.

A luminometer is a device for measuring the amount of light produced by this chemical reaction and reports the information in relative light units (RLU). The more ATP present, the higher the RLU value, therefore the more organic matter on the sampled surface (Figure 4).

To determine the hygienic status of a surface using ATP, pass/fail values can be

Figure 2. ATP content in different cell types



established based on the RLU value. These results do not indicate the source of the ATP contamination but do measure the overall cleanliness of the surface being tested. ATP tests are commonly used for surface testing, but water ATP tests are also available for facilities using, for example, a clean-in-place (CIP) system.

Different manufacturers of ATP swabs and luminometers use varying sensitivities and technologies, so there is no universal RLU reading across systems. It is important to thoroughly evaluate the accuracy, precision, and robustness of each system to ensure optimal performance, especially in the challenging conditions of a manufacturing environment.

Figure 3. Measuring ATP with bioluminescence

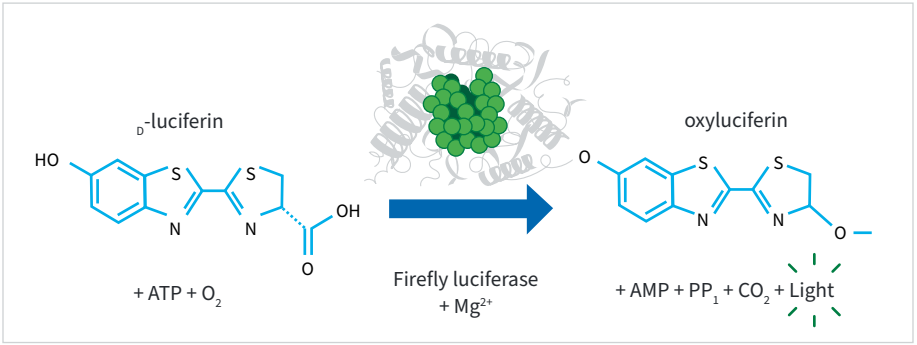
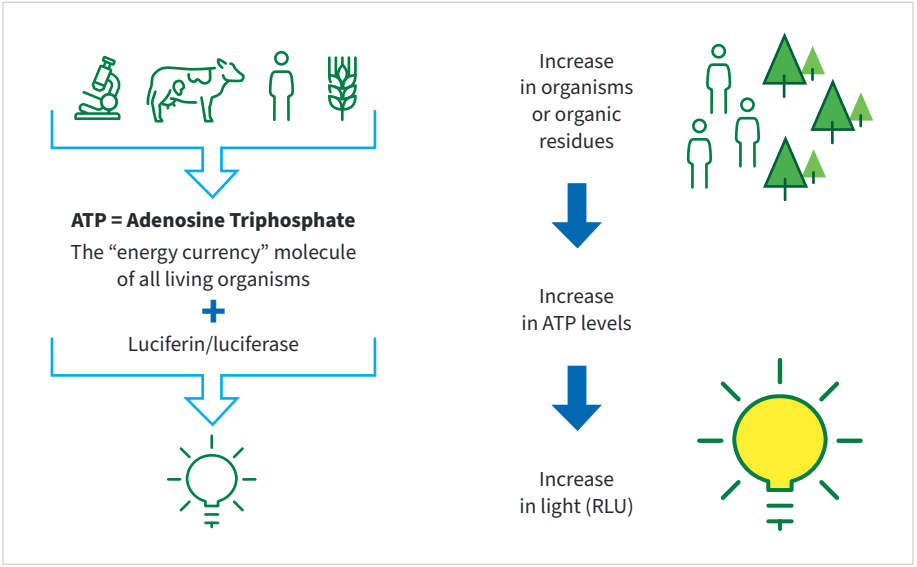


Figure 4. Principle behind ATP bioluminescence



4.2.2 Principle of protein testing

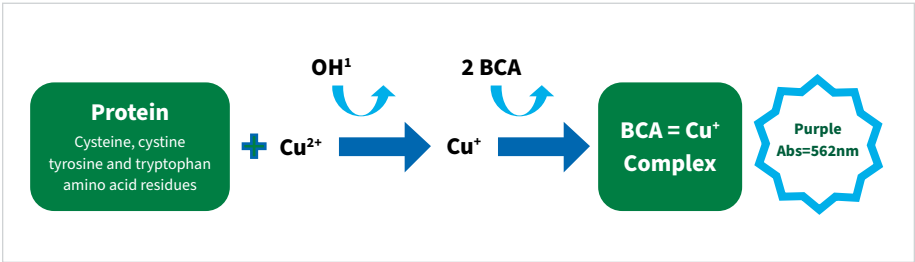
Protein-based tests can be used as an alternative method to ATP tests. Protein testing is a qualitative or semi-quantitative color-based test to detect the presence of protein residue and therefore determine cleanliness.

The depth of color produced indicates the level of protein present. However, as with ATP testing, the technology cannot indicate whether the source of the protein is microbial, food residue or comes from another source.

Protein-based tests generally utilize the copper-based Biuret reaction (Figure 5). In this reaction, cupric ions (Cu2+) form a complex with the peptide bonds of protein, reducing the Cupric ions to cuprous ions (Cu+). Bicinchoninic acid (BCA) can then form a complex with the Cu+ ions, resulting in a color change.[2] For example, a green color as a test result may indicate the surface is clean, whereas a purple color may indicate the surface is unclean.

Protein-based tests may be less preferred compared to ATP tests due to the longer time to result and limited data trending capabilities. Results from protein-based tests are generally available within several minutes (versus seconds for ATP-based tests) and may be less sensitive than results from ATP tests. Generally, qualitative or semi-quantitative results limit their usefulness in data analysis and trending. However, protein tests offer benefits and often can be performed without specialized equipment. They are also temperature-stable, making them useful for facilities that may have limited refrigerated storage space or other limitations, such as food service outlets.

Figure 5. The Biuret reaction utilized in protein tests



4.3 When to conduct ATP or protein tests

The sanitation process includes two principal steps: cleaning and sanitation (or application of sanitizer). Both work together to provide a safe and hygienic environment, thus reducing the risk of contamination. The cleaning step is used to remove debris such as dirt or food product residue from surfaces. Cleaning can include actions such as spraying water, applying detergent and using a mechanical force like scrubbing or spraying to loosen and remove debris. Of the two steps, the cleaning step should involve most of the work.

During sanitation, chemicals are used to inactivate or kill remaining microorganisms on surfaces. First, however, it is essential to have effective cleaning so sanitation can be successful. Insufficient cleaning of a surface may leave debris, which can act as a barrier or inactivate sanitizing agents and thereby reduce the efficacy of sanitization.

The purpose of ATP and protein tests is to determine the effectiveness of the cleaning process in removing debris. Good practice for routine cleaning verification is to conduct ATP and protein tests between the cleaning and sanitation steps. This assesses the effectiveness of cleaning before the sanitation process begins, ensuring any residual debris is identified and addressed. In the case of processing equipment which utilizes CIP, a sample of the final rinse water can be analyzed for ATP utilizing a collection device specific for this purpose.

Furthermore, application of a sanitizer does not remove remaining debris on a surface. So, if a failing ATP or protein result occurs after the sanitation process, additional cleaning and sanitation steps typically need to be taken to address the inadequate hygienic conditions. If a facility can identify a failing result before application of a sanitizer, time and money can be saved.

ATP and protein methods can experience sanitizer interference if testing occurs after sanitizer application. Therefore, to ensure the most accurate results, it is important to talk with the ATP/protein swab or device manufacturer to verify the impact of specific sanitizers and their concentrations on the system being used.

If it is not possible to test before sanitization, some ATP and protein-based systems have flexibility to accommodate differences in cleaning and sanitation processes. However, if testing after the sanitation step occurs, it is still important to verify the impact of the sanitizers on the results of the method.

Furthermore, when conducting ATP and protein tests, it is critical to be consistent in sample collection timing, technique and test point locations to help ensure accurate and comparable results over time.

4.4 ATP vs. microbiological results

While ATP and protein testing are well established methods for measuring hygiene, it is important to note the technologies cannot be used as replacements for traditional microbiological testing.

ATP and protein tests are not as sensitive for the detection of microorganisms as microbial tests. The amount of ATP or protein in a single microbial cell is far below the detection levels of ATP or protein tests.

In addition, not all ATP and proteins come from microbial cells. So, higher ATP results do not necessarily indicate a high concentration of living microorganisms. Instead, the source could be food product residue on a surface that if not cleaned properly may serve as a niche for microbial proliferation.

Furthermore, depending on the growth phase of the microorganism, various amounts of ATP may be present. For example, a microbial cell in a dormant phase will have less ATP than a cell in an active growth phase.

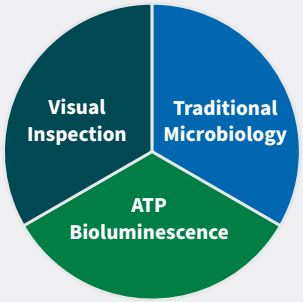
Therefore, ATP and protein testing cannot be used to quantify microbes or directly correlate with microbiology results. While there may be some alignment in results such as when performing a cleaning validation, it may not be consistent due to the factors outlined above.

An advantage of ATP and protein tests is that results are quickly actionable compared to microbial tests, which can take a day or more to complete. ATP and protein methods are relatively simple, easy to use and do not require laboratory equipment nor laboratory staff to complete. These benefits can help alleviate challenges such as employee training or turnover.

The role of ATP and protein-based tests is to assess effectiveness of cleaning procedures, which then relate to risk levels of contamination. Microbial tests are intended to verify sanitation processes in Sanitation Standard Operating Procedures (SSOPs) and overall hygienic conditions of the site, and will be discussed in later chapters.

ATP or protein tests and microbial tests have different roles but work together to verify a surface is properly cleaned and sanitized to reduce the risk of microbial contamination. A comparison of these different environmental monitoring test methods can be found in Figure 6. An effective environmental monitoring program will incorporate a combination of these technologies in a methodically planned and well-justified manner.

Figure 6. Comparison of environmental monitoring test methods



All methods have their place within an environmental monitoring program

	Visual	Microbiological	ATP
Real time			
Sensitivity			
Quantitative			
Detection of organic matter			
Simple			
Monitors cleaning			
Verifies sanitation			

4.5 Development of an ATP or protein program

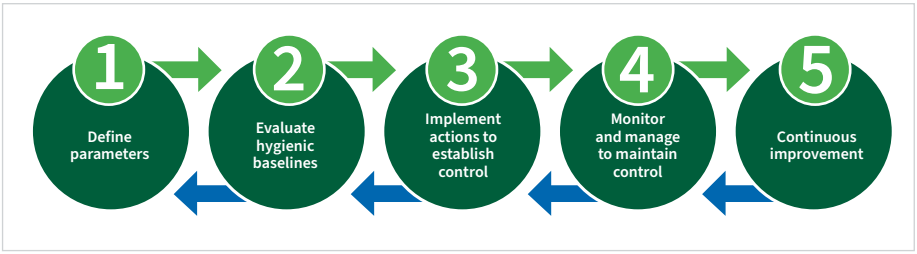
Development of an environmental hygiene monitoring program can be divided into five phases (Figure 7). The first three phases focus on defining parameters and monitoring for control. The last two phases focus on managing the program to maintain control and drive continuous improvement. Facilities may find they move back and forth between the phases, depending on the maturity of their ATP and protein testing programs and changes in their operations.

When Phase 1 is implemented, parameters for the program will be defined. These include activities such as identifying test points and test methods using a risk-based approach, defining initial pass/fail values and determining an action plan and corrective actions when failures occur.

Activities during Phase 2 will assess and evaluate initial hygienic pass/fail values by testing at high frequency across all the selected test points. From the data collected, modifications can be made to the initial pass/fail values. Additionally, it may be possible to identify test sites that are consistently failing and thus may require targeted cleaning and sanitation (i.e. additional dismantling, changes to cleaning SSOP, etc.). Microbiological tests should be included as part of the sanitation validation and to demonstrate alignment with ATP or protein results, and overall cleaning and sanitation requirements.

Phase 3 involves implementing actions to establish control. Test points that were identified as consistently failing in Phase 2 will be addressed with high frequency testing and evaluation of the results. Additionally, consistently passing test points can be reviewed and analyzed to determine if control parameters such as pass/fail values are still appropriate or if they need to be tightened because they are potentially too lenient. Adjustments to the pass/fail values can be made based on the data analyzed.

Figure 7. Framework to implement ATP or protein testing program



In Phase 4, steps will be taken to monitor and manage the program to maintain control. Data will continue to be collected and analyzed to identify trends and problematic testing sites. Appropriate action will address failing test results to correct and improve the hygienic conditions of surfaces. Randomization may also be implemented to reduce testing frequency for the sites that are in control or help manage the testing program more efficiently based on the risk assessment.

In Phase 5, the program and data will be routinely analyzed to identify opportunities for improvement. These could include activities such as adjustment of parameters or inclusion of additional hygiene management elements in the program. Phases 2 and 3 should be repeated when new elements are added or changes made, such as the introduction of new cleaning chemicals or processes, new equipment or the manufacture of new food products.

The effectiveness of each phase is based on collecting and analyzing data to determine the parameters to be controlled and taking corrective actions to maintain control.

4.5.1 Selection of sampling sites

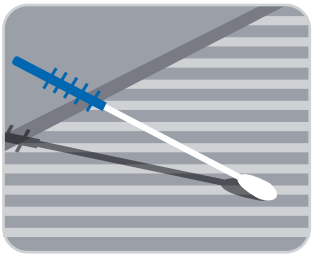
Selection of sampling sites should begin with a mapping exercise that can provide an overview of the facility and food production process. This will involve dividing (or mapping) the facility into several areas (zones) based on the extent of microbial risk to the product (Figure 8).[3, 4, 5, 6]

Once the environment has been mapped, the most appropriate test points can be identified for assessing cleanliness and controlling risks posed by unclean surfaces.

Points selected for ATP testing may differ from microbiological sampling sites.

This mapping process is best conducted by a team, with input from the cleaning and quality departments. This can combine their knowledge of ATP testing and a risk-based approach to sampling.

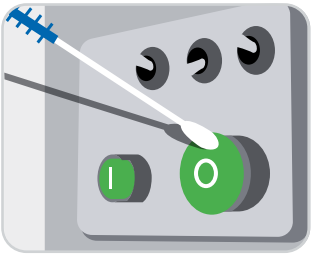
Figure 8. Environmental monitoring sampling zones



ZONE 1

Product Contact Surfaces

Slicers, peelers, fillers, hoppers, screens, conveyor belts, air blowers, employee hands, knives, racks, worktables



ZONE 2

Non-Food Contact Surfaces in Close Proximity to Food and Food Contact Surfaces

Processing equipment exterior and framework, refrigeration units, equipment control panels, switches

ZONE 3

More Remote Non-Food Contact Surfaces Located In or Near the Processing Areas

Forklifts, hand trucks, carts, wheels, air return covers, hoses, walls, floors, drains

Not typically tested for ATP

ZONE 4

Non-Food Contact Surfaces Outside of the Processing Areas

Locker rooms, cafeterias, entry/access ways, loading bays, finished product storage areas, maintenance areas

In selecting test points, the following parameters should be considered:

1 Stage of food processing

Any manufacturing step that reduces microbial contamination will impact the degree of microbial risk in prior or subsequent manufacturing steps or environments. Microbial reduction steps can take many forms, from pasteurization to peeling of fruit.

Processing steps that occur after microbial reduction steps can be considered higher priority for testing due to the potential for post-processing contamination. Any processing step (or environment) prior to the microbial reduction step can be considered lower priority for testing since it precedes the hazard control point.

Lower risk ratings assigned to areas prior to microbial reduction steps must be viewed in context of the validated microbial reduction steps. Insufficient cleaning of these areas may lead to cumulative microbial contamination, rendering later processing steps insufficient.

2 Proximity to food and the potential for cross-contamination

Generally, a high-risk surface has direct contact with a product line that will not be processed further to eliminate microbial risk. In contrast, a low-risk surface will not have contact with a product and/or is a location where the product will be processed further to eliminate microbial risk.

In addition to direct contact surfaces, the potential for cross contamination should also be considered including:

- Proximity of the surface to the product, for example, whether the equipment is above the product or whether there is risk of contamination such as water droplets in a humid environment.
- Location of control panels, utensils or tools and whether there is a risk of cross contamination by operators.

3 Ease of cleaning and condition of the surface to be tested

While sanitary design and good maintenance should be fundamental in any facility, circumstances may arise where these activities are less than optimal.

To address the risk and degree of difficulty in cleaning, a surface must be assessed to determine if its material or condition can reduce the effectiveness of cleaning. The level of risk associated with the surface may increase where cleaning is difficult. Examples include older equipment, porous surfaces, scratched or marked surfaces, and poor accessibility for cleaning and sampling.

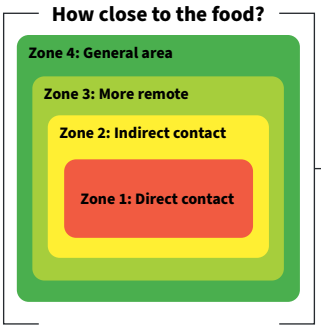
A simple and convenient way to conduct the risk analysis and understand the potential risk to be mitigated through the use of hygiene monitoring can be summarized as follows (Figure 9):

Risk analysis:

- How significant is the hazard? = How close is the surface to the food?
- What is the probability the hazard will occur? = How hard is it to clean the surface?

Based on these principles, the use of hygiene monitoring technologies such as ATP and protein-based swabs is typically

Figure 9. Identification of high-risk sampling sites



Zone	How hard is it to clean?		
	Low	Medium	High
High (Typically Zone 1)	Mid-level priority	High priority	High priority
Medium (Typically Zone 2 and 3)	Low priority	Mid-level priority	High priority
Low (Typically Zone 4)	Low priority	Low priority	Mid-level priority

directed towards Zone 1 (product or packaging contact) and Zone 2 (indirect contact to product or packaging) test points. In a facility that is "under control," Zone 1 areas will be free of pathogens and have low levels of indicator organisms (both discussed in other chapters). With the reduced likelihood of direct risks at these points, the primary focus should be to control indirect risks such as unclean surfaces that can lead to the development of direct risks or impact product quality. Zone 2 locations can pose a risk for cross-contamination so should be monitored appropriately based on the risk analysis.

The approach can be used for any facility, although in facilities that utilize a CIP cleaning system, the ability to access higher-risk surfaces may be limited.

Additional test points may also be needed as a result of corrective and preventive action (CAPA) activities or during validation activities that follow a process change such as construction or modification of existing equipment.

4.5.2 Sampling frequency and number of test points sampled

Once sampling sites have been identified, a combination of the testing aims (cleaning validation or ongoing verification) and outcomes of the previously conducted risk rating exercise should be used to determine the number of test points to sample and the sampling frequency.

The primary factors determining the number of test points to sample are the physical size of the manufacturing operation and the complexity or number of steps involved in the manufacturing process. For example, where several manufacturing steps or pieces of machinery are utilized and considered a risk, each should be sampled. In cases of complex or large machinery, use of multiple test points should be considered.

Highly manual production processes may warrant more Zone 2 test points to be included in the sampling plan, as hands-on operations pose an increased risk of cross contamination by production staff.

Zone 1 areas should have the highest sampling frequency and testing should be conducted daily or before each production shift, ideally during every cleaning and sanitation process and possibly also as part of the production start-up routine. This ensures that corrective actions can be undertaken before the finished product is compromised. Where there are a large number of test points, it may be more economical to randomize or rotate a portion of testing based on a risk assessment, but careful consideration should be given to ensure overall hygiene is still being achieved.

For Zone 2 or lower risk areas, the sampling regimen may occur at a lower frequency but should still be sufficient to ensure cleaning and hygiene levels are maintained before initial problems can lead to more extensive issues. Sampling frequencies for Zone 2 could include rotating sampling throughout a given time frame until all areas are tested, a periodic (weekly, for example) check of all test points or a daily randomized selection. However, for harder-to-clean areas in Zone 2, adequate monitoring should be conducted to ensure and verify hygienic conditions are being maintained.

For any test points where an ATP or protein-based test is not performed, for example due to rotated sampling, a visual inspection should still be conducted and findings or corrective actions undertaken recorded. A visual inspection can also be used prior to ATP or protein testing.

4.5.3 Determination of pass/fail values for ATP

For hygiene monitoring, pass/fail values must be established to determine the amount of ATP (RLU values) that defines an acceptable hygienic limit, and thus determine if a corrective action is necessary when ATP results are outside these criteria. While some test methods may have well-established or regulated limits at which corrective action must be taken, acceptable hygienic limits are dependent of a variety of intrinsic factors present at a particular processing facility (i.e. equipment age and substrate, product manufactured, chemical and cleaning methods used, etc.). Thus, acceptable hygienic limits should reflect the needs of the individual facility or processing environment.

The use of meaningful pass/fail values is critical to ensure that unclean surfaces are not approved for production, posing a risk for contamination. In addition, well-defined pass/fail values help avoid unnecessary delays in manufacturing by preventing failing results on surfaces that are already clean.

Many ATP systems allow setting a caution range that falls between a pass value and a fail value. Caution ranges provide an opportunity to identify negative trends and take corrective action to prevent failures before they occur.

For ATP testing, multiple steps are used to determine and adjust pass/fail values, with latter steps increasing the level of complexity and accuracy of those limits. The basic process is shown in Figure 10 with a more detailed description in Figure 11. Good practice for setting pass/fail values is to continuously refine them through each step in the process, as more data becomes available for analysis. This can help ensure the most accurate and meaningful pass/fail values will be utilized to evaluate hygienic conditions.

It has become increasingly important for facilities to communicate and justify the process used for determining pass/fail values in audits. A framework to establish robust pass/fail values can be defined in the steps that follow.

Figure 10. An overview of the process for determining ATP pass/fail values

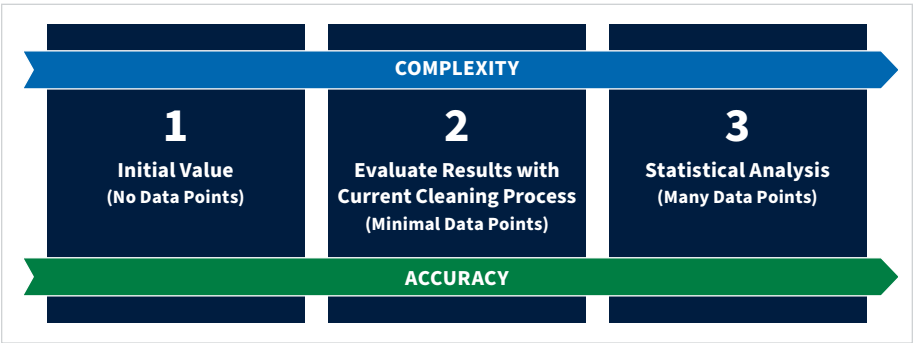
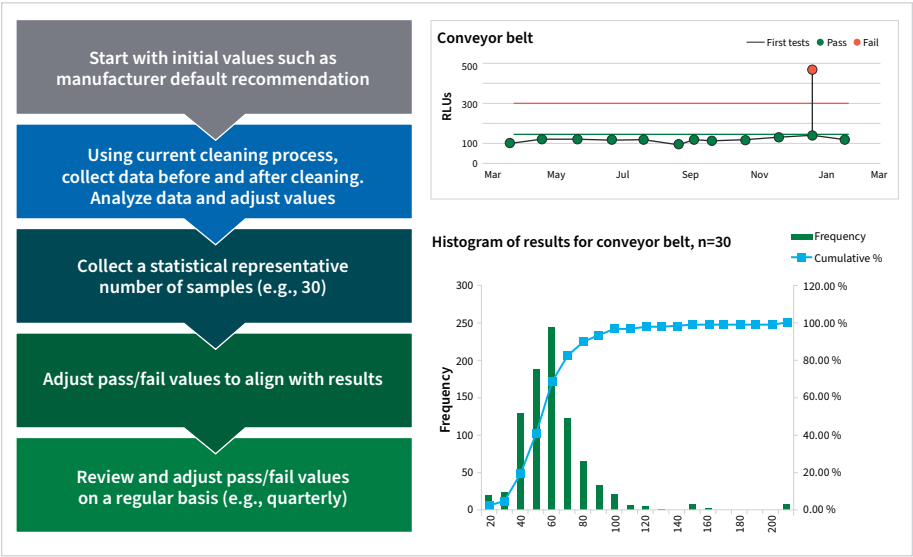


Figure 11. Details of the process for determining ATP pass/fail values



1 Starting point (initial values)

The first step to determine pass/fail values is to start with an initial value; this might include seeking guidance from the manufacturer of the ATP system being used, industry experts, and/or publications. This will help to develop a starting point for pass/fail values to build upon. The guidance should reflect the types of food products manufactured and/or the types of equipment or surfaces being sampled.

Regardless of the source, the pass/fail values should be reviewed as soon as data is available to assess if this initial value is suitable to evaluate cleanliness on a surface. After selecting initial values, it is important to move to step 2 which will help refine the pass/fail values. During step 2, it is important to involve testing of both clean and unclean surfaces to verify that the ATP values are reflecting the status of the equipment.

Be aware that manufacturers of ATP testing systems use different RLU measurement scales, so pass/fail values cannot be used or compared interchangeably between one ATP testing system and another.

2 Evaluate results with current cleaning process

The next step is to conduct ATP testing to assess and evaluate the current cleaning processes. This will involve measuring representative test points over several days before and after cleaning. It is important to conduct sufficient tests that reflect normal variations in the cleaning process such as different cleaning teams, production running times, cleaning chemicals,or various foods being manufactured. This step may also involve taking several measurements following a deep clean to show the results that may be achieved.

After data has been collected from step 2, pass/fail values can be adjusted if required.

For example, if initial pass/fail values in step 1 were set at 300 RLU, and after step 2 the average RLU values after cleaning are 150 RLU, it might indicate that the pass/fail value is too broad and reducing it would be appropriate. If the aim is to achieve an immediate improvement in hygiene rather than maintain current levels, clean levels may be based on results from deep cleaning rather than routine cleaning. For example, if the data in step 2 is showing average values around 75 RLU after a deep cleaning and considering that these were obtained on a variety of conditions, then that pass/fail value for those surfaces might need to be reduced further.

Furthermore, facilities might require different ATP pass/fail values for various test points, based on the achievable cleanliness for those surfaces. Differences in pass/fail values across test points may be due to various surface materials used in the facility or equipment, differences in the age and condition of a surface, changes in the food products that are manufactured on each piece of equipment or line, etc. To help monitor and record the pass/fail values, some ATP systems can specify different limits for the various test points. This allows for better and easier management of the results and necessary actions.

Continuous evaluation of average ATP results from step 2 can help refine pass/fail values to be more robust to the facility and processing environment.

3 Statistical analysis

In this step, pass/fail values are further defined after collecting enough data for statistical significance. Using statistical analysis will result in the most meaningful pass/fail values. Performing a statistical analysis will involve the collection of a larger number of results (data points) from cleaned surfaces, with a minimum of 30 points required. Ideally, 30 data points will be collected from each test point and analyzed individually, although it is possible to group similar test points (in terms of surface type, product, and risk, etc.) to obtain the 30 data points for analysis.

The statistics used can vary, although two common approaches use either standard normal distribution or an accepted percentage of pass/fails. Both are described below. For more detailed guidance and tools to help determine pass/fail values, the ATP system manufacturer should be contacted.

For both types of analysis described here, an initial review should be conducted to confirm the data set is acceptable. This can be achieved by performing a simple plot of the relative light unit (RLU) values over time followed by a review to exclude any obvious outliers (high or low RLU values) that may skew the results. This review should be performed using a scale that considers the results that would be expected from an unclean surface. If the results are erratic, it indicates the cleaning process is highly variable and should be investigated and stabilized.

Once an acceptable data set has been obtained, the pass/fail values can be statistically determined. To use a method based on standard normal distribution, the mean and standard deviation must be calculated. The fail value can then be determined by adding two or three standard deviations to the mean, corresponding to ~95 percent or ~99 percent of results respectively.

An alternate method utilizes an accepted level of cleaning efficacy that the company would like to achieve as a target (e.g., 95 percent) or can be viewed as the percentage improvement in cleaning they would like to achieve (e.g., 5 percent). To use this method, a histogram of the results is generated and the level at which the required number of pass/fails is reached (e.g., 95 percent) is determined to be the pass/fail value.

Once pass and fail values have been adjusted from step 2, they should be continuously reviewed to ensure they are reflective of actual cleaning performance.

Where ATP testing is used effectively and a CAPA process implemented, there will typically be an improvement in hygiene levels and a subsequent lowering of average ATP results within a short space of time.

To take into account the improved hygienic conditions that may occur over time, the pass and fail levels should be reviewed as soon as sufficient additional data is available. Subsequently, ongoing periodic reviews should be completed as part of a continuous improvement approach.

4.6 Corrective actions based on ATP or protein sampling results

A key benefit of ATP and protein-based hygiene monitoring systems is the speed at which results become available, thereby allowing corrective actions to be taken immediately.

A failing result should be addressed with corrective actions, which should be documented as part of a quality control program. These actions can also help prevent a recurrence. In the case of hygiene monitoring, a failed test outcome will typically result in recleaning and retesting until a pass is achieved.

In some cases, a caution range may be utilized in the ATP testing system and immediate corrective action may not be warranted. Instead, more thorough cleaning and/or increased scrutiny before the next production run may be sufficient to maintain control.

Repeated failures or caution results should be investigated as a priority by those on-site who have knowledge of the process and appropriate preventive actions should be taken. Data trending and analysis can help identify these locations, which are discussed in more detail in the following section.

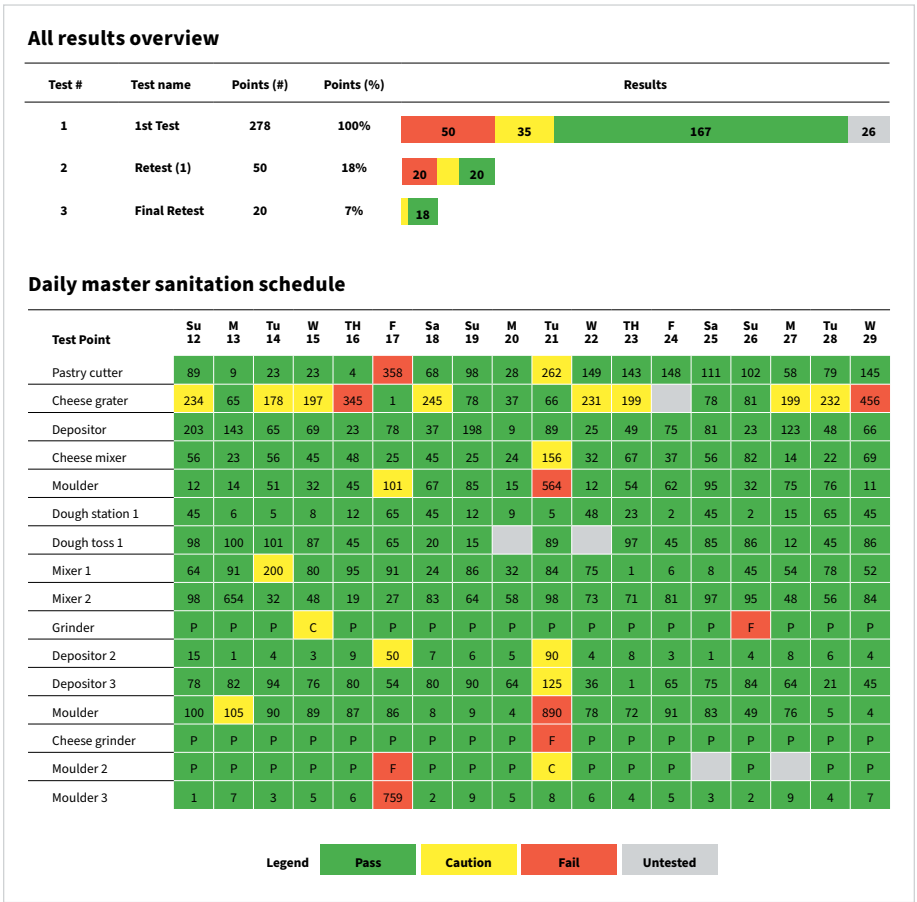
Another key benefit of ATP hygiene monitoring is the ability to trend and analyze data generated over time because results are quantitative with pass/fail values to monitor performance. This can provide better understanding — and ultimately control — of the facility's hygiene and production processes. Additionally, facilities can be more proactive in monitoring and making improvements to sites before failures start to occur.

4.7 Data trending and analysis

ATP system manufacturers will typically provide software for managing data, although the data analysis capabilities and ability to present meaningful data varies with each system (Figure 12). Data analysis is an important tool in a hygiene monitoring program because it allows immediate results to be transformed into actionable information and drives continuous improvement.

When trending and analyzing data, aspects that should be routinely monitored include cleaning consistency, pass/fail values, trends or patterns and areas of concern.

Figure 12. Data trending software for cleaning consistency



All results overview

Test #	Test name	Points (#)	Points (%)	Results
1	1st Test	278	100%	50 / 35 / 167 / 26
2	Retest (1)	50	18%	20 / 20
3	Final Retest	20	7%	18

Daily master sanitation schedule

Test Point	Su 12	M 13	Tu 14	W 15	TH 16	F 17	Sa 18	Su 19	M 20	Tu 21	W 22	TH 23	F 24	Sa 25	Su 26	M 27	Tu 28	W 29
Pastry cutter	89	9	23	23	4	358	68	98	28	262	149	143	148	111	102	58	79	145
Cheese grater	234	65	178	197	345	1	245	78	37	66	231	199		78	81	199	232	456
Depositor	203	143	65	69	23	78	37	198	9	89	25	49	75	81	23	123	48	66
Cheese mixer	56	23	56	45	48	25	45	25	24	156	32	67	37	56	82	14	22	69
Moulder	12	14	51	32	45	101	67	85	15	564	12	54	62	95	32	75	76	11
Dough station 1	45	6	5	8	12	65	45	12	9	5	48	23	2	45	2	15	65	45
Dough toss 1	98	100	101	87	45	65	20	15		89		97	45	85	86	12	45	86
Mixer 1	64	91	200	80	95	91	24	86	32	84	75	1	6	8	45	54	78	52
Mixer 2	98	654	32	48	19	27	83	64	58	98	73	71	81	97	95	48	56	84
Grinder	P	P	P	C	P	P	P	P	P	P	P	P	P	P	F	P	P	P
Depositor 2	15	1	4	3	9	50	7	6	5	90	4	8	3	1	4	8	6	4
Depositor 3	78	82	94	76	80	54	80	90	64	125	36	1	65	75	84	64	21	45
Moulder	100	105	90	89	87	86	8	9	4	890	78	72	91	83	49	76	5	4
Cheese grinder	P	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P	P
Moulder 2	P	P	P	P	P	F	P	P	P	C	P	P	P		P		P	P
Moulder 3	1	7	3	5	6	759	2	9	5	8	6	4	5	3	2	9	4	7

Legend: Pass / Caution / Fail / Untested

Cleaning consistency can be assessed using a simple line graph where the results are plotted over time with the corresponding pass/fail values (Figure 13). If the graph shows a high degree of variability, it indicates that the cleaning process is not under control.

To understand the root cause of poor cleaning control, common areas to investigate include staff discussion and training, cleaning methods/tools, variations in the products produced and sampling technique.

Trends or patterns can also be identified using longer term data sets plotted in line graphs (Figure 14). Such trends can reflect improving or declining hygiene levels at plant-level or test point-level and can be used to demonstrate improved hygiene practices or identify areas needing investigation.

Figure 13. High degree of variance/inconsistency in results over time; cleaning not in control

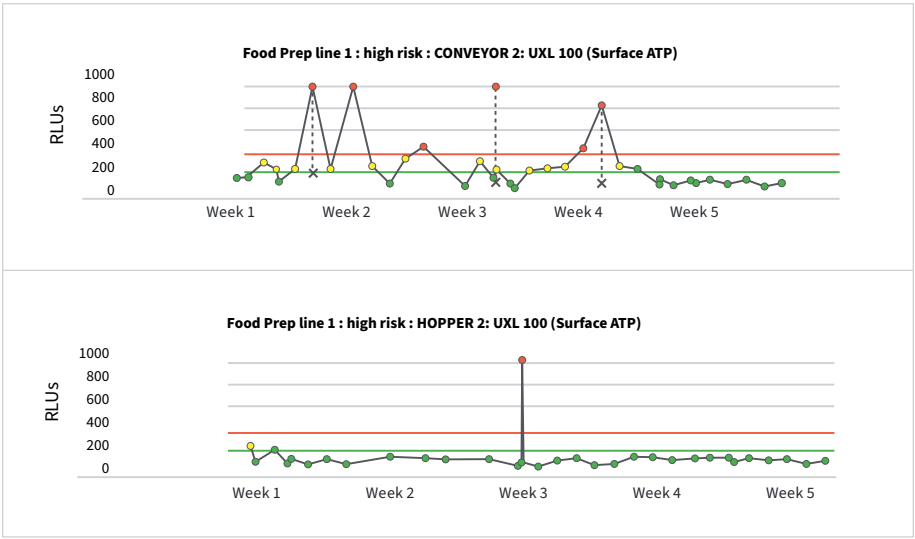
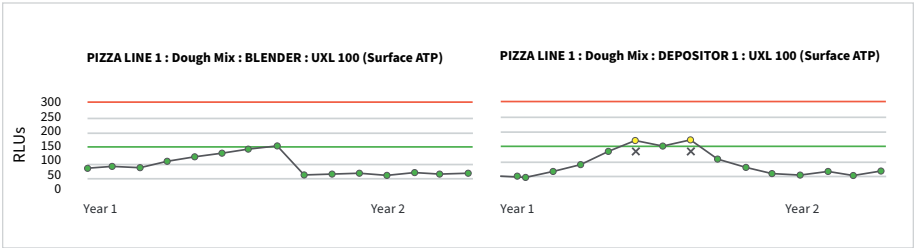


Figure 14. Cleaning trend identification from long-term ATP data



Adverse trends should be investigated to understand their root causes. For instance:

- Long-term trends may be associated with seasonal variation or equipment surfaces becoming worn. In these cases, hygiene could be improved through increased cleaning at appropriate times of the year or worn equipment could be replaced.
- Regularly occurring patterns may be linked to scheduled production of different products or changes in cleaning crews.
- Step-changes may indicate a change in cleaning practices, chemicals or equipment.

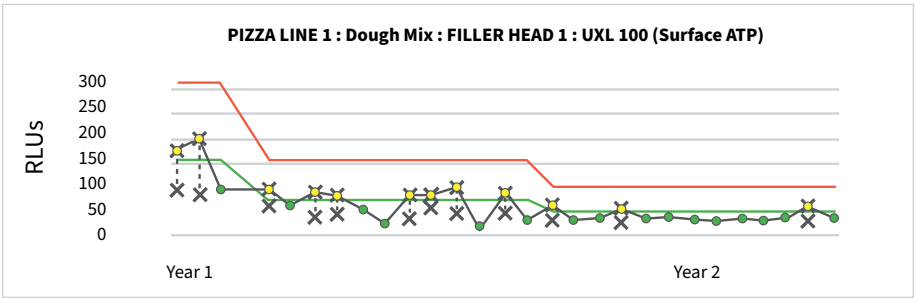
Areas of concern can be identified by analyzing test points for frequency of failing results, indicating those test points are consistently difficult to clean and may require more dedicated resources or process changes to improve conditions. This analysis can be more complex and generally requires a software system capable of performing the analysis automatically.

Pass/fail values should also be reviewed on an ongoing basis (Figure 15). Assessing whether values are appropriate can be managed in several ways but can include evaluating each test point for its history of failing. If over an extended period of time or many measurements, the test point has never failed, it is likely that either the pass/fail levels are too lenient or the level of risk associated with that test point should be reviewed. Following the review, appropriate pass/fail values may be set as discussed previously. A system of continuous improvement through regular lowering of pass/fail values is also a common practice.

Overall, the use of ATP tests should be seen not only as a convenient tool for quickly determining the hygiene levels of a surface before starting production but also as an investment in data related to the production process.

Once generated, the data should be analyzed and used as a tool to manage the plant in a more effective manner and demonstrate that hygiene targets are being achieved. Using the data can lead to an informed, focused approach to managing the hygiene of any given area. The data can also be used for training, optimizing cleaning regimens and production run times, or modifying use of cleaning chemicals and sanitizers.

Figure 15. Adoption of more stringent pass/fail values for continuous improvement of hygiene control



4.8 Additional considerations

A trial of any rapid hygiene monitoring system is strongly recommended and should mimic a part of the full sampling schedule. As previously mentioned, it is critical to note that while all ATP systems provide results in relative light units ("RLU"), those readings from different manufacturers are not interchangeable. For instance, a reading of 10 RLU from one manufacturer may be equivalent to 50 RLU for a different manufacturer, so pass/fail values must be independently determined for every system.

ATP testing can be complemented by visual inspection beforehand. Visual inspection can quickly give a big-picture view about the effectiveness of cleaning processes but it has limitations because some organic debris or microorganisms cannot be seen by the naked eye.

Microbiological testing can enumerate organisms that may cause contamination; however, it cannot provide immediate results on the manufacturing floor. A robust hygiene monitoring program would utilize all three complementary methods.

Learn more about environmental monitoring

info.neogen.com/Environmental-Monitoring

Connect with a Food Safety expert

info.neogen.com/Environmental-Monitoring-Support

Neogen would like to thank Gareth Lang and Burcu Yordem for their contributions to this chapter in the First Edition.

CASE STUDY

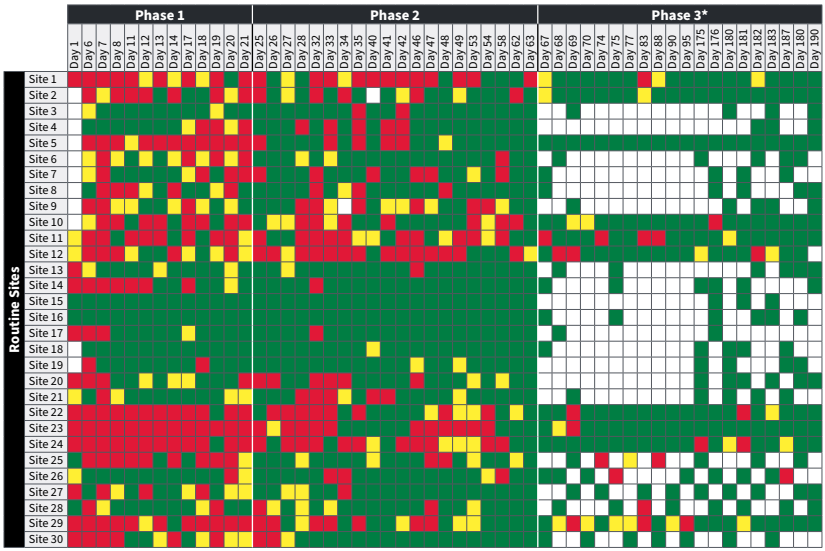
This case study provides an example of improved environmental cleanliness and microbiological product quality when implementing a robust hygiene monitoring and management program that utilizes both ATP and microbiological testing.

The Cornell University Department of Food Science and Neogen Food Safety conducted a three-phase study in a soy-based, ready-to-eat (RTE) food manufacturing facility. In each phase of this study, cleaning and sanitation operations were monitored and assessed for efficacy by conducting ATP and microbial indicator tests on identified testing sites with a focus on harder-to-clean locations. Additionally, product samples were tested and analyzed in Phase 1 and 3 to determine product quality, utilizing results from total aerobic microorganisms, yeasts and mold, and lactic acid bacteria methods.

1	**During Phase 1**, 30 test points were sampled every day for 3 weeks for ATP results to verify cleaning procedures and establish baseline values without targeted cleaning. Microbial testing of the environment was performed on surfaces adjacent to ATP test points for yeast and mold, lactic acid bacteria and aerobic microorganisms.
2	**In Phase 2**, ATP and microbial testing continued to be sampled every day for the 30 test points across 6 weeks to verify cleaning and sanitation efficacy. Furthermore, results from Phase 1 were used to identify test points that needed targeted and enhanced cleaning due to consistent failures.
3	**In Phase 3**, the ATP and microbial tests were maintained every day for 16 weeks, but with reduced testing of 18 test points per day for both ATP and microbial tests. The Hygiene Monitoring and Management software was used to implement randomization of test points for sampling optimization. Additionally, enhanced cleaning continued to be utilized for the test points identified in Phase 2.

After completion of the study, the results for both ATP and microbial tests were analyzed for quality of environmental cleanliness and microbial product quality. For the ATP testing results, the highest rate of daily failure occurred during Phase 1, with a steady decrease during Phase 2, and then stabilized in Phase 3 (Figure 16). From Phase 1 to Phase 3, ATP swab failures decreased by 26.5% for Zone 1 sites and by 51.0% for Zone 2 sites, indicating that targeted cleaning was improving the cleanliness of the facility.[7] Microbial test results showed a significant decrease in failures from Phase 1 to Phase 3 for aerobic count and lactic acid bacteria methods ($p < 0.001$), demonstrating that targeted cleaning was also improving the facility's hygienic conditions. However, yeast and mold failures did not significantly change with targeted cleaning efforts in Zone 1 and Zone 2 ($p < 0.05$), which

Figure 16. Reduction in ATP test failures over 3-phase study[8]



ATP test showed a failed result
ATP test showed a caution result
ATP test showed a pass result

	Phase 1	Phase 2	Phase 3
Randomized sampling	No	No	Yes
Number of weeks per phase	3	6	16
Number of sites per day	30	30	18

*Testing was conducted over a 16-week period, in order to fit results for this document, ATP test results between days 95 and 175 are not shown, but had similar trend to the results shown for this phase. Some days may not be represented because there were no operations in the manufacturing line.

may be attributed to additional sources of these organisms in the facility that were not impacted by improvements in cleaning and sanitation.[7] For microbial product quality results, pre-pasteurized food products showed a significant decrease in the microbial load from Phase 1 to Phase 3, demonstrating improvement in microbiological quality.

In summary, this study demonstrated the significant impact that combined ATP testing and microbiological indicator testing, along with Hygiene Monitoring and Management software, can have on assessing and improving the hygienic conditions of a food manufacturing environment and microbial product quality. As the study progressed and the facility became cleaner, fewer failures occurred, which enabled the team to confidently decrease the number of test sites while keeping the cleaning and sanitation process under control.[7] This enabled establishing a framework for implementing a hygiene monitoring program so the processor can identify problems and implement appropriate actions to reestablish control of their cleaning and sanitation program.

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CHAPTER 5

Environmental monitoring for allergens

By

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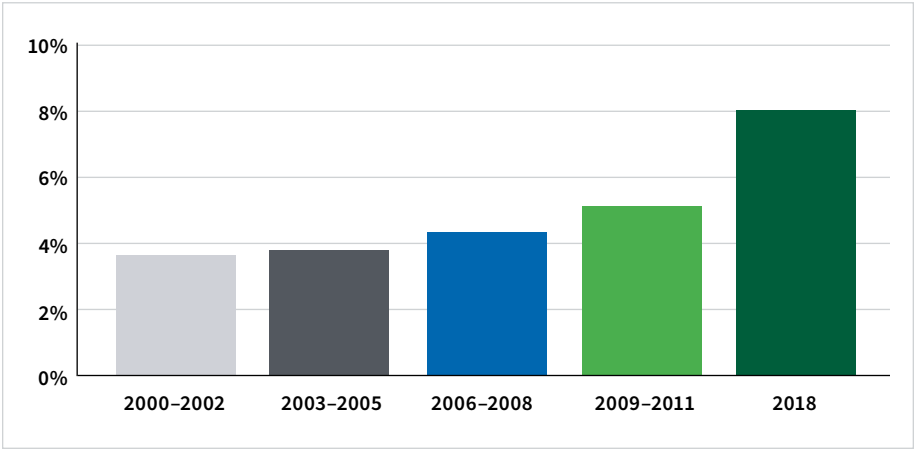
5.1 Purpose of environmental monitoring for allergens

Food allergens have increasingly become major concerns for food and beverage manufacturers. By 2018, it was estimated the incidence of food allergies among infants and young children in the United States had risen to eight percent. (Figure 1).[1] However, some studies put the estimates as high as 10 percent.[1,2]

Further, the number of people diagnosed with food allergies has increased significantly over the past several years along with the number of related hospital visits. This has a direct impact on public health expenditures and lost productivity.[3,4] At the same time, the presence of allergens that are not declared on food and beverage labels have consistently been among the leading causes of food recalls in the U.S., which significantly impacts food manufacturers.[5]

While having dedicated facilities for allergen-containing and allergen-free manufacturing would be ideal, the reality is that food not intended to contain particular allergens may be manufactured in the same facility and, often, on the same equipment as allergen-containing foods. Consequently, a robust environmental monitoring program should include considerations for allergen detection on manufacturing equipment after cleaning and before production of the next commodity. Also, the presence of allergens should be assessed in the environment to prevent cross-contact of food with allergens.[6]

Figure 1. Percentage of U.S. children with food allergies over time[1]



5.2 Allergens and their significance in the food processing environment

Foods that can cause allergic reactions are varied and wide-ranging with the most common grouped into several categories. However, these categories are not consistent across regulatory agencies, which adds complexity to the classification process (Table 1).[7–10]

Also, the specificity of the category definitions can determine the number of foods included.

Table 1. Regulated allergen foods in the United States, Canada, Australia/New Zealand and European Union (EU)*[7–10]

United States	Canada	Australia/New Zealand	EU
Milk	Milk	Milk	Milk
Eggs	Eggs	Eggs	Eggs
Peanuts	Peanuts	Peanuts	Peanuts
Soybeans	Soybeans	Soybeans	Soybeans
Wheat	Wheat or triticale	Cereals containing gluten (Including wheat, barley, rye, etc.)	Cereals containing gluten (Including wheat, barley, rye, etc.)
Tree nuts (almonds, Brazil nuts, cashews, filbert/hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, walnuts [black, California, heartnut, Japanese, English, Persian])	Cereals containing gluten (Including wheat, barley, rye, etc.)	Tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pistachios, pine nuts, walnuts)	Tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts or Queensland nuts, pecan nuts, pistachio nuts, walnuts)
Fish	Tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, walnuts)	Fish	Fish
Crustacean shellfish	Fish	Crustaceans	Crustaceans
Sesame	Crustaceans	Sesame	Molluscs
	Molluscs	Lupin	Mustard
	Mustard		Sesame
	Sesame		Lupin
			Celery

*Sulphites are not classified as food allergens but are regulated in a similar way as adverse reactions can occur in some individuals. Therefore, they may be listed with true food allergens in specific regulatory documents.

For information on additional country regulations and up to date information, please visit the FARRP International Regulatory Chart at **farrp.unl.edu/IRChart/**

The Food Safety Modernization Act (FSMA) requires manufacturers in the U.S. or companies that export to the U.S. to include allergen controls in their food safety plans.[11] Similarly, schemes commonly employed for compliance with the Global Food Safety Initiative (GFSI) also require allergen controls to be identified and monitored. While not explicitly required in Hazard Analysis and Critical Control Points (HACCP) plans, there is an implicit expectation that allergens should be identified as hazards, and that critical controls should be in place to prevent inadvertent contamination of products with allergens.

For facilities and production lines that manufacture products with different allergen profiles, it is essential to take appropriate actions to ensure there is no cross-contact between foods. In some cases, scheduling of manufacturing operations can limit that risk. Also, when changeovers are required between products with different allergen profiles, collection of environmental samples is important to determine the effectiveness of cleaning procedures.

In the context of food allergen controls, environmental monitoring can be thought of as including both the initial validation of cleaning procedures and the ongoing verification of allergen cleaning controls.

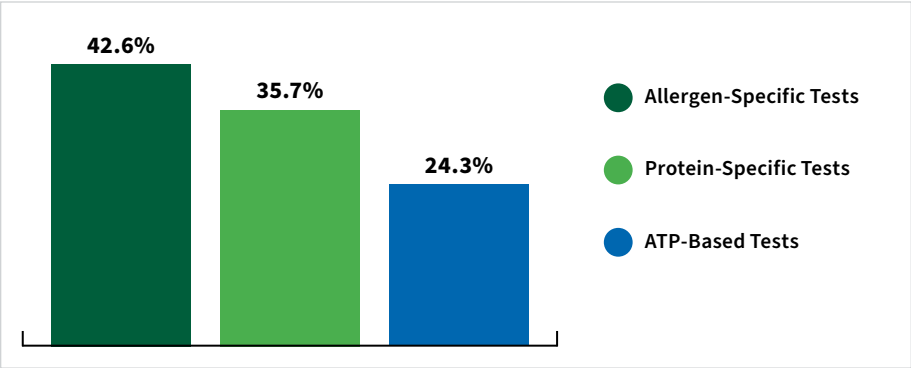
5.3 Selecting a fit-for-purpose analytical method

5.3.1 Specific vs. non-specific allergen testing

Food and beverage manufacturers use a variety of approaches and tests in food safety allergen programs (Figure 2) with 30 percent reporting use of multiple allergen tests.[6]

Two general approaches for allergen testing that have traditionally been employed for cleaning verification: specific and non-specific allergen tests.

Figure 2. Food manufacturers' allergen testing by method[6]



Specific allergen tests use a target recognition approach to detect proteins within allergenic food and identify and/or quantify the amount of allergenic food that may be present in a sample. For example, a facility that makes both peanut butter ice cream and vanilla ice cream needs to ensure the peanut butter ice cream is completely removed from the manufacturing equipment before producing vanilla ice cream. They could use an antibody-based test such as a lateral flow device (LFD) or an enzyme-linked immunosorbent assay (ELISA) to detect and/or quantify peanut proteins, using antibodies raised against the allergenic source (i.e., peanut).

The use of an allergen test based on the application of specific antibodies has an advantage in its high specificity. For example, if an antibody-based test produces a positive result for peanut there is a high-degree of certainty that the surface or rinse water sample is contaminated with peanut. Due to this selectivity, specific allergen tests, such as LFD or ELISA methods, are required by many third-party food safety certification schemes.[12]

If a cleaning process is designed to remove residual milk from processing equipment prior to manufacturing soy milk, then a milk-specific ELISA or LFD is needed to validate the process is capable of removing milk. This is typically done by testing before and after cleaning to specifically show that milk residues have been effectively removed. LFDs and ELISAs can help define a HACCP system by surveying the processing equipment and finding the "hot spots." This approach can identify areas that will need future monitoring (e.g., valves and equipment interfaces) or need optimized clean-in-place cycles.

After validation, routine testing following cleaning can allow users to verify validated cleaning is being conducted effectively. For example, following routine cleaning during a line changeover, obtaining results that indicate allergenic residues are at low or undetectable levels would serve as a useful verification.

While most companies are aware of specific allergens they need to monitor, the specificity of ELISAs and LFDs also present a drawback when managing foods that contain multiple allergens. For example, a production line for salad dressing containing egg, milk, gluten, and soy scheduled to next produce a vinaigrette lacking all these allergens would require verification the allergens have been removed by using tests specific for egg, milk, gluten, and soy. Alternatively, one may choose a single target allergen representative of all four allergens that can indicate there is no residue present from the previous salad dressing. In this instance, one might choose the highest concentration in the matrix, e.g., milk, or the allergen that is most difficult to remove, e.g., egg.

In these situations, a non-specific allergen test may be useful for a first-pass assessment of cleaning or as an alternative to ELISAs and LFDs. Non-specific allergen tests include ATP and protein surface swabs. While ATP testing does not directly measure allergens, if a surface is cleaned sufficiently to remove or reduce ATP to a low level then it can reasonably be expected that cleaning has been adequate to remove allergens.

However, it is recognized that the solubility of ATP, a small negatively charged molecule, can be very different from that of allergenic proteins in food that may be baked onto a surface. Additionally, some allergenic food sources like egg white have low ATP levels, making ATP a poor surrogate to confirm removal of these allergenic proteins. For these reasons, highly sensitive protein swabs offer a direct assessment of success in removing allergenic proteins from surfaces during cleaning.

This rationale suggests that if proteins have been removed to an undetectable level (e.g., fewer than 3 micrograms per 100 square centimeters), then allergenic proteins have also been removed to a very low level. In situations with multiple allergens, such as the salad dressing example, determining in a single test that fewer than 3 micrograms of total protein are present directly demonstrates that fewer than 3 micrograms of protein are present from any and all allergenic food sources.

In some cases, non-specific tests can be more practical for allergen changeover verification activities after a cleaning validation has been performed with allergen-specific tests. In this approach, conducting side-by-side tests during validation with allergen-specific and non-specific tests can be useful to ensure the non-specific tests are sufficiently sensitive.

Ultimately, choosing whether to use an allergen-specific test or non-specific test depends on many factors. Among these are the difference in the number and types of allergens in the products produced within the same area or production line, the time required for testing, the necessity for quantitative results, the relative technical aptitude of the technician, and the requirements from customers for whom the products are being produced.

Developing a cost-effective cleaning verification regimen can be challenging for food manufacturers. It is critical that all standard operating procedures designed to achieve a clean state through effective allergen removal are validated, monitored, and verified at appropriate frequency to ensure the established procedures are working as intended. It is also critical to select appropriate methods for validation and routine verification to obtain adequate information about the effectiveness of a cleaning standard operating procedure.

5.3.2 Method target and allergen source considerations

Selecting the proper allergen detection test may require detailed knowledge about the targets of the test. For example, many commercial milk assays target the casein protein fraction, which comprises approximately 80 percent of the protein in cow's milk. Therefore, this can be a good indicator for manufacturers using products containing whole-milk or cheese powders.

However, if milk-containing products only contain whey powder, the casein test will not detect residues from those products, as the content of casein in whey is very low. For companies with whey- or whey protein isolate-containing products, tests that target beta-lactoglobulin (the major protein in whey) would be required to measure the carryover of whey protein in non-milk labeled products. Similar concerns exist for food containing egg yolk or white, as most tests for egg proteins focus on the ovalbumin from egg white but would be ineffective in detecting the presence of egg yolk.

One of the interesting "quirks" of allergen categories in the U.S. and other regions is the grouping of certain allergen sources into large categories, such as the seafood or fish or shellfish categories. Some antibody sources and, in turn, ELISAs and LFDs may be specific for certain species within a category, while others may be more broadly applicable to a wide range of species. It is important to perform a verification for any selected test to ensure the test is fit for purpose and can reliably detect the allergen source present in the specific food matrix. For environmental samples, this verification can include taking a swab from dirty equipment following production of the allergen-containing food product to ensure a positive result is obtained with a given test kit.

Additionally, processing can affect the recognition of target proteins by antibodies in the test. For example, heat treatment of a food (e.g., boiling, baking, roasting, etc.) or even the temperatures used during cleaning (e.g., steam-cleaning) may alter the sensitivity of a test to allergen residues in the environment. Therefore, it is important to ensure that the test selected for environmental monitoring can detect both non-processed (i.e., raw soy flour) as well as processed (i.e., ultra-high pasteurized soy milk) food allergens.

Users should take extra caution with foods that go through fermentation (e.g., soy sauce, wheat beer) or enzymatic/chemical digestion (e.g., hydrolyzed proteins used in some infant formula). Food processes during which proteins may be severely fragmented into small peptides may make allergenic foods undetectable by traditional ELISA or lateral flow tests. However, these protein fragments may still be capable of causing reactions in allergic individuals. For this reason, it is important that the selected method used for cleaning verification is fit for purpose and therefore capable of detecting the allergens of concern in the users' process.

The detection of gluten and wheat also poses a number of challenges. Gluten is the protein that triggers celiac disease (an immune response different from other allergies) and can trigger symptoms in those people with gluten sensitivity. Gluten is also the principal protein found in a wide range of grains including wheat, barley, and rye, and their sub-cultivars.

In contrast to celiac disease, there are people who have a specific allergy to wheat proteins including gluten. To complicate matters further, some test

methods employ gluten antibodies that are highly specific for wheat gluten but have low affinity for barley gluten, while other test methods can have more than a four-fold greater reaction to barley gluten than to wheat gluten. The wheat-specific gluten antibodies may indicate there is no gluten present when, in fact, there are significant amounts contributed by barley contamination.

In contrast, barley-specific gluten antibodies may indicate there is 40 ppm of gluten present when, in reality, the concentration is only 10 ppm of barley gluten. In this case therefore, it would be important to verify that the selected method can specifically detect and quantify rye, barley, and wheat.

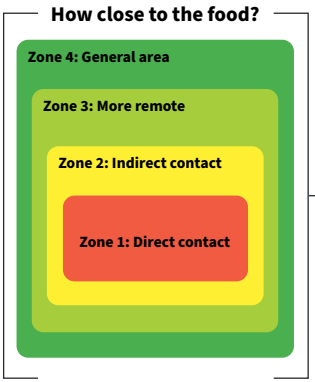
5.4 Development of an allergen sampling program

5.4.1 Selection of sampling sites

Most of the testing focus for allergens should be on immediate post-cleaning validation and verification of Zone 1 test points (direct contact surfaces) prior to release of the line for production. Depending on the facility there may be value in periodic testing of other environmental sampling zones to identify areas of dust, liquid, and other residue build-up that might lead to cross-contact.

For routine cleaning verification, a risk-based approach should be used that examines both the impact on the food should a surface be contaminated (the hazard) and the level of difficulty in getting the surface cleaned properly (the probability) (Figure 3).

Figure 3. Identification of high-risk areas for allergen testing



Zone	How hard is it to clean?	Low	Medium	High
High (Typically Zone 1)		Mid-level priority	High priority	High priority
Medium (Typically Zone 2 and 3)		Low priority	Mid-level priority	High priority
Low (Typically Zone 4)		Low priority	Low priority	Mid-level priority

5.4.2 Sampling frequency and number of samples

Those areas with direct food contact (Zone 1) and very close indirect contact (Zone 2) that are judged to be difficult to clean should be prioritized for most frequent testing. Those areas that are distant from food (Zones 3 and 4) or are very easy to clean (smooth, flat surfaces with easy access) should be prioritized lower.

High priority areas (red, in Figure 3) should be tested every time the line is cleaned or perhaps at a high frequency such as once per week, depending on production scheduling. Medium priority areas (yellow) could be tested periodically, at a medium frequency, such as once per week to once per month. Low priority areas (green) should be tested at a low frequency of, perhaps, once per month or once per quarter. By modifying testing frequency based on risk assessment, food and beverage manufacturers can ensure they obtain the greatest amount of risk-reduction for the resources invested in testing.

The number of samples depends on both the complexity of the manufacturing equipment/line and the practical considerations of a testing budget. Some manufacturers find it useful to select a fixed number of sites to be tested for verification after each allergen changeover, but rotate the sites selected during each round of testing. The number of samples and strategy, however, should be left to the discretion of the quality team, and the rationale should be planned and documented in the facility's food safety plan or HACCP plan.

5.4.3 Determination of allergen thresholds

Allergen thresholds have been the focus of debate over the last decade. Gluten thresholds in a finished product seem to be generally accepted at less than 20 parts per million (20 ppm or 20 µg/g).[13] However, special interest groups that support celiac and gluten-sensitive communities are advocating for lower thresholds (5 ppm–10 ppm) than those required by regulations.

To address the question of food allergens doses, an expert consultation was convened by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) at the request of the Codex Committee on Food Labeling. They developed consensus recommendations for reference doses to be used in risk-based frameworks for precautionary allergen labeling and labeling exemptions.[14-16] While the recommendations represent consensus by experts representing a wide range of stakeholders, they have not yet been adopted by Codex. Some countries and regulatory authorities have begun to utilize the recommended reference doses and risk-based approaches, but there has not yet been widespread regulatory adoption of thresholds for food allergens.

There is growing consensus on thresholds relevant for finished food, however there is little understanding of acceptable values for equipment surfaces and environmental samples. This is further compounded by concentration units of measure for food (ppm) being improperly applied to surfaces, where weight or per weight volume units of measure have no meaning. Historically, this likely came about through the use of ELISA methods that give results

as ppm to analyze environmental swabs. Regardless of the source, this practice has produced additional confusion in the marketplace.

Still, the current best practice is that a result of "not-detected" with rapid test kits used for environmental sampling (i.e., LFDs) is sufficiently sensitive to protect allergic consumers, given typical detection capabilities at concentrations equivalent to approximately 1–5 ppm total protein from the allergenic source. This provides a very practical approach to setting environmental thresholds for testing systems, despite the lack of clarity from regulatory bodies.

5.5 Corrective actions based on allergen testing results

When an allergen test result from an environmental monitoring program is above the designated threshold, the immediate corrective actions to be taken depend on the risk of the sample, as illustrated in Figure 3.

- High priority (red) samples that are positive require re-cleaning of the equipment and re-testing prior to clearing the line for production.
- For medium priority (yellow) samples that are positive, depending on the type of product produced, a small amount of discretion may be used in determining corrective actions. Ideally, the area should be re-cleaned prior to production. However, increased monitoring and/or deep cleaning of the area in the future might be an acceptable response.
- Low priority (green) samples that are positive should be scheduled for additional cleaning at a future date, followed by post-cleaning testing.

Longer-term corrective actions should include root-cause analysis to determine the sources of allergen contamination or the causes for failure of the cleaning procedure.

Additional long-term corrective actions could include:

- Changing the cleaning frequency.
- Revalidating the cleaning procedure.
- Changing the cleaning process to remove variability or increase effectiveness.
- Assessing equipment for upgrades or replacement.
- Upgrading plant design to improve cleaning.
- Improving raw material/ingredient segregation.

5.6 Identifying sources of allergen contamination

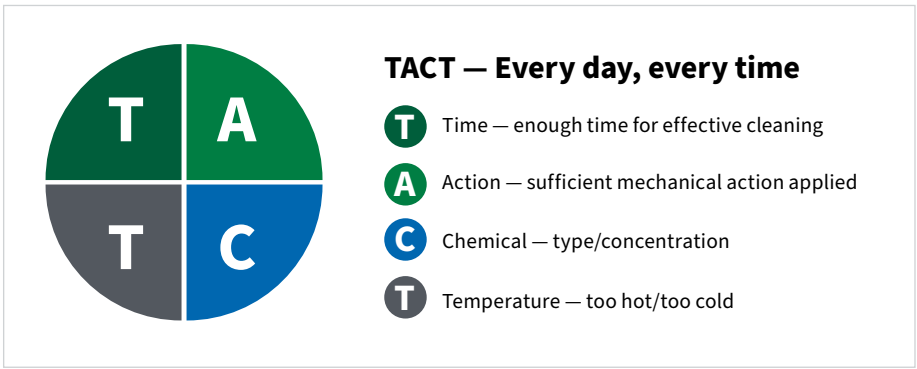
As with any food safety failure, a root-cause analysis is required to determine the source of allergens or origin of the failure, then follow-up action is needed to ensure the failure is not repeated. There may be situations where source of the contamination is not known. In these cases, use of tests for specific allergen residues will likely be more valuable in a root-cause analysis than use of non-specific tests such as ATP or protein swabs.

If the failure occurred in Zone 1 or Zone 2 where the source of allergens is obvious (i.e., they were present in the previous product run on the equipment), the focus of the root-cause analysis should be determining why food residues from the previous run were not adequately removed. Emphasis should be on the cleaning process and on a potential failure in the Time, mechanical Action, concentration of Chemicals, or the Temperature of the process, commonly referred to as TACT (Figure 4).

Additional considerations could include intentional or unintentional changes to the manufacturing process, such as excessive cooking that can make food residues more difficult to remove, equipment failures that can cause spattering or product accumulation, or changes in the raw materials.

If the failures occurred in Zone 3 or Zone 4, the focus of the root-cause analysis should be on the source of the allergenic materials and their potential transportation to those zones. Movement of people, spatter from the manufacturing process, fine powder drift, traffic patterns for fork-lifts and other causes may result in the migration of allergen-containing residues from the manufacturing area to Zones 3 and 4. Air-handling equipment, fans, and construction may also cause inadvertent transport of allergen residues.

Figure 4. TACT approach to evaluate root-cause failure of the cleaning process



5.7 Summary

- Food allergies have increased over the years, which may have a severe impact on public health, especially in infants and young children.
- Current food demand may require sharing of production facilities to manufacture foods containing different allergen profiles. Therefore, robust food safety programs that consider environmental monitoring and allergen control are essential.
- An effective allergen control program in a food manufacturing facility should identify and monitor areas of potential cross-contact and utilize comprehensive validation to ensure a cleaning process effectively minimizes contamination with food allergens.
- Verification of allergen control measurements can be achieved through allergen testing. Two general approaches can be used:

  (1) Highly specific allergen testing that relies on recognition of specific proteins, yielding a qualitative or quantitative result. This approach is recommended for cleaning process validation, to test for allergen-free final product, and for environmental monitoring.

  (2) Non-specific allergen testing that generally detects ATP and proteins whose presence may indicate an inadequate cleaning process. This approach is useful for food manufacturing that includes products containing various allergens in a single product or for overall assessment of cleaning processes, when necessary.
- Selection of a testing method should be supported by a risk-based analysis to help confirm verification measurements will support allergen control plans.
- Testing methods for allergen detection are often based on specific recognition of a particular protein. Therefore, it is important to perform a validation for any selected test to ensure the test is fit for purpose and can reliably detect the allergen source present in the specific food matrix.
- Currently, allergen thresholds are a rapidly developing focus. Based on available information, the detection capabilities commonly seen with allergen test kits for environmental samples are sufficiently sensitive to protect allergic consumers.
- Environmental monitoring for allergen control should include a sampling plan that supports verification of food safety or HACCP plans. High-risk areas (Zones 1 and 2) should be prioritized for greater testing frequency. Consideration can also be given to moderate- and low-risk areas (Zones 3 and 4), which may be tested with lower frequency.
- A complete allergen control strategy should consider short-term and long-term corrective actions within an environmental monitoring program. Also, root-cause analysis should be used to determine potential sources of allergens and identify risks for failure of allergen removal during cleaning and potential failures to exclude allergens in final product.

Learn more about environmental monitoring

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CHAPTER 6

Environmental monitoring for indicator organisms

By

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6.1 Purpose of environmental monitoring for indicator organisms

The term "indicator organism" is defined as an organism or group of organisms that reflects the general microbiological condition of a food or an environment.[1] The presence of indicator organisms does not provide any information on the potential presence or absence of a specific pathogen, nor does it provide an assessment of potential public health risk. However, data from environmental monitoring programs that incorporate indicator organisms can be used to:

- Determine the hygienic status of the processing equipment and environment.
- Understand the microbial ecology of the processing environment.
- Validate and/or verify cleaning and sanitation.*
- Verify process control steps.
- Assess post-processing contamination risk.

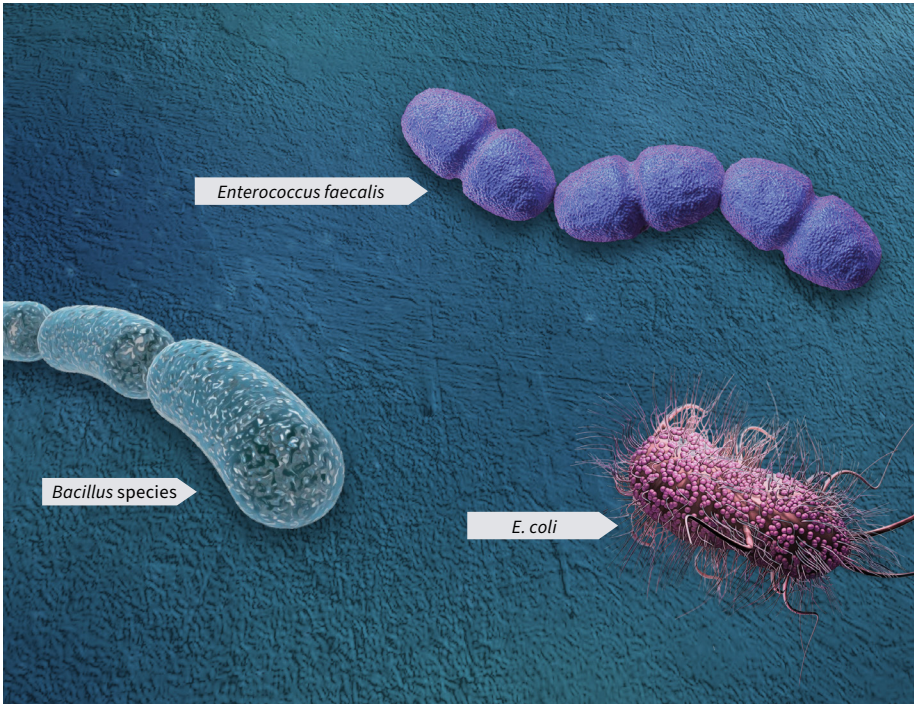
The role of testing for indicator organisms is still often misunderstood by food microbiologists, quality assurance personnel and others. Many incorrectly assume that detection of indicator organisms above a certain level suggests the presence of pathogens. In fact, those organisms whose presence (or detection above a threshold) suggest an increased risk for the presence of an ecologically similar pathogen are referred to as "index organisms". There is, however, considerable skepticism whether any organisms can accurately be considered as "index organisms," with the possible exception of *Listeria* spp.

*Typically testing for indicator organisms would validate or verify sanitation, while ATP testing (see Chapter 4) would be used to validate or verify cleaning.

6.2 Indicator organisms and their significance in the food processing environment

Once thought to be an indication of fecal contamination or potential pathogenic contamination, indicator organisms were incorporated into food microbiological testing during the beginning of the 20th century. Testing for these organisms gave a broader view of organisms in ingredients, finished product and the environment, rather than looking for a specific species.

Microbiologists knew if the manufacturing process was truly under control, the number of indicator organisms would also be under control. Indicator organisms that can be used for environmental monitoring programs include those found by Total Plate Count, coliform, and *Enterobacteriaceae* tests.



6.2.1 Total Plate Count

Total Plate Count (TPC), also referred to as Aerobic Plate Count (APC), Standard Plate Count (SPC), Total Viable Count (TVC) or Mesophilic Count (MC), represents one of the most common indicator tests, although methods used throughout the world vary slightly. At their core, these methods have a key commonality: a non-selective nutrient medium incubated under aerobic conditions used for enumeration. These methods provide information on the total population of bacteria present, capable of growing in the presence of oxygen at mesophilic temperatures.

TPC has many applications. For instance, the total number of organisms present can affect both the quality and potential spoilage risk of a finished product. In its application as an indicator organism, TPC is used to provide an indication of the total microbial population on a surface or in a sample. The use of the word “indication” is important here as not all microorganisms will be recovered under the conditions specified in TPC methods.[2] Moreover, TPC is an extremely valuable method for validating and verifying sanitation procedures. A TPC result above a certain threshold would typically suggest that sanitation of the specific environment or equipment was ineffective or improperly performed.

Utilizing coliforms as an indicator for environmental monitoring

While there is general agreement that coliform detection does not provide evidence of fecal contamination, some countries (e.g., Japan) and industries (e.g., the dairy industry in the United States) have requirements about coliforms in food. For example, in Japan, coliforms are historically well-recognized in several regulations for food industries. Therefore, coliforms are generally used as indicators in Japan for monitoring production environments.

Environmental monitoring for coliforms is considered valuable since coliform presence in finished products would typically result from environmental sources after critical control points (CCP), usually the heat treatment step, except in rare instances where coliform presence may indicate a failure of a CCP. When coliforms are used in environmental monitoring, high levels of coliforms may sometimes lead to additional follow-up pathogen testing. Therefore, despite an increasing preference for *Enterobacteriaceae* testing, coliform testing of environmental samples may still be common in a number of countries and industries.

6.2.2 Coliforms

Coliforms are a diverse group of Gram-negative, non spore-forming rods that are defined by their ability to ferment lactose to produce acid and/or carbon dioxide gas. The precise definition varies by internationally accepted standard methods. Traditionally, testing of coliforms derived from the search for *E. coli*, and presence of coliforms had long been thought to indicate fecal contamination. However, decades of research regarding this diverse group of bacteria indicates that only a fraction are fecal in origin, while the majority are environmental contaminants.[3]

Coliform testing is used as an indication of improper cleaning, insanitary conditions or post-process contamination. Importantly, however, coliform testing only detects a subset of the organisms that may be present in a food processing facility. For example, members of the genus *Pseudomonas*, which represent important spoilage organisms for many foods, are not detected with coliform tests. For this reason, a coliform test may not detect certain problems with a sanitation program, which could be detected with another test (e.g., TPC). Therefore, coliform tests are best used in conjunction with other tests, such as TPC, to validate or verify sanitation procedures and protocols.

6.2.3 *Enterobacteriaceae*

Enterobacteriaceae represent a diverse group of Gram-negative bacteria, which includes all coliform bacteria. *Enterobacteriaceae* are non spore-forming, oxidase-negative rods that ferment glucose to acid and/or carbon dioxide gas. Although the *Enterobacteriaceae* group includes genera known to be pathogenic, such as *Salmonella,* it is considered an indicator test group and not a method for monitoring the presence of pathogens. If information regarding the presence or absence of a specific pathogen is required, it is advisable to perform a specific test for that organism as opposed to relying on indicator tests.

Enterobacteriaceae testing serves the same purpose as coliform testing in that it indicates improper cleaning, insanitary conditions or post-process contamination. Similar to coliform testing procedures, *Enterobacteriaceae* tests will not detect all Gram-negative bacteria, for example, *Pseudomonas* species.

6.3 Development of an indicator sampling program

Development of an indicator sampling plan should be initiated under the direction of a person trained in and experienced with microbiological indicators, testing methodology, sampling methodology and interpretation of microbiological results, who is also knowledgeable about the processing system that will be sampled. Sampling sites, sampling frequency and collection times should be determined based on risk and processing schedules. Once the sampling plan is fully developed, training and documentation should be facilitated.

Sample collectors and data reviewers should always be trained prior to collection of samples or analysis of data from environmental monitoring programs. Training should include aseptic technique, as well as, proper collection of samples, correct swabbing method, and an understanding of safety considerations for each location tested. To observe how each operator collects samples, training and evaluation of their technique would ideally be hands-on, rather than classroom training. Collectors should be retrained if there are incidents or indications of improper handling or swabbing. Training should take place annually to ensure that proper technique and sampling are maintained year after year.

6.3.1 Selection of sampling sites

The first step when selecting sampling sites should be mapping the manufacturing process and identifying the processing steps (e.g., filling, freezing, slicing), functional units (e.g., processing lines, which typically consist of multiple pieces of equipment), and equipment, noting the construction materials used (e.g., stainless steel, rubber, high-density polyethylene [HDPE]). Mapping and sampling of sites should focus on Zone 1 (product contact surfaces) and Zone 2 (surfaces adjacent to product contact surfaces), as indicator testing in these areas provides the most value in terms of sanitation effectiveness. In-process sampling of Zone 1 sites also provides quantifiable data that can be used to indicate possible loss of process control or conditions that could lead to product contamination. In-process sampling of Zone 1 sites for indicator organisms can also be used to define appropriate run times for different lines and could be used to provide scientific support for extended run times.

Additionally, indicator testing for Zone 1 and Zone 2 sites provides a supplementary method for monitoring the condition of equipment and prescribing the frequency of preventative maintenance or repairs. For example, trends towards higher numbers of indicator organisms in certain sites may point towards the need for (more frequent) replacement of gaskets or other rubber and plastic parts.

Incorporation of Zone 3 sites into the indicator sampling plan may be valuable during investigations or root-cause analysis, as these sites are likely to have fluctuating levels of various target bacteria, which may result in erratic trends.

Similar to pathogen testing sampling sites (see Chapter 8), indicator sampling sites should be selected to identify potential issues rather than selected to be easily cleaned and sanitized or always meet acceptable limits. For example, large, flat stainless-steel surfaces are typically easy to clean and sanitize (and therefore tend not to be the best sampling sites, particularly if they are the only sites tested) while a fabric-backed belt is more difficult to clean and sanitize. A sampling plan should include a representative site from each processing step as well as sites that include each of the different types of material used in the construction of equipment.

Once sites are selected, the appropriate tool for sampling each should also be determined. If the site is a small niche or crevice that is difficult to access, a swab may be the best option. For areas that are larger, a sponge would be best, as it allows for more effective collection through greater mechanical action. On easy-to-clean, flat surfaces in which a higher sensitivity test method is desired (as low counts are expected), direct contact of a microbiological growth medium to the surface may be used (Figure 1).

Figure 1. Examples of direct contact and swab sampling using Neogen® Petrifilm® Plates

6.3.2 Sampling frequency and number of samples

In an environmental monitoring program (which is typically designed as a verification activity), sampling frequency for indicators should be risk-based and consider the type of product being produced (ready-to-eat, ready-to-cook or raw; high or low-water activity), the level of risk at each process step and other factors specific to the processing environment such as:

- Processing lethality.
- Sanitation frequency.
- Facility characteristics.
- Potential for cross contamination.

The frequency of in-process sampling will also be influenced by the microbial susceptibility of the product being produced, and the microbial load of ingredients and normal flora of ingredients.

A risk-based sample selection approach should allow one to test only a portion of all the available sampling sites, but still be able to verify control of the environment or sanitation procedures.

When used for verification of sanitation efficacy, sampling should take place after every sanitation cycle and prior to production startup to allow for trending of results and early identification of issues. If the production equipment is complex or contains areas that are difficult to access, it may be helpful to take samples while the equipment is operating but before starting processing of food. Therefore, some equipment (e.g., conveyor belts) may need to be run for a certain time period (e.g., 15 minutes) before sampling. This can increase the likelihood that residual microbial populations, which remained in place after sanitation, become accessible to sampling.

Increased sampling should also take place following an out-of-specification result, particularly for coliforms and *Enterobacteriaceae*.

This section has discussed considerations for sampling frequency in routine (verification) environmental monitoring programs that utilize indicator organism tests. However, indicator organism testing is also an essential tool for validation of sanitation procedures, for instance with specific high-risk pieces of equipment. As detailed in other chapters, validation of cleaning and sanitation may include multiple testing methods (e.g., ATP, indicator and possibly pathogen tests).

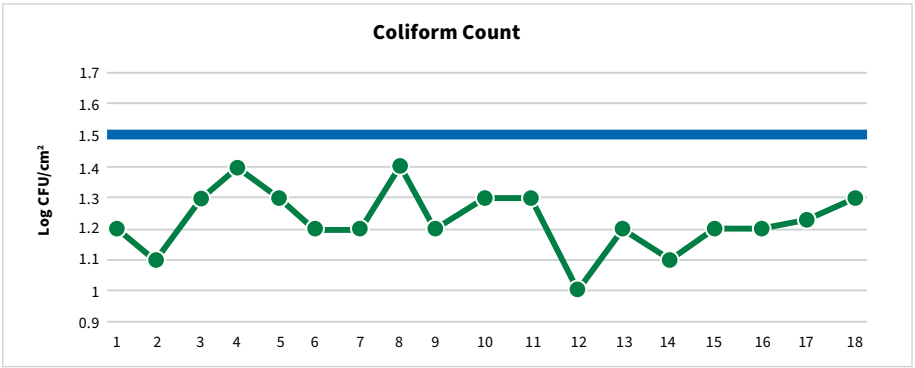
6.3.3 Data trending, analysis and establishing a baseline for indicator organisms

Quantitative results that can be obtained from indicator organism testing are particularly useful, as they can be further analyzed and used to determine baseline levels and visualize trends. Analysis of the data should take place regularly to identify trends and specific issues and allow for appropriate corrections and corrective actions. For example, frequent analysis can help identify a trend of increasing indicator organism numbers, which may then allow facilities to take action before a failure point is reached. Longer term analysis can also foster understanding of seasonality effects and identify opportunities for operational and product improvements.

Baseline levels represent the results that a sanitation program can deliver on a consistent basis throughout the process/facility and can therefore be used to expose results that are out of specification, in terms of sanitation effectiveness. The baseline can be determined in several ways, including collection of samples after consecutive sanitation cycles from each test point. The results can then be plotted in a process control chart to establish the baseline (Figure 2).

Importantly, standard operating procedures (SOPs) for indicator testing should include specific instructions for trending, including the frequency of formal reviews of indicator testing data.

Figure 2. Example of coliform counts and baseline-level post-sanitation



6.3.4 Determination of cut-off levels for indicator organisms

Acceptable limits for indicator organisms should be established for each sampling location. Limits can be determined in several ways, including utilizing the baseline levels and leveraging historical data.

Following sanitation, detecting low levels of indicator organisms on surfaces is expected. Guidance from the Almond Board of California (Table 1) suggests appropriate and achievable levels for indicator organisms in their respective industry. However, the presence of levels of indicator organisms above acceptable limits demonstrates conditions that could lead to loss of process control and potentially result in product contamination.

Table 1. Recommended microbiological indicator limits for equipment cleaning before and after application of sanitizer provided by Almond Board of California[4]

Quantitative Microbiological Indicator Test	Target/ Acceptable Limits	Post-Heat Treatment Taken Before Sanitizer (CFU/40 in² [250 cm²])	Post-Heat Treatment — Pre-op Taken After Sanitizer (CFU/40 in² [250 cm²])
Aerobic Plate Count	Target	<100	<10
	Acceptable	<500	<100
Coliforms	Target	<10	<10
	Acceptable	<100	<50
Total *Enterobacteriaceae*	Target	<10	<10
	Acceptable	<100	<50

Improvements to sanitation, repair of equipment and changes in processes may allow a new baseline and lower acceptable limits to be applied.

6.4 Corrective actions based on indicator organism results

Documentation of corrective actions should include actions taken, results of those actions, dates of actions and names of people involved. Significant deviations should trigger re-evaluation of the sampling plan and retraining of personnel in sample collection methods. Once corrective actions have been implemented, additional sampling at strategic locations in the area of the failure should be undertaken to ensure the effectiveness of the corrective actions. In addition, corrective actions should be taken in locations or production lines where similar conditions or risks may occur.

Corrective actions may not always be a consequence of a failure; they may also be taken for quality or process improvement. Results of in-process sampling may reflect seasonal effects, abnormal microbial load of an ingredient or equipment in need of repair.

Consider the following as an example of corrective action resulting from a seasonal effect. In winter, after eight hours of operation, the bioburden will become elevated on a line to a level that requires cleaning of the equipment. However, in summer, after only four hours, the same level of bioburden will occur at that location. Therefore, the process improvement may be increasing the frequency of cleaning during the summer on that production line.

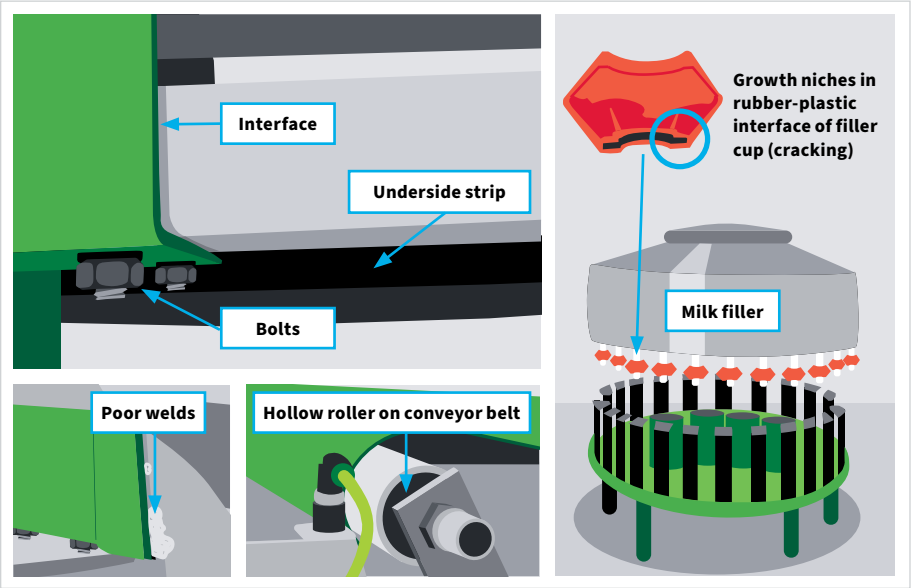
6.5 Identifying sources of indicator organisms

Indicator organisms are commonly present in food production environments, and more broadly in nature. Generally, sources of indicator organisms are cross-contamination from outside the production area or process (potable) water. Indicator organisms introduced to Zone 1 and Zone 2 areas are most likely to stem from ingredients and raw materials. When assessing sources of elevated indicator organism levels, it is also important to consider any atypical activity that may be occurring in the facility, such as construction or new product runs on an adjacent line. New activities, equipment, or changes in sanitation chemicals or personnel could also contribute to increases in indicator organism counts.

Sporadic increases in indicator organism counts could also stem from equipment failures or improper cleaning. Equipment failures could include cracks in gaskets or fractures in conveyor belts which can create growth niches or harborage sites (Figure 3).

Furthermore, if all equipment and machinery is not dismantled for cleaning on a regular basis, or if difficult-to-clean areas exist, biofilms may form and cause product contamination. While these issues should typically be identified during validation of cleaning and sanitation SOPs, they may sometimes be identified through verification sampling that targets indicator organisms.

Figure 3. Examples of potential growth niches in equipment



6.6 Summary

A robust environmental monitoring program should include testing for indicator organisms, especially post-sanitation and on Zone 1 and 2 surfaces. Indicator tests TPC, coliforms, and *Enterobacteriaceae* may be used to verify the efficacy of sanitation activities and confirm that plant operating conditions are under control. The presence of indicator organisms does not indicate the presence of a pathogen, but their levels above defined acceptable limits can indicate insufficient cleaning and sanitation or operating conditions. Use of indicator testing can act as an early warning system to identify and prevent potential product contamination issues. If results exceed established control limits, facilities are expected to take appropriate corrective actions and document those actions and the results obtained.

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CHAPTER 7

Environmental monitoring for spoilage organisms

By

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7.1 Purpose of environmental monitoring for spoilage organisms

Environmental monitoring allows companies to take a proactive approach to microbial spoilage, rather than retrospectively address failures as they arise. This is particularly useful in quality management systems, as spoilage incidents often arise sporadically and, without consistent baseline measurements, underlying or chronic issues can go unnoticed.

Environmental monitoring is often used as a verification activity for sanitation regimes since the processing environment is one of the main contributors to microbial quality failures that manufacturers seek to control. Poor environmental sanitation increases the risk of an unintended microbial spoilage incident.

7.2 Spoilage organisms and their significance in the food processing environment

Food processing environments are non-sterile and the microorganisms that colonize these environments are often well-adapted to using the manufactured food product as a substrate for growth. This adaptation increases the risk of spoilage if cross-contamination occurs.

Moreover, facility-specific spoilage microbes are often adapted to withstand facility-specific production controls. For example, heat-tolerant bacteria and fungi are more frequently isolated from products and facilities with thermal processes. Preservative-resistant yeast are more frequently isolated from facilities employing those preservatives. Long-term use of sanitizers that are not broad-spectrum, or poor cleaning practices, can also result in higher levels of environmental spoilage organisms. In addition, the power of selective pressure can lead to environmental harborage of troublesome spoilage microbes.

Microbial spoilage can result in decreased shelf-life, inferior organoleptic properties, and in some instances, product recalls and withdrawals.[1] These outcomes have significant economic and consumer perception consequences (Figure 1).

Particular production methods or product types are associated with certain spoilage organisms. Therefore, facilities should consider the spoilage organisms most pertinent to their operations and determine which environmental monitoring strategy is more appropriate: a targeted strategy that focuses on a specific type or class of organisms, or a more generalized strategy that relies on relevant indicators. For example, hot-fill facilities would likely address heat-resistant molds as part of their environmental monitoring program.

The spoilage organism's resilience to the inactivation process, tolerance to formulation conditions, and affinity for the facility's raw ingredients should be evaluated in identification of specific spoilage organisms. Relevant spoilage organisms are often grouped using a combination of taxonomy, function, and detection methods. Commonly used groups include yeast and molds, Total Plate Count, and lactic acid bacteria.

7.2.1 Yeast and molds

Yeast and molds are fungi, eukaryotic spoilage organisms that are highly resistant to many processing and formulation controls.[1] Yeast and molds reportedly persist and propagate even under extremely harsh environmental conditions.

Yeast, single-celled eukaryotes that appear similar to bacteria on a Petri dish or under a microscope, are resilient to low pH and are particularly associated with the spoilage of high-water activity and/or high-sugar foods such as pasteurized juices, syrups, fresh-cut fruit and yogurt. Yeast transmission often occurs through food, beverage, or processing/cleaning water vectors, or can be due to insufficient sanitation practices.

Filamentous fungi (molds) are resilient to low pH and water activity, and some are extremely heat-resistant. They are particularly associated with shelf-stable or extended shelf-life (ESL) food products, that have been formulated and processed in a manner that can control other, faster growing spoilage organisms. Mold transmission frequently occurs through air due to the high aerosolization potential of spores, in addition to other mechanisms relevant to all spoilage organisms.

7.2.2 Total Plate Count

Total Plate Count (TPC), or more accurately, total aerobic plate count, refers to all culturable microorganisms recovered on a non-selective growth medium with appropriate nutrients under aerobic conditions at a mesophilic temperature.[2] TPC may be used as an indicator of general sanitation and used to evaluate the total microbial load in the processing environment.

Detection using a Total Plate Count method may be particularly relevant to highly perishable products subject to spoilage from a diverse array of commensal organisms, rather than products that support growth of a few spoilage organisms. TPC results are usually dominated by bacterial growth, which out-competes slower growing fungi.

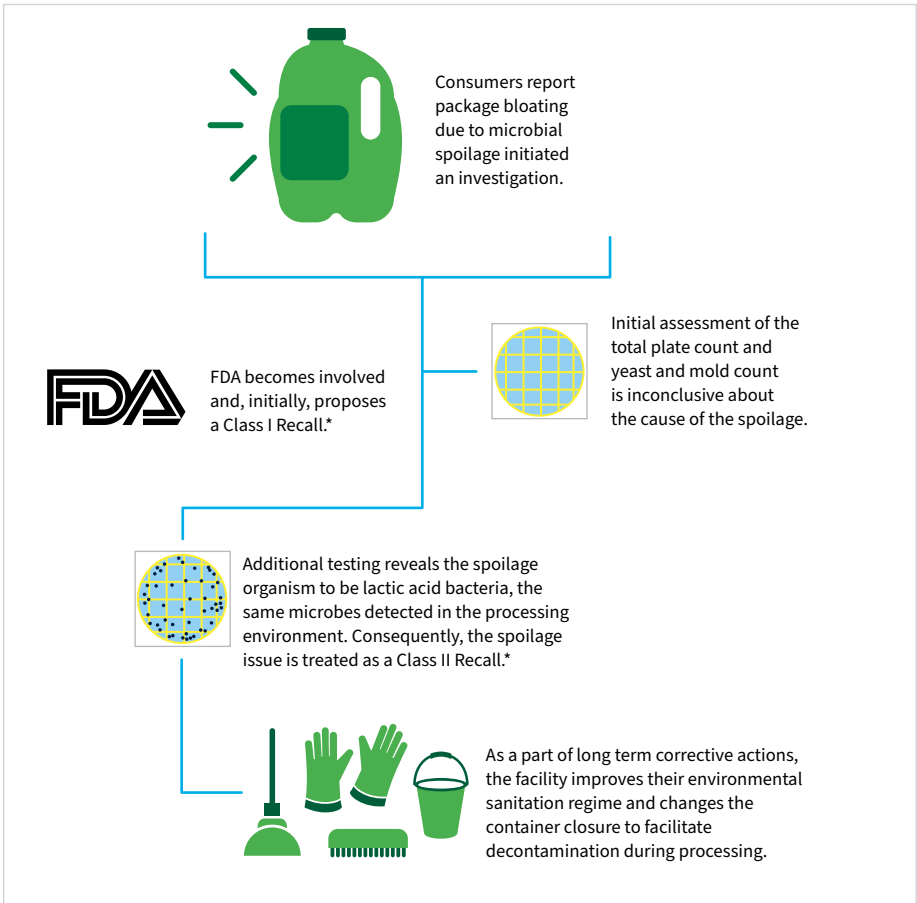
Processing environment designs that are sensitive to environmental contamination may also benefit from TPC evaluation. Examples include filler areas, cooling water reservoirs, and hard-to-clean niches in production lines.

7.2.3 Lactic acid bacteria

Lactic acid bacteria represent a diverse, functional collection of bacteria that cause spoilage of fresh meat and meat products, ready-to-eat (RTE) products like fresh-cut fruit, and modified atmosphere packed (MAP) lunch meats, beer, and wine.[2] Spoilage is characterized by off-flavor metabolites produced during microbial growth — notably, lactic acid. Homofermentative lactic acid bacteria exclusively produce lactic acid as a byproduct of their metabolic activity, whereas heterofermentative lactic acid bacteria variably synthesize lactic acid, acetic acid, carbon dioxide, and other organoleptic metabolites.

Lactic acid bacteria present a significant challenge to the meat industry. Meat is a high-value, highly perishable product that is commonly associated with lactic acid bacteria spoilage. It is one of the best-studied product/spoilage relationships and its quality defects are well-characterized. Spoilage due to lactic acid bacteria outgrowth can be recognized by off-flavors and aromas, slime (dextran) formation, and package bloating due to carbon dioxide production among heterofermentative strains. Lactic acid bacteria are ubiquitous; contamination comes from the environment but can be mitigated through strong environmental sanitation and utensil sanitation practices, along with control of storage conditions during shelf-life.

Figure 1. Chronology of spoilage-induced recall for a hypothetical U.S. food manufacturer



***Class I Recall:** Dangerous or defective products that predictably could cause serious health problems or death. **Class II Recall:** Products that might cause a temporary health problem, or pose only a slight threat of a serious nature.[3]

7.3 Development of a spoilage organism sampling program

A prescriptive environmental monitoring program can target problem areas to reduce spoilage in the short term and allow tracking and trending to control quality threats in the long term. This aids in root-cause analysis and can help distinguish between failures in policy versus failures in the execution of policies. Sampling plans should be structured around the following factors:

- Identification of an appropriate microbial target.
- Selection of sampling sites.
- Determination of sampling frequency.
- Establishment of actionable cut-off levels and associated corrections.

Sampling programs should be feasible for the facility. Decisions regarding these parameters may necessarily involve multiple members of the food quality team.

Facilities should also consider the method most appropriate for their spoilage organism of concern. Facilities targeting molds that produce readily aerosolized spores may consider air sampling methods to monitor spore load (Figure 2).

Microbial air quality can be evaluated using quantitative air sampling or using the settle plate method. Sampling locations and times should be considered in developing a monitoring plan. Areas of high-air circulation, high-sensitivity (i.e., exposed product), or high-microbial prevalence (e.g., a depalletizing area) are relevant air-sampling locations.

Environmental monitoring of surfaces can be accomplished by direct plating and indirect plating by using sponges.

Figure 2. Example of air sampling using Neogen® Petrifilm® Plates

Direct contact plating is a rapid, easy-to-use method for detecting low levels of microbes from non-food contact surfaces. However, if sampling of larger surface areas is required, common indirect contact methods with swabs or sponges can be used.

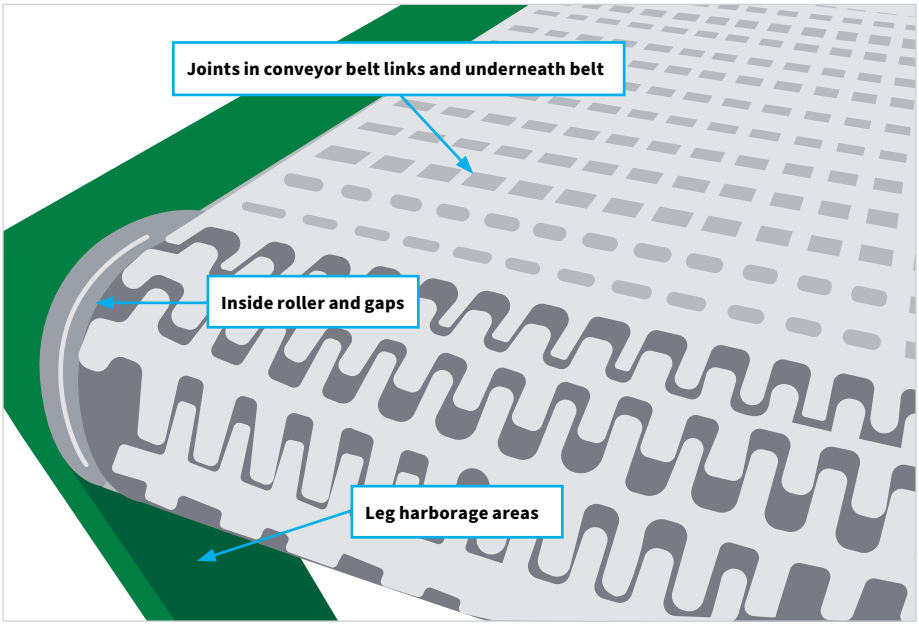
Indirect plating also allows additional sample processing. For selection of heat-resistant spoilage organisms, a heat shock can be applied to the sample before plating to reduce background microbiota. This method also allows for plating on multiple media if several microbial targets are of interest.

7.3.1 Selection of sampling sites

Site selection should be based on the goals and targets of the environmental sampling plan. Environmental monitoring plans targeting spoilage organisms can be used to verify sanitation procedures or as a "seek and destroy" technique for targeting specific spoilage organisms in the environment. Both goals can be addressed through the same plan, but the primary goal may influence aspects of the procedure.

A master list of sampling sites should be developed and a subset of those sites should be tested each monitoring day. If particular sites are notoriously problematic or indicative of sanitation efficacy, the facility may choose to incorporate those sites more frequently into the rotation within a randomized subset. It is advisable to periodically re-evaluate the master list and invite alternative opinions about relevant sampling sites that should be added to the list. Moreover, employees should be trained about where, specifically, to collect samples, based on descriptions in the sampling plan. Figure 3 illustrates how multiple, highly relevant sites can be identified on the same piece of equipment.

Figure 3. Example of multiple sampling sites from one piece of equipment



Verification of sanitation is supported by selection of a diverse array of changing sites, along with targeted checks of difficult-to-clean sites. Seek and destroy approaches to eliminate specific spoilage organisms from the environment should be informed by the transmission mechanism and probable sources associated with the organism, as described earlier.

Generally, swabbing larger areas, has been shown to prevent spoilage, compared to the investigation of small niches often sampled in *Listeria*-targeting programs. Environmental swabs may serve a dual purpose since spoilage organisms and pathogens, or their indicators, can be detected from a single sample. However, with a swab, or more likely a sponge being used for an enrichment as part of pathogen detection, the sample should not be split and used for indicator and/or spoilage test methods. Furthermore, in some instances, sampling site selection may vary between pathogen and spoilage environmental monitoring programs, based on the zones that are selected.

For control of spoilage organisms, facilities may choose to direct sampling activity to surfaces increasingly distant from food production as they can contribute to cross-contamination. Zone 2 surface such as overhead pipes directly above food contact surfaces and cooling water reservoirs, Zone 3 surfaces such as fan blades and air intake vents all represent areas prone to harboring problematic spoilage organisms, depending on the facility.

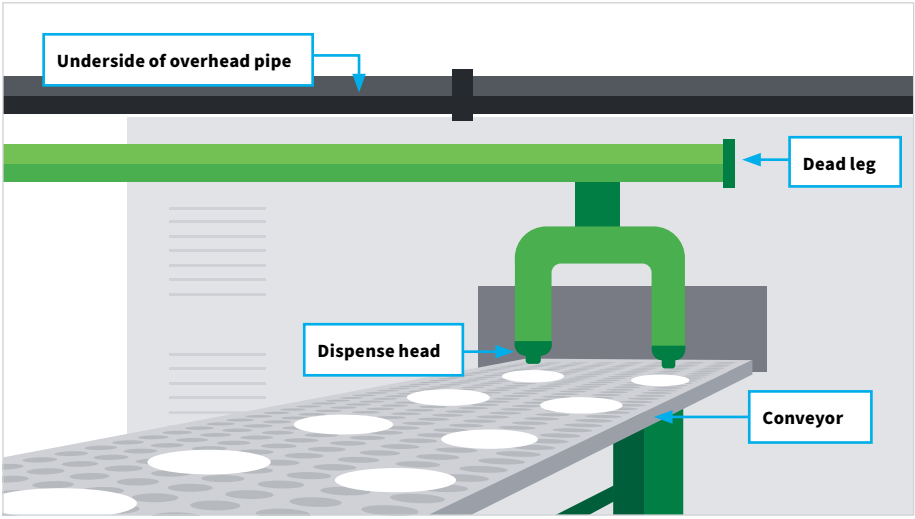
Moreover, Zone 1 surfaces are easily included in an environmental monitoring program that targets spoilage organisms and findings may inform sanitation interventions which pertain to safety. Table 1 lists common problematic areas in food processing facilities, which arise in all four zones.

Table 1. Example sample sites often associated with spoilage organism harborage

Site	Quality Threat	Zone
Dead leg	Lack of turbulent flow leads to accumulation and growth of spoilage bacteria and yeast.	1
Conveyor	Complex equipment that may directly contact product and may also include hollow rollers, rough welds, and microcracks. Moreover, employee overspray during sanitation can contaminate this equipment and contribute to cross contamination.	1+
Cooling water reservoirs	Biofilm development contributes to post-processing contamination of hot-filled or retorted product.	2
Fan blades	Accumulation of fungal spores and dust particulate leads to circulation through air streams in the production environment.	3
Air vent		
Cooler seals/ gaskets	Harborage site, particularly associated with machinery mold, that is difficult to clean without dedicated attention in the master sanitation schedule.	3

In Figure 4 below, a baked product exits an oven on a conveyor while a topping is added from a dispenser. Above the line is an overhead pipe on which condensation forms during production. The arrows in Figure 4 indicate potential sampling sites for spoilage organisms in this area of production.

Figure 4. Example of spoilage organism sample sites in Zones 1 and 2



7.3.2 Sampling frequency and number of samples

The number of samples taken on each monitoring day should be based on the size and complexity of the facility, in addition to the practicality of implementing the program. The frequency of sampling should be evaluated in accordance with the relative risk of a quality failure, should pre-established cut-off levels be exceeded.

Facilities in which environmental monitoring results frequently reveal poor sanitation or emerging microbial harborage sites should increase the frequency of sampling. The same risk evaluation should be used to determine how frequently results should be evaluated by a food safety and quality team. Alternatively, sampling frequency may be established in relationship to the timing of a sanitation event or high-risk processing activity, which may require additional monitoring to prevent quality deviations.

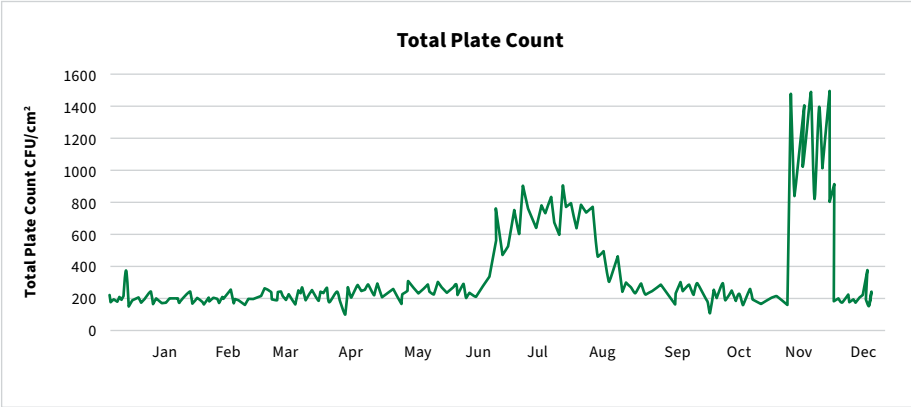
Depending on the facility, sampling may need to be adjusted seasonally or in response to intermittent events. For example, the concentration of airborne fungal spores increases during spring and manufacturers with products sensitive to mold spoilage at the fill step may adjust accordingly. Alternatively, co-packers and facilities handling multiple SKUs on shared processing lines may consider their sampling schedule to be part of a mitigation strategy for preventing the introduction of problematic spoilage organisms, or their growth substrates, into sensitive products.

Generally, one swab per 1,000 square feet (roughly 100 square meters) of processing space may be used as a baseline for quality management, although utilizing more swabs may be increasingly informative. In most cases, sampling frequency should occur at least monthly. Both frequency and the number of sites will increase as size of the facility, pace of production, age of the facility and equipment, and quality threat risk aversion increase.

7.3.3 Data trending and analysis for spoilage organisms

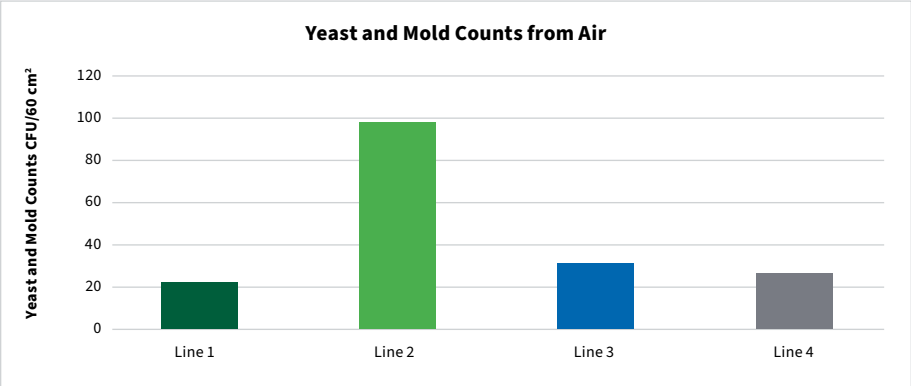
Different visualization methods allow the food quality team to address different questions. A graph can provide useful for presenting environmental monitoring data from an extended period of time. It can make trends and patterns more apparent than visualizing them in a spreadsheet or collection of sampling reports. Sorting data based on date, location, or type of sampling site can address various issues that can arise in a production facility. Manufacturers should take time to analyze results to gain the full benefit of instituting an environmental monitoring program for spoilage organisms. Figures 5a–c illustrate how a company may choose to analyze their environmental monitoring data.

Figure 5a. Example of environmental monitoring data visualization: Total Plate Count



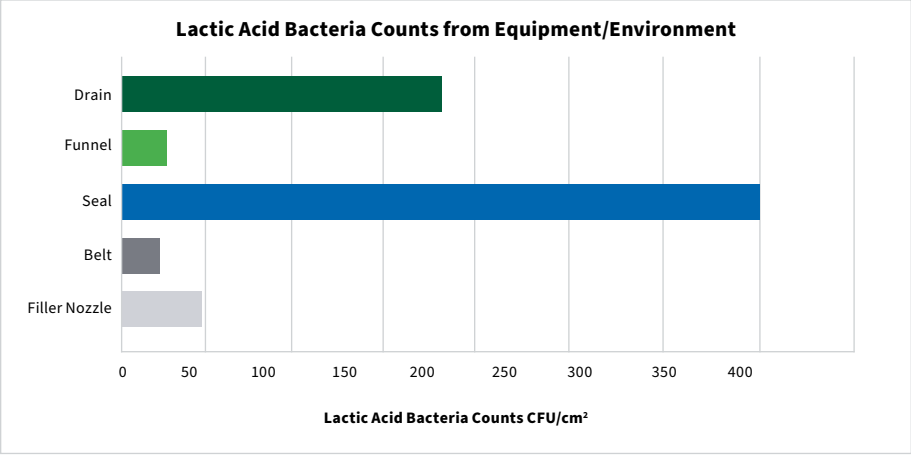
This chart is an illustration of Total Plate Count results in one location over the course of a year. During the warmer summer months (June, July, August), counts increase due to the season. A sharp, significant increase can be seen in late November, which is unusual for the season. A root-cause investigation would need to be conducted to understand the cause of these irregular results.

Figure 5b. Example of environmental monitoring data visualization: yeast and mold



Yeast and mold counts from air samples from multiple locations in a facility can be monitored by comparing counts side-by-side using a bar graph. In this example, the counts on Line 2 are higher than the other locations, thereby putting the food produced on this line at a higher risk for yeast and mold contamination. Steps can be taken to mitigate the risk by determining the source of the yeast and mold, putting equipment in place to shield the product from contamination or implementing a process to eliminate the yeast and mold after this point in production.

Figure 5c. Example of environmental monitoring data visualization: lactic acid bacteria



In this chart, lactic acid bacteria counts have been monitored at various locations. If a finished product is contaminated, this information may be useful to start an investigation to determine the root-cause of the failure. In this example, the lactic acid bacteria counts on a seal are higher than expected and the seal should be checked for cracks or improper cleaning.

7.3.4 Determination of cut-off levels for spoilage organisms

Cut-offs are the quantitative standards that delimit acceptable results for the environmental monitoring program and are best established through in-plant experience. To make an informed decision and set cut-offs at appropriate levels, a facility should conduct 10-20 rounds of monitoring and track the results. This data will create a baseline from which normal variation can be observed, and cut-offs can be extrapolated.

The baseline method is particularly suited for establishing cut-offs for indicator and spoilage organisms, and quantitative microbial evaluations. In contrast, environmental monitoring programs that target specific spoilage organisms, with the goal of their total exclusion from the processing environment (e.g., heat-resistant molds), may determine any detectable target sufficient to initiate a correction.

A facility may choose to stratify their cut-offs, and corrections, based on the type of surface from which the sample was taken. For example, a baseline appropriate TPC for a drain will likely differ from that of a food-contact surface. The initiation of a corrective response should be appropriate for the findings.

Environmental monitoring programs often become burdensome for companies when ineffective cut-offs or overzealous corrective actions are mandated. Since environmental monitoring programs are largely preventive instead of reactionary, sustained trends in microbial detection may also warrant investigation. Again, slight variations in counts are expected and guide baseline calculations. However, if an upward trend of 5–10 consecutive sampling events occurs, a facility may trigger a correction prior to reaching cut-off levels.

7.4 Corrective actions based on spoilage organism results

When cut-off levels are exceeded, short-term and long-term corrective actions must be initiated. Immediate corrections universally include a sanitation step, which either targets a particular location or is a general deep cleaning.

The steps and focus of the cleaning practices initiated after exceeding the established environmental monitoring cut-offs should be documented. The employees responsible for interpretation of the environmental monitoring results and initiation of corrections should be trained for those responsibilities. Many facilities opt to include a re-sampling step in their corrections following the sanitation procedure to verify the contaminant was removed or reduced to an acceptable level. In addition, it is advisable to include the site, in the next monitoring cycle to determine if the source or cause of the contamination was removed or if the same location has become re-contaminated.

Root-cause analysis and long-term solutions should be based on data from several observation cycles and consider cleaning and sanitation procedures, sanitation schedules, and pertinent changes in production. Corrective actions may be dependent on the extent of risk aversion within the company and the probability that a spoilage issue will result subsequent to the environmental monitoring observations.

Both the probability and severity of potential product spoilage should be considered in determining if finished product needs to be reprocessed or destroyed. This should be recorded in a policy within the environmental monitoring program before any breach of established cut-off levels occurs. Following a violation of cut-off levels, some facilities will also increase their sampling frequency or number of sites tested. Theoretically this approach, could direct targeted sanitation towards the contamination source through vector swabbing from ATP tests. It would also increase the degree of control a facility has in maintaining an acceptable sanitary level in their production environment.

7.5 Identifying sources of spoilage organisms

Identifying areas in the processing facility that are contaminated with spoilage organisms is a useful quality management strategy. However, spoilage organisms may be continuously reintroduced into the system if the point source is not eliminated. Continually detecting problematic levels of spoilage organisms from the same site may indicate additional underlying issues are not being addressed through routine or specialized sanitation of the site.

Facilities should consider their risk of introducing spoilage organisms from various sources. Common sources include poor-quality raw ingredients, which can continuously reintroduce microbes into the environment during every production run. Equipment selection and design may also work against the environmental monitoring system if they allow cross-contamination, or fail to actively exclude contaminants. Isolation of activities, age of equipment, age of the building, and the degree to which processes are enclosed all impact the environmental microbiota and the probability of contamination. Therefore, consider long-term findings for spoilage organisms from the environmental monitoring program when developing preventative maintenance and approved supplier programs.

7.6 Additional aspects to consider

Environmental monitoring for spoilage organisms is diagnostic, and should not be considered a standalone system for control. Microbial analysis of cooling water, ingredients, and pressurized air may be important supporting analyses for an environmental monitoring program. Rigorous good manufacturing practices (GMPs) also serve to control spoilage microbiota. However, only GMP violations that directly contribute to changes in surface or air contamination may be detected by the environmental monitoring program and other avenues of contamination should be considered.

A concerted effort across a broad-based team is the best strategy to minimize the risk of a spoilage incident. Additionally, data from the program may be broadly beneficial as the food safety and quality team evaluates various systems. An increase in spoilage potential may signal systemic problems, potentially leading to future food safety failures. Environmental monitoring systems that detect spoilage organisms support proactive responses, but companies need to be prepared and have personnel with sufficient time to evaluate the results from these programs in order to leverage the findings.

Employees should also consider the impact of GMPs on spoilage microbiota in the facility. Re-evaluation of the environmental monitoring program itself should be conducted every 1–3 years. Changes to the processing system or formulation may not only change the level of spoilage risk, but also impact the type of spoilage organisms relevant for a given manufacturer.

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CHAPTER 8

Environmental monitoring for pathogens

By

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Rocio Foncea | Neogen

8.1 Purpose of environmental monitoring for pathogens

In general, environmental monitoring programs are implemented by companies in processing and food-handling facilities to identify and address sources of foodborne pathogens, consequently reducing the risk of food contamination and the associated risks of recalls, foodborne illness cases, and outbreaks. Pathogen environmental monitoring (PEM) programs therefore are often considered a proactive approach to microbial food safety. They may identify challenges and pathogen sources before contamination of finished food products can occur. PEM programs are particularly important since foodborne pathogen contamination of finished products typically occurs at low frequency, which makes finished product testing alone an ineffective strategy for ensuring food safety.

More specifically, PEM programs are typically used to (1) verify an overall food safety system (or specific components of a food safety system) and (2) provide early indication of potential food safety hazards.[1] However, testing of environmental samples for pathogens is usually not an effective means for validation of food safety practices, pre-requisite programs, and "non-process preventive controls" (e.g., sanitation standard operating procedures (SSOPs)). This is because the absence of pathogens may suggest a control strategy was effective when, in fact, the target pathogen was simply not present, even before the control strategy (e.g., sanitation) was applied.

Validation of sanitation procedures and other control strategies typically requires use of multiple environmental monitoring approaches, including ATP testing, to validate cleaning and Total Plate Count (TPC) methods to validate sanitation. Often, use of these tests is supplemented with pathogen testing to identify specific harborage sites that allow for pathogen growth or survival. The process used to identify specific harborage sites or niches (e.g., as part of validation or similar type efforts) is often referred to as the "seek and destroy" technique.[2] In addition to validation and verification, testing of environmental samples for pathogens is used to support root-cause analysis efforts and to verify that corrective actions have been effective in addressing specific pathogen-related problems. These activities may be part of "for-cause" and "not-for-cause" investigations.

PEM programs are most commonly used in processing facilities that manufacture ready-to-eat (RTE) products. Examples include use in produce packing-houses to aid in the control of *L. monocytogenes* and use in powdered infant formula (PIF) manufacturing facilities to manage *Cronobacter*. PEM programs may also be valuable in verifying pathogen control strategies in other establishments that handle RTE food, such as institutional kitchens that serve high-risk populations.

Importantly, there are a number of industry and commodity-specific guidance documents for establishing and implementing PEM programs (in particular for *Listeria*) that should be consulted (Table 1).[1-8] These guidance documents typically provide a level of detail that considerably exceeds the content offered in this handbook.

Table 1. Examples of guidance documents for the establishment of pathogen environmental monitoring programs

Document Title	Organization	Target Industry	Target Pathogen
Compendium of Microbiological Criteria for Food[3]	Dairy Food Safety Victoria (Australia)	Dairy	*Salmonella and L. monocytogenes*
***Listeria monocytogenes* Guidance on Environmental Monitoring and Corrective Actions in At-Risk Foods**[4]	Grocery Manufacturers Association	RTE	*L. monocytogenes*
Control of *Listeria monocytogenes* in Ready-to-Eat Foods: Guidance for Industry[5]	U.S. Food and Drug Administration	RTE	*L. monocytogenes*
Control of *Listeria monocytogenes*: Guidance for the U.S. Dairy Industry[6]	Innovation Center for U.S. Dairy	Dairy	*L. monocytogenes*
Guidance on Environmental Monitoring and Control of *Listeria* for the Fresh Produce Industry[7]	United Fresh Produce Association	Fresh Produce	*L. monocytogenes*
FSIS Compliance Guideline: Controlling *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Meat and Poultry Products[8]	U.S. Department of Agriculture Food Safety and Inspection Service	RTE	*L. monocytogenes*

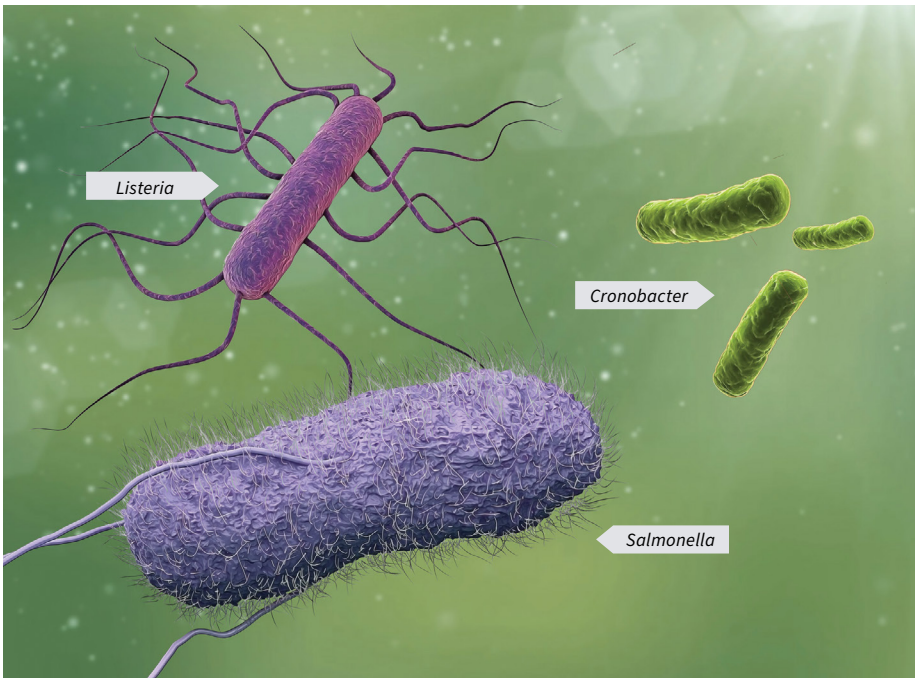
RTE = Ready-to-eat foods

8.2 Pathogens of concern and their significance in the food processing environment

While a considerable number of pathogens cause foodborne illness, there are only a few pathogens for which sources in food processing and handling environments have been linked to foodborne illness cases and outbreaks. Key pathogens targeted in PEM programs include *L. monocytogenes*, with testing typically targeting all *Listeria* spp., rather than the specific pathogenic species *L. monocytogenes,* and *Salmonella*. In addition, environmental sources of *Cronobacter* spp. are a concern in manufacturing powdered infant formula.

L. monocytogenes (in the meat, fish and seafood, dairy, and fruit and vegetable sectors), *Salmonella enterica* (in the feed, meat, egg, and low moisture food sectors) and *Cronobacter* spp. (in the low moisture food sector) have been identified as the bacterial food safety hazards most relevant to public health, which are associated with persistence in the food and feed processing environment.[9]

Although not discussed in detail here, food processing environments cannot be excluded as the source of other foodborne pathogens, including Gram-negative pathogens in the *Enterobacteriaceae* family (e.g., pathogenic *E. coli*, such as *Enterohemorrhagic E. coli* (EHEC)) and even *Yersinia*. Therefore, some facilities may include pathogenic *E. coli* as a target in their PEM programs.



8.2.1 *Listeria* and *Listeria monocytogenes*

Listeria is a bacterial genus classified into two groups: *sensu stricto* and *sensu lato*. Currently, *Listeria sensu stricto* refers to 10 species that share common phenotypic characteristics (e.g., ability to grow at low temperature); this group includes the human pathogen *Listeria monocytogenes*. The other group, *Listeria sensu lato*, currently includes 18 *Listeria* species that are less phylogenetically related to *L. monocytogenes*.[10,11] It is important to select a method that has been tested for its ability to detect all *Listeria* species. However, methods that do not detect some *Listeria* species (in particularly those classified as *Listeria sensu lato*) generally would still be appropriate for use, particularly because *Listeria sensu lato* species usually are phenotypically distinct from *L. monocytogenes* and other *Listeria sensu stricto* species. (For example, some *Listeria sensu lato* species do not grow well at low temperatures.)[12]

Although *L. monocytogenes* is the pathogen of concern, PEM programs typically test for *Listeria* spp. because doing so allows monitoring of processing facility environments for the presence of conditions that would facilitate the presence, survival, and/or growth of *L. monocytogenes*. This strategy provides a more sensitive approach to identify (1) conditions that allow *L. monocytogenes* presence or introduction and (2) harborage sites that could support *L. monocytogenes*. However, a site that is positive for *Listeria* species could also be positive for *L. monocytogenes* so follow-up on each *Listeria* positive sample should be conducted "as if the site were positive for *L. monocytogenes*." If this approach is followed consistently and appropriately, it can provide a more sensitive approach to food safety and environmental monitoring than specific testing for *L. monocytogenes*.

There are specific situations, however, where testing of environmental samples to specifically find *L. monocytogenes* may be appropriate, for example in a for-cause investigation triggered by a finished product testing positive for *L. monocytogenes*. Furthermore, it is important to emphasize that finished product testing approaches for *Listeria* spp. or *L. monocytogenes* may differ considerably by country and region. For example, in the United States, one would virtually always test for *Listeria monocytogenes* and not for *Listeria* spp., as regulatory agencies typically expect speciation of *Listeria* spp., isolated from finished RTE products. In other countries however, screening of finished products for *Listeria* spp. may be the more common approach.

8.2.2 *Salmonella*

The genus *Salmonella* includes two species, *Salmonella enterica* and *Salmonella bongori*. PEM testing in facilities where *Salmonella* has been identified as a hazard reasonably likely to occur from environmental sources will virtually always target *Salmonella* spp., using tests that detect both species.

While *Salmonella* is typically considered a fecally transmitted pathogen, evidence clearly shows that the environment of processing facilities and other food-associated environments can be an important source of *Salmonella*, particularly, but not limited, to dry

environments. For example, *Salmonella* has been shown to persist for at least 10 years in dry food processing facilities.[13] Also, there is evidence that *S. enterica* can persist for long periods of time in the processing environment for egg product, even after pasteurization, which may indicate post-process contamination from the production environment.[14] Therefore, the identification of *Salmonella* harborage sites is important for certain types of RTE food facilities.

Be aware of the square inch or cm² mentality

Many training materials and even government guidance documents specify a certain area that should be sampled when environmental pathogen monitoring is performed. Areas of 12 inches by 12 inches or 30 centimeters by 30 centimeters are often mentioned.[8] However, areas that should be tested are not always defined by a numerical size.[15] Consequently, these numerical recommendations are problematic, as virtually all potential niches that should be sampled as part of an environmental monitoring program are not square or flat areas. Examples are hollow table legs or rollers, floor-wall junctures, or floor cracks.

Therefore, it is important to provide training on sampling that emphasizes the need to sample potential niches in irregular areas, rather than only flat surfaces. Hard-to-reach places such as holes or crevices in difficult-to-clean equipment, rusting, and hollow materials, are potential harborage sites that should be sampled. In some instances, a good sample may be 600 centimeters (6 meters) of a floor seam that is 0.5 centimeters wide. In other situations, one may have to test any surface of a hollow table leg that is accessible for sampling.

8.2.3 *Cronobacter*

The genus *Cronobacter* (formerly *Enterobacter sakazakii*) has been amended over the last several decades due to the continued genotypic and phenotypic investigation of various strains that have emerged over time.[16,17] *Cronobacter* species are opportunistic pathogens known to cause life-threatening illnesses in neonates, infants, and immunocompromised older individuals. *Cronobacter* has been primarily associated with plant-based foods (corn, soy, wheat, rice, herbs, and spices), as well as milk powder and powdered infant formula.[18]

Contamination of powdered infant formula has been a primary cause for infections in neonates and infants, resulting in many outbreaks worldwide and associated recalls of PIF.[19] Also, there is sufficient evidence that *Cronobacter* spp. can persist in dry food processing and preparation environments, particularly in infant formula processing plants. In addition to finished product, *Cronobacter* has been isolated from milk powder and powdered infant formula plant environments (including roller dryers, drying towers, and tanker bays) and has been shown to persist

in these environments for long periods of time due to its resistance to desiccation.[18,20,21] Monitoring for *Cronobacter* in milk powder and powdered infant formula plant environments is critical to prevent contamination of finished product. Due to the changes in taxonomical classification, it is especially important to select a method that reliably detects all species of *Cronobacter*.[22]

8.3 Development of a pathogen sampling program for verification of environmental pathogen control strategies

This section will focus on the development of PEM programs that provide verification of safety systems. It will not cover sampling strategies for validation of food safety programs, pre-requisite programs, and non-process preventive controls (such as sanitation procedures), as this would typically involve a combination of multiple testing approaches, including ATP, TPC, and, potentially, pathogen tests. Development of a PEM program and associated sampling plans involves multiple steps. A possible step-wise framework for this is detailed in Table 2; however, individual facilities must typically refine and expand this framework.

A well-designed environmental pathogen sampling and testing program can allow manufacturers to detect hazards that have become persistent and verify that existing control measures are effective to eliminate sources of contamination and prevent persistence. Moreover, ongoing analysis of results can serve as an early warning, which can allow producers to rectify problems before they become a serious risk.[23]

The selection of sampling points should incorporate areas that have previously tested positive or are likely to be contaminated, such as wet areas, hard-to-reach places, and difficult-to-clean equipment. It is recommended that facilities develop a comprehensive sample site list, with sites sampled at a specific sampling event, conduct testing at sites randomly selected from the list; some facilities may also choose to include some sites that are sampled at each sampling event. Furthermore, for special events (e.g., construction), a specific sampling plan should be developed to investigate the potential presence of harborage niches that could become accessible due to the event. Likewise, a specific sampling plan should be established following "non-conformities", where intensified samplings around the initial contamination site, (considering also different categories of product proximity), should be performed to assess how far the contamination has spread and identify potential harborage sites.

Importantly, a key challenge with PEM programs is that the specifics of sample collection, including the pressure applied to a sponge and the specific locations tested (e.g., a floor crack versus an adjacent uncracked floor section) can have a major impact on whether pathogens are detected. Therefore, it is important to design a sampling plan and an overall PEM program to avoid intentionally or unintentionally providing incentives for sample collectors not to collect samples that would likely yield positive pathogen results. For example, setting numeric

targets or key performance indicators for the percentage of positive PEM samples may simply lead to team members not collecting samples that would likely yield positive results. The goal of a PEM program is to find and eliminate pathogen contamination in the processing environment and this goal cannot be achieved if there are incentives against collecting a positive sample.

Table 2. Key steps for development of a PEM program

Steps		Comments and Suggestions
1	Assemble PEM team	Should be a cross-functional team that, at minimum, includes representation of quality assurance, microbiologist, sanitation and plant management functions
2	Assemble documentation and information needed for PEM program development	Includes floor plans, details on equipment and equipment location, PEM results obtained previously in same facility, other environmental programs already implemented (e.g., ATP testing), and validation data for food safety programs (if available)
3	Identify regulatory and customer requirements for PEM (if any)	Should include identification of industry and regulatory guidance documents
4	Select key parameters of PEM program	Includes target organisms, testing procedures, sample sites, sampling frequency, number of samples collected per week or month, sampling time and day and testing lab (in-house versus third-party lab)
5	Develop written documentation	Includes record-keeping system, SOPs and written guidance for follow-up on positive results (corrective actions). All tasks need to be assigned to specific individuals with written records of those assignments
6	Train sample collectors	Includes training SOPs, records of training and results for tests. Training should be delivered in a form that is easily understandable by all personnel
7	Schedule regular review	Regular review of sampling plans, results, and corrective actions should occur every 6 to 12 months and needs to include complete PEM team (Step 1). This may include a regular (e.g., yearly) PEM sampling performed by an independent or outside group (e.g., consultants or a corporate food safety team), which may collect a large set of environmental samples to assess whether implemented routine sampling plan is effective at detecting target pathogens

8.3.1 Zoning and selection of sampling sites

Virtually all PEM programs use the concept of sampling "zones" when developing a sampling plan. In most countries and regions, sampling sites in processing facilities are assigned to one of four zones (see Figure 1) with Zone 1 representing food contact surfaces (i.e., surfaces directly contacted by an exposed RTE food) and Zone 4 representing areas outside the RTE area (such as locker rooms, loading docks, etc.).[3,5,7,24] In some countries, sampling sites may be classified into three zones. Under this scheme, Zones 2 and 3 of the "four-zone" scheme are typically combined into one zone.

Assignment of sampling sites to zones is not always straightforward. For example, surfaces above exposed RTE foods, which show condensation that can drop onto the exposed food, would typically be considered Zone 1, but may be classified into Zone 2 if zone classification is performed during a time of low humidity when no visible condensation is present and when team may not be aware of the potential for condensation. Additionally, while drains are typically classified into Zone 3, drains located immediately under food contact surfaces may be considered Zone 2 sites.

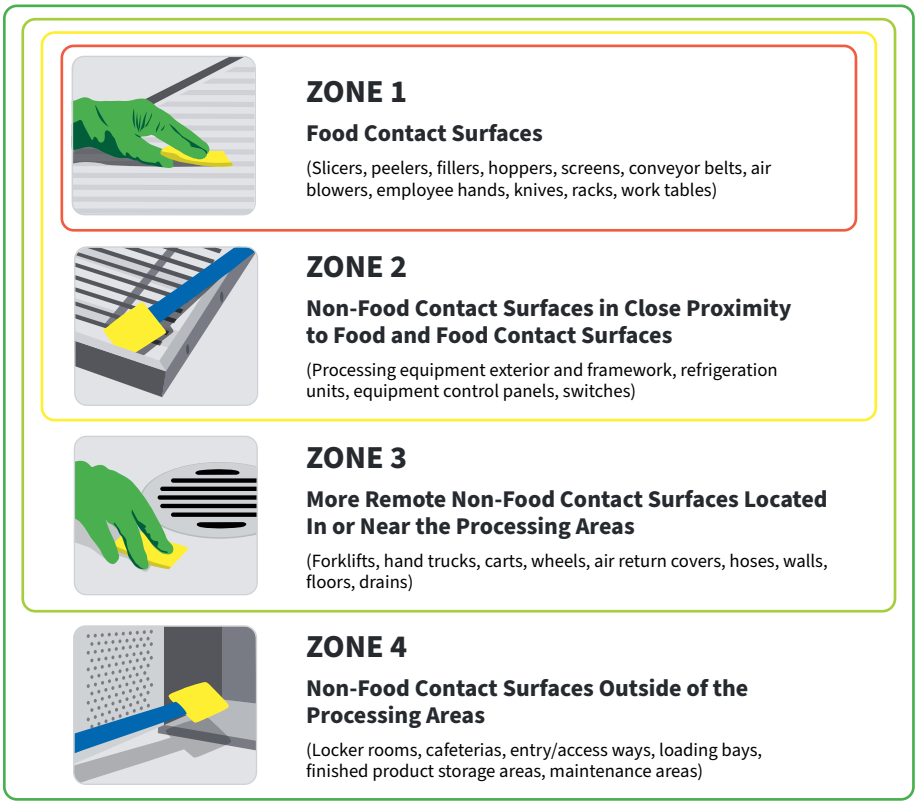
Typically, an initial step in the design of a PEM program is selection of possible PEM sites. The result is usually a master list of sampling sites with a unique identifier for each sampling site. Sufficiently detailed descriptions should be included to ensure subsequent sampling of the same site can be reproduced. Preferably, the list would be created and maintained in an appropriate database compatible with other databases such as laboratory information management systems (LIMS). Selection of sampling sites typically involves a walk-through by the PEM team (Table 2, Step 1), to identify sampling sites. These can include hard-to-clean areas, potential niches, harborage sites, high-traffic areas, and pathways that may facilitate pathogen movement in the facility.

Disassembling equipment to sample actual harborage sites is not feasible for routine verification sampling. Instead, companies may select representative sampling sites contiguous to or adjacent to potential harborage areas. For example, disassembling drains by removing drain covers can be useful. Sampling of harborage sites after disassembly is typically performed during validation sampling or as part of Seek-and-Destroy missions triggered by events such as positive findings in verification sampling.

Each sample site will be assigned a zone while zone definitions may differ by country, region, and even regulatory agency. A written definition of what constitutes a given zone should be included in each sampling plan. Importantly, while this list represents all potential verification sampling sites, samples from all sites will not be taken during each sample collection.

For example, it would not be unusual for a medium-sized food processing facility to have a master list of 400 to 500 sites but only collect samples from 40 to 50 of those sites per week. Moreover, it is important that individuals responsible for sample collection are given the freedom to also collect samples not included in the list. This allows collection from high-risk sites such as pooled water, drain back-ups, or new floor cracks that may become apparent during sample collection.

Figure 1. Environmental monitoring sampling zones



8.3.2 Sampling frequency and number of samples

The standard guidance for sampling frequency and number of samples suggests both should be determined "based on risk." However, this definition has limited value because there are few, if any, guidance documents that specify how to quantitatively assess the risk associated with environmental pathogens. Generally, facilities where RTE foods are exposed to the environment are considered high-risk and would require, at minimum, weekly sampling for target pathogens. Specifically, *Listeria* would be a target pathogen in weekly sampling for any facility that either produces RTE foods that allow *Listeria* growth (e.g., cheese, fluid milk, RTE deli meats, or RTE seafood) or foods that have been linked to listeriosis outbreaks, regardless of whether they typically would not allow for *Listeria* growth. (Ice cream is a good example of this latter case.)

Sampling frequency can be reduced to monthly frequency (or less, in rare cases) if there is substantial evidence of a low risk of *Listeria* contamination. For example, a very small facility that processes less than 3 to 4 days per week may be able to justify

a lower sampling frequency. Similarly, facilities that only produce RTE foods that undergo in-package listeriocidal treatment and do not allow *Listeria* growth may be able to justify sampling less than weekly. *Salmonella* would be a target pathogen for weekly sampling in any facility that produces RTE foods that are exposed to the processing facility environment and have previously been associated with either salmonellosis cases or outbreaks, or contamination events that could be linked to sources in the processing facilities. Facilities that would typically be required to execute stringent *Salmonella* sampling plans involving weekly sampling include, but are not limited to, those that produce chocolate, dry cereals, dairy powders, and many other low-moisture RTE food products.

As with sampling frequency, there are few, if any, recommendations for the number of samples to be taken as part of PEM programs, other than that sample number determinations should be "risk-based." One of the few documents that provides guidance on sampling frequency is a United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) *Listeria* guidance document that suggests collection of 3 to 5 food contact surface (Zone 1) samples per production line per sampling.[8] This could range from weekly to every 6 months for extremely low-risk facilities (Table 3), but only covers Zone 1 (food contact surfaces).

Table 3. Description of sampling frequency for the different food processing facility alternatives classified by USDA FSIS[8]

USDA FSIS Alternatives	Description*	HACCP Classification Size	Production Volume/Day (lbs.)	Minimum Frequency of Food Contact Surface Testing**
Alternative 1 (Alt. 1)	PLT and AMAP	n/a	n/a	2 times/year/line (every 6 months)
Alternative 2, Choice 1 (Alt. 2a)	PLT only	n/a	n/a	4 times/year/line (quarterly)
Alternative 2, Choice 2 (Alt. 2b)	AMAP only	n/a	n/a	4 times/year/line (quarterly)
Alternative 3 (Alt. 3); non-deli or non-hotdogs	Sanitation only (neither PLT nor AMAP)	n/a	n/a	1 time/month/line (monthly)
Alternative 3 (Alt. 3); deli or hotdogs	Sanitation only (neither PLT nor AMAP)	Very small	1–6,000	1 time/month/line (monthly)
		Small	6,001–50,000	2 times/month/line (every 2 weeks)
		Large	50,001– > 600,000	4 times/month/line (weekly)

**Post-lethality treatment (PLT): a process used to reduce or eliminate *L. monocytogene* in the product; examples include pasteurization and high-pressure processing. Antimicrobial agent or process (AMAP): an agent or process used to limit or suppress the growth of *L. monocytogenes* in the product.

**3–5 samples should be collected per line

8.4 Corrective actions based on pathogen testing results

For a routine verification program, which is described here, it is essential to have a clear, written plan and an outline for corrective actions. These should include details on:

- Minimum number of vector swabs to be collected after an initial positive test result, including a protocol to determine specific vector swabbing procedures.
- Deep-cleaning procedures to be used in following-up to a positive test result.
- Root-cause analyses procedures to be used, including details on the team that will conduct these analyses.
- Procedures to be used to translate findings into a corrective and preventive action (CAPA) plan, including requirements for CAPA close-out.

Vector swab sites should be selected to represent areas and sites that could be the source of the initial positive findings. These could be nearby potential harborage sites, such as floor-wall junctures, drains, overhead drip pans, or traffic path sites that intersect with the initial positive site to which the organism could have spread. If routine ("verification") environmental sampling is used to verify a validated food safety program, pre-requisite program or non-process preventive control (e.g., a set of SSOPs, sanitation procedures), the written plan should also include details on the procedures to be used for revalidation of the affected non-process preventive controls.

Air testing for pathogens

A frequent question is whether air should be tested for pathogens. Unlike mold spores, there is no evidence that vegetative bacterial pathogens are transmitted by air in food processing facilities. However, aerosols (extremely fine aerosols and small water aerosols suspended in air) can be a very effective vehicle for transmission of pathogens in processing facilities. Confusion about the role of air versus aerosols may explain why questions about air sampling for pathogens are frequently raised.

Rather than air testing to identify the role of aerosols in pathogen transmission in a processing facility, testing the sources and deposition areas of aerosols would be a more appropriate strategy to address this concern. In addition, minimizing aerosolization (for example, by removing high-pressure hoses in processing facilities and minimizing water use during processing) is essential to reduce pathogen transmission in processing facilities.

Another air-associated potential source of pathogens maybe high-pressure air because air hoses may be a niche for pathogens. Therefore, testing high-pressure air may be advised in facilities, particularly if it is used to clean food contact surfaces.

8.5 Identifying sources of pathogens and development of preventive controls

A key part of a PEM program is identifying harborage sites where pathogens survive and grow, often because they are protected from sanitizers. The food safety goal is identifying and eliminating growth niches (i.e., areas that support general bacterial growth) as well as potential harborage sites during validation of sanitation procedures and before those sites become contaminated. Routine verification PEM programs confirm the effectiveness of sanitation procedures and other preventive controls that have been implemented.

The initial objective of a PEM program is to identify and eliminate harborage sites within an exposed product area. However, niches may be overlooked during a validation process (and subsequent Seek and Destroy missions), as sometimes they may only be identified by ongoing verification sampling. An example would be a niche that was not present at the time of validation sampling but developed over time. In addition, areas that initially do not have potential niches and harborage sites may develop them as equipment and equipment parts, such as gaskets, wear out. Well-designed and implemented verification PEM programs can and should detect these issues. However, detection of pathogen sources can sometimes be hindered if follow-up activities after an initial positive test result (e.g., vector swabbing or deep cleaning) are not executed correctly.

For example, excessive sanitizer use (including floor sanitation) performed immediately prior to vector swabbing may yield negative results, when in reality it simply led to a situation where persistent pathogens, index organisms, or harborage sites were “covered up” rather than truly eliminated. This approach can cause situations where a pathogen or index organism positive sample may be misinterpreted as a sporadic positive result, when in fact, it is an indication of pathogen persistence. Appropriate, well-planned and executed follow-up for each pathogen or index organism positive sample is therefore essential for effective PEM programs.

8.6 Advanced sampling approaches to control environmentally transmitted foodborne pathogens

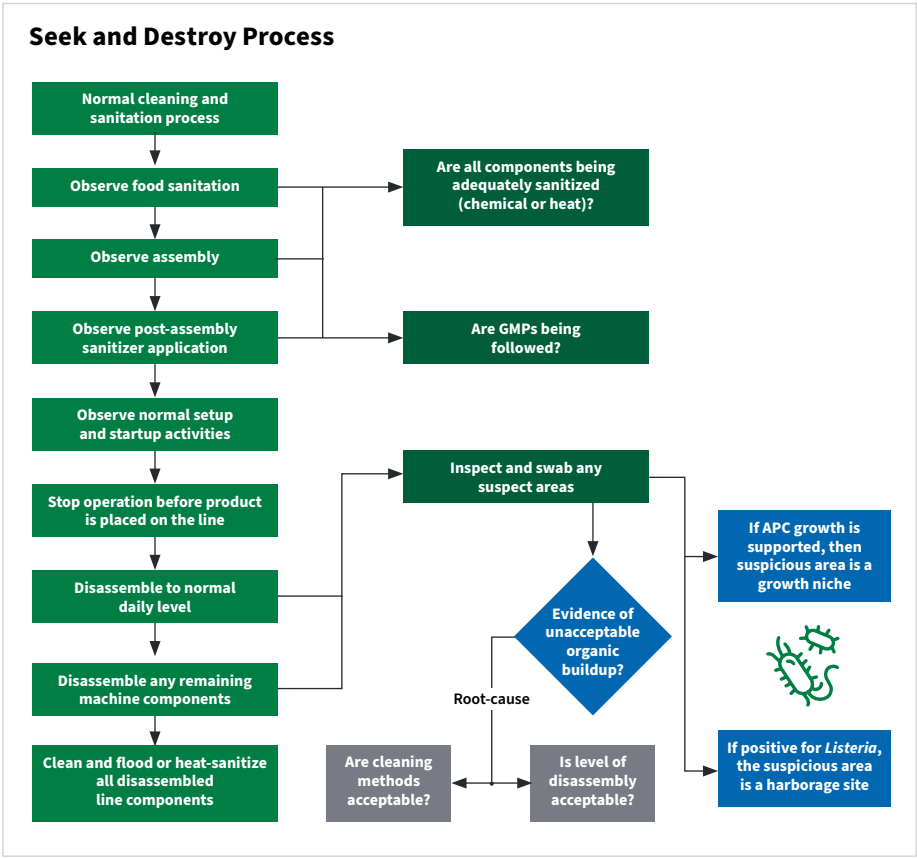
As detailed throughout this chapter, basic environmental pathogen monitoring programs generate data needed to validate and verify environmental pathogen control strategies. This includes "for-cause" investigative sampling after verification samples yield positive results.

Food processing facilities that have robust validation and verification sampling strategies often develop and implement advanced sampling strategies. These sampling methods enable the creation of preventive controls and other strategies that further improve the ability of these facilities to prevent microbial contamination events from environmental sources. For example, some RTE meat processors in the U.S. perform "process control" sampling in addition to verification and validation sampling activities, with validation sampling using the Seek and Destroy approach to find and eliminate niches and harborage sites (Figure 2).

Process control sampling utilizes "indicator sites" (not to be confused with indicator organisms) for sampling, which are early warning sites where pathogen detection does not yet indicate an acute food safety issue. These include areas in a facility and equipment with sanitary design concerns as well as Zone 4 to Zone 3 transfer pathways where presence or ingression of a target pathogen can be identified before it reaches a verification sampling site. Indicator sites are typically located near hurdles and barriers to measure the effectiveness of the obstacle, or at or near a growth niche to measure the level of control exerted by the sanitation process control system. In addition to pathogen detection, indicator site sampling can also use TPC, ATP and other analysis methods.

Investigative sampling following pathogen detection in an indicator site would be considered "not-for-cause," as this is conducted as part of a sanitation process control program but is not necessarily a component of a regulatory compliance program. Employing process control sampling that utilizes indicator sites provides facilities not only with an "early warning system," but can also encourage stringent testing strategies, as positive results at an indicator site would not necessarily indicate a systematic failure of a food safety system that requires "for-cause" investigative sampling.

Figure 2. Example of the Seek and Destroy process[2]



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CHAPTER 9

Principles and methods for conducting root cause analysis

By

Gustavo Gonzalez | Neogen

9.1 Problem solving framework

9.1.1 Total Quality Management

The framework of the root cause analysis (RCA) approach outlined in this chapter is based on W. Edwards Deming's problem-solving method, within the Total Quality Management system (also known as the quality circle or quality cycle). "Total Quality Management" or TQM is a powerful tool because of the broadness of its application and the framework's simplicity. Yet this does not mean TQM is the "best" method for all cases or situations.

Most quality management methodologies can be used to determine the root cause of an issue. However, the intention of this chapter is to provide a standard, documented, and systematic problem-solving framework that is simple to learn and can be used for both temporary and recurrent (chronic) issues.

This guide may be used by an internal quality or food safety team when a process-control issue or "near miss" occurs. For example, when environmental monitoring results are out-of-specification or when quality criteria for finished product is not met, root cause analyses may be required.

Table 1 illustrates a problem-solving approach using eight steps (including RCA), grouped into four stages, which repeat themselves to form the "quality circle" or "quality cycle."

Table 1. The quality cycle stages and steps

Stage	Step
Plan	1 Clarify the problem
	2 Break down the problem
	3 Set targets
	4 Root cause analysis (RCA)
	5 Develop corrective and preventive actions
Do	6 See corrective actions in operation
Check	7 Monitor processes and results
Act	8 Standardize successful processes

9.1.2 Assemble the team

Throughout the progression of this step, the goal is to gain a comprehensive understanding of the situation. Questions will be formed and posed to the team to provide answers which help get closer to a clearly detailed problem statement. Several tools may be useful for forming these questions, such as the 5W2H tool shown in Table 2.

The methodology is not intended to be applied by an individual; assembling a team is typically a best practice for problem solving (an organization may elect that a root cause analysis for low impact issues, such as an isolated Zone 4 *Listeria* positive, may typically only involve one individual). Ideally, the team will be comprised of committed people, covering all departments found to be essential to the investigation. This may include, but is not limited to, quality and food safety, quality assurance, maintenance, and cleaning and sanitation. It is good practice for the core team to have no less than three people and no more than seven.

A team of this type will be better-equipped to support the project's initiatives, increase awareness, and serve as a change management tool.

Be aware that systems and products are interconnected, therefore, improvements in some processes may lead to challenges in others.

Table 2. 5W2H tool

Who	Who discovered the issue: internal control, supplier, operator, auditor, customer?
What	Describe the issue: add pictures, videos, data that show differences among standard and defective products or conditions.
When	Where is the issue located: line or lines, facilities, etc.
Why	Why is the issue a problem? Quality? Food safety? Regulatory?
How	How was the issue detected: organoleptic evaluation, microbial test, other assay?
How many/much	Describe, when is possible, all the lots, batches, units involved.

9.2 Understand the problem

9.2.1 **Step 1.** Clarify the problem

At this step in the approach, the team might work to describe the problem realistically and transparently. This may include performing several rounds of questions to get at "What is our purpose?" and answering, in increasing detail, to avoid as much ambiguity as possible.

If available, visualization of data using charts and graphs may be used to benefit the understanding of the situation and help to prevent subjectivity.

Charts, graphs, and historical data might include:

- microbiological findings and trends from raw material samples (Figure 1)
- findings and trends based on units produced
- failures by SKU
- environmental sample results
- comparisons among days (or processes) that had defective control versus successful control
- correlation coefficients (Figure 2)

Using maps and floor plans that indicate findings can be helpful in determining a potential root cause (Figure 3). Since most microbiological issues require physical contact or proximity, identifying if there are "hot areas" or equipment where problems occur more frequently may lead to a potential root cause. Sophisticated software systems may help make connections among product, the environment and raw materials, which can quickly help delimit a situation. Chapter 10 of this handbook, Data Management and Utilization, describes additional ways to visualize data and its power to help tell a story.

To aid in simplifying and understanding the problem and its relative importance, the team may use a Pareto analysis (Figure 4). This analysis explains, in general terms, the importance or magnitude of the problem compared to other situations that the company is undergoing. This can help keep the team focused during meetings or in conversations if attention becomes diverted.

The team might use this to determine the magnitude of the problem in order for individuals to prioritize it accordingly in their day-to-day activities. The problem's magnitude can range from internal issues to supply disruption, quality complaints, damage to company image, regulatory non-compliance, consumer health concerns, plant closure, and so on. When possible, the team may estimate the monetary impact of the problem.

If the team is working through a food safety issue, the importance of the problem should be emphasized to the team and other stakeholders, reminding them that other problems (such as operational challenges) may need to be considered lower priority.

Classic error detected: Jumping to conclusions
In a busy environment where haste is expected, teams often find themselves moving to a resolution after only one or two working sessions, creating false confidence in their processes and a high probability of failure.

Figure 1. Example of a box plot chart

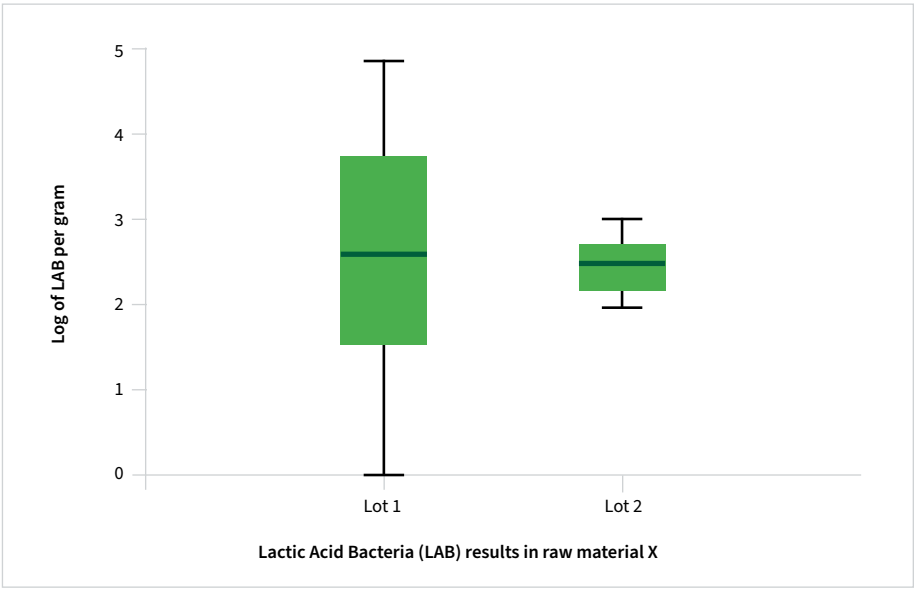


Figure 2. Example of a correlation coefficient chart

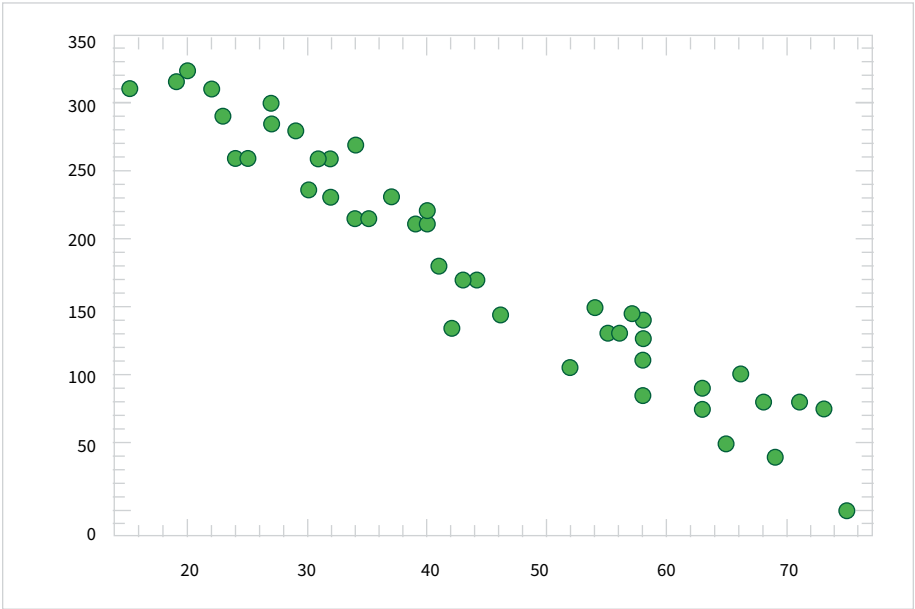


Figure 3. Neogen Analytics software digital floor map of processing environment

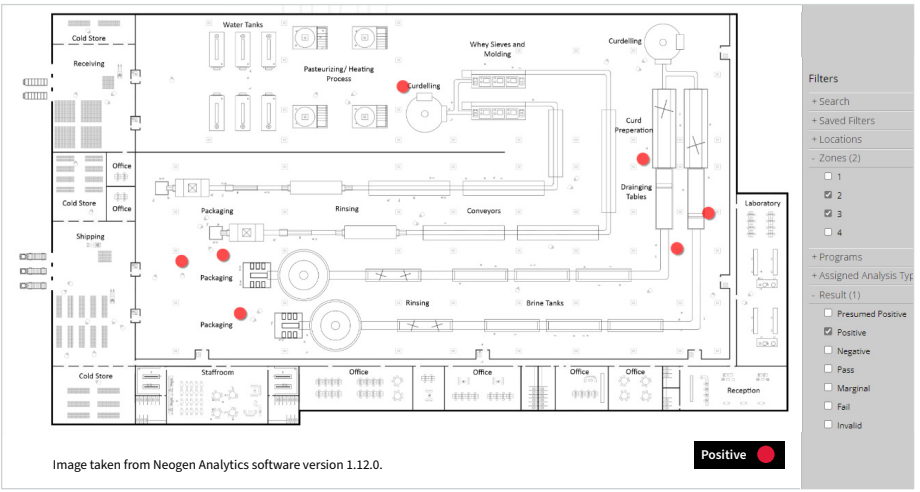
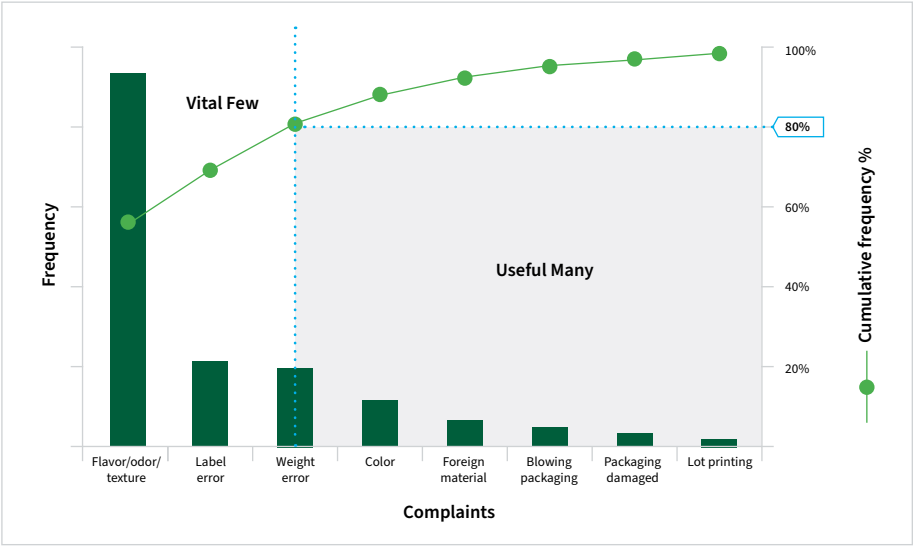


Image taken from Neogen Analytics software version 1.12.0.

Figure 4. Example of a Pareto analysis chart



9.2.2 **Step 2.** Break down the problem

The objective of this step is stopping the issue and preventing potentially large-scale problems. Temporary solutions may be needed and implemented to reduce and contain the problem until the root cause can be identified. While these temporary solutions may not always require deep analysis, it may still include identification of individuals that should be involved, the actions they will take, and when the effort to implement the solution should take place. The goal in putting a temporary solution in place is to gain time until the root cause is found and addressed and reducing the impact in terms of time and cost, while preserving food quality and safety (whenever possible).

At this step, a company may take important actions such as: determining when action is needed, identifying all affected product, identifying manufacturing lines, product, processes, and customers who may be affected, notifying customers, quarantining product, issuing recalls, and disposing of product.

While some immediate actions can be taken to correct or contain an issue; these are reactive steps and generally do not fully address the issue, which means the problem will likely remain.

9.2.3 **Step 3.** Set targets

From this point in time onward, the focus will be on causes, not effects. In each meeting, the target or objective is to find and eliminate the source of the problem and avoid re-engaging in analyzing effects (there will be a time for such analysis when the cycle repeats). At this step, some questions can be answered such as: What is the final objective for the future status? What will be achieved by solving this problem? What is the desired timeline to resolve the situation and what is the target date for achieving the goal?

Sample targets:

- Find the cause of the presence of *Salmonella* in finished product
- Find and control the cause for an increase in the number of positive environmental samples for *Listeria*
- Control the number of shelf-life related non-compliant product
- Find the reason for repeat failure of ATP tests on a specific piece of equipment

Classic error detected: Attempting to solve other situations the company is undergoing, which are different than problems the team planned to address.

9.3 Step 4. Root cause analysis (RCA)

The following sections — on root cause analysis (RCA) — contain the key messages of this chapter. Content will be divided into four steps: Reviewing historic data, Observe, List findings, and Select RCAs to address.

9.3.1 Reviewing historical data

Because the team members worked together to gather all the available information describing the situation, ideally, they should continue working as a unit to conduct the root cause analysis.

Examples of questions to ask include:

- Are there relationships between two or more events?
- Are there patterns or trends in control indicators such ATP, microbiological enumerations, or presence of pathogens on environmental samples?
- Did any raw materials that were received have indicators above specifications?
- Were there any new suppliers who were not fully evaluated?
- Have new testing programs been implemented?
- Are there any new processes that have not been validated?
- Have any pest incidents been reported?
- Could any recent activities have caused the contamination?

Many activities can lead to microbial contamination such as moving or modifying a packaging line, resurfacing floors, cutting drains, equipment breakdowns, new employees, a heavy production shift with reduced cleaning and sanitation, recent leaking water or leaking steam valves in dry environments, or leaks from roofs, to name a few. Some guidance documents outline various activities by food, by pathogen and by type of facilities.[1,2,3]

The team may need to create new charts or graphs to illustrate the data gathered in this step or possibly return to section 9.2.1. “Step 1. Clarify the problem” during the discussion.

Insights or leads may be followed up to either confirm or discard theories about the situation. And remember correlation does not imply causation.

9.3.2 Observe

Observation of plant activities and operations may verify any insights or theories stemming from the review of historical data. It may also provide insight to aspects not previously considered or procedures which were assumed to be conducted in a specific way. The intention behind the plant activity review is to observe but avoid interfering with and thereby changing those activities (i.e., to avoid the observer’s paradox). During a review, it may be appropriate to enter the production area. Keeping the problem, background, and objectives in mind, the team should make their observations as detailed as possible, and may, for instance,

focus on where and how equipment and products are moved, which personnel adjust machinery and/or conditions, the presence of leaks or openings, unused equipment, evidence of condensation, maintenance activities, surfaces employees touch with their hands, etc.

It may be necessary to wait for a cleaning and sanitation process to be routinely carried out, at night, in the early morning or during a weekend. Meanwhile, the team can prepare by reviewing the previously validated SSOP; and when food production resumes observe conditions before cleaning and determine if the SSOP was correctly followed. The team should observe the way the equipment is disassembled, where pieces are placed once they are disassembled, how cleaning is conducted, how the mechanical actions are carried out, and which personnel perform cleaning and sanitation monitoring or verification. In cases where the team believes it necessary, they may request a more intensive disassembly.

If violations are found, it is important not to wait for the entire, formal root cause analysis to be completed, but to make the corrections immediately and record findings and reasons for those corrections. If the team determines it is appropriate, they can remove product, waste, debris, equipment parts, and perform environmental swabbing. The items can then be analyzed to provide a source of information.

Prior to donning uniforms and boots and entering the production floor, it is helpful to gather various devices (and label them) for use in microbiological sampling. These devices may include bags, extra swabs, sponges that can remove firmly attached material, neutralizing solutions, spatulas, dustpans, etc. Microbiological results can assist in directing the team's efforts. Vector swabbing may help detect sources of contamination and/or paths followed by contaminants to reach "hot spots" and/or adjacent areas.

When a company and an internal team are heavily involved in a problem, a normalcy bias can occur. In other words, abnormal conditions can be regarded as normal. In contrast, external specialists can provide fresh vision, avoid internal biases, and detect a situation that the internal team may overlook.

9.3.3 List findings

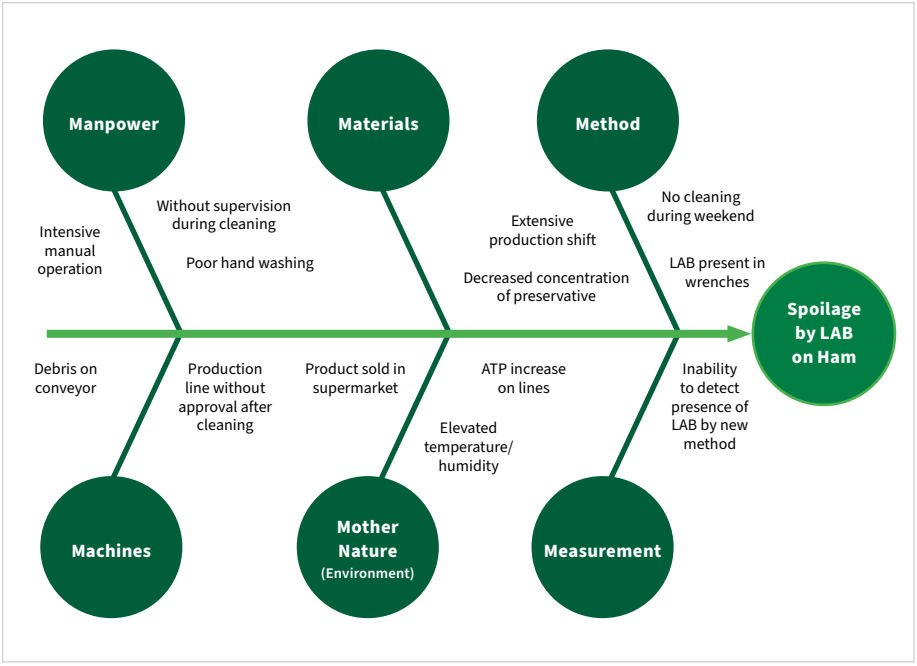
As soon as possible after the observation or floor check inspection, the team should meet to list or describe their findings. All information that may indicate possible causes should be pursued. The team's diversity should be considered a strength in identifying findings that may be overlooked by others, while avoiding personal biases and passing subjective judgments. Listing involves paying attention to unusual pieces of information, following clues, and listening to all sources.

There are various ways to list possible causes, including but not limited to: Ishikawa (6M method, Process flow, and Stratification or enumeration of causes), Brainstorming, KNOW & 4 P's analysis, and Fault tree analysis.

Ishikawa (cause-effect or fishbone): The Ishikawa method relates problems or situations with the factors or causes that generate the problems or situations (Figure 5). This type of diagram is critical because it supports identification of the root causes of problems, preventing immediate conclusions or selection of solutions without understanding underlying issues. Therefore, this methodology is probably the most common approach used. Three types of Ishikawa diagram exist: 6M method, Process flow, and Stratification or enumeration of causes.

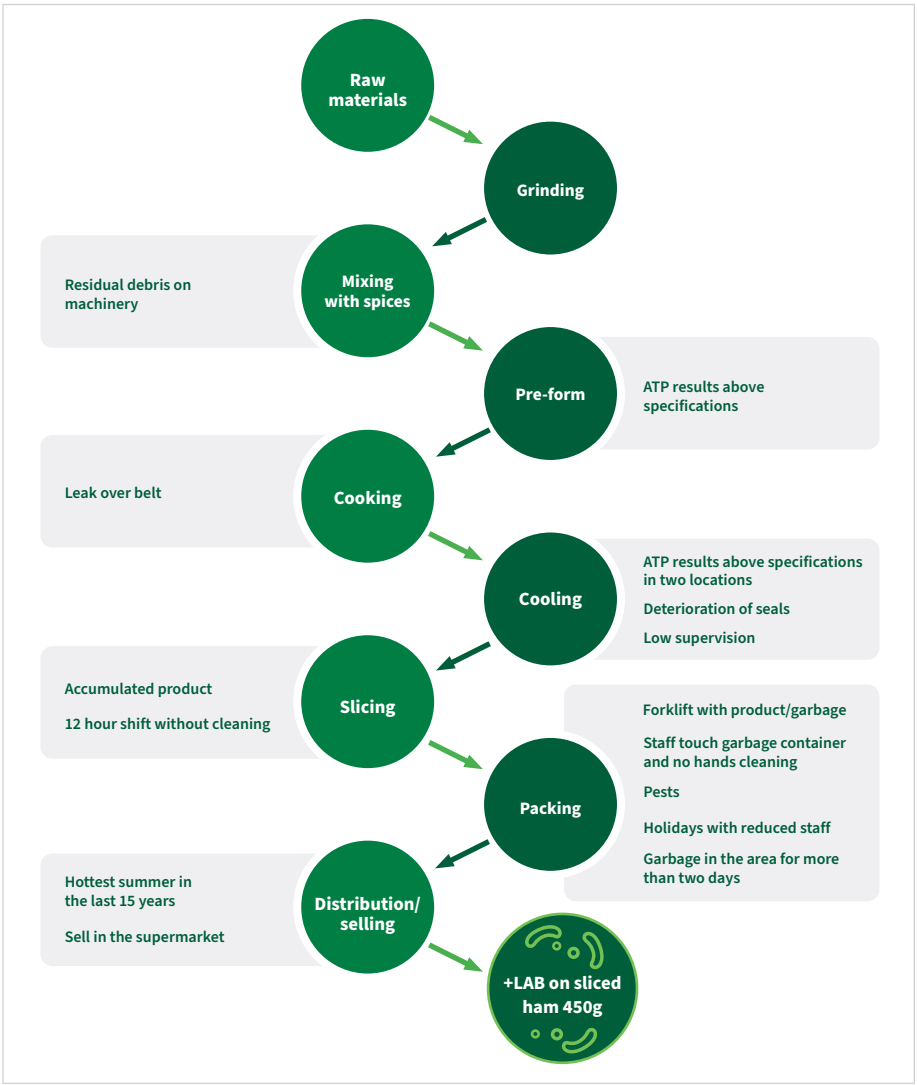
The 6M method: A system or methodology in which every problem (or variability) is attributed to or associated with the following six sources: manpower, method, materials, machines, measurement, and work environment. Some authors believe that policies, food, management and economics can be used as categories to group sources.[1] The 6M method has the advantage of focusing on the process, not the product. If one does not have enough content on a single branch, too many potential causes and the tendency to concentrate on small details of the process will appear. This methodology requires considerable knowledge of process and interactions and is not very illustrative for those who are unfamiliar with a given process. Another notable feature of the method is that "human error" is not acceptable as a cause of failure, as it is too imprecise.

Figure 5. Example of an Ishikawa or fishbone diagram



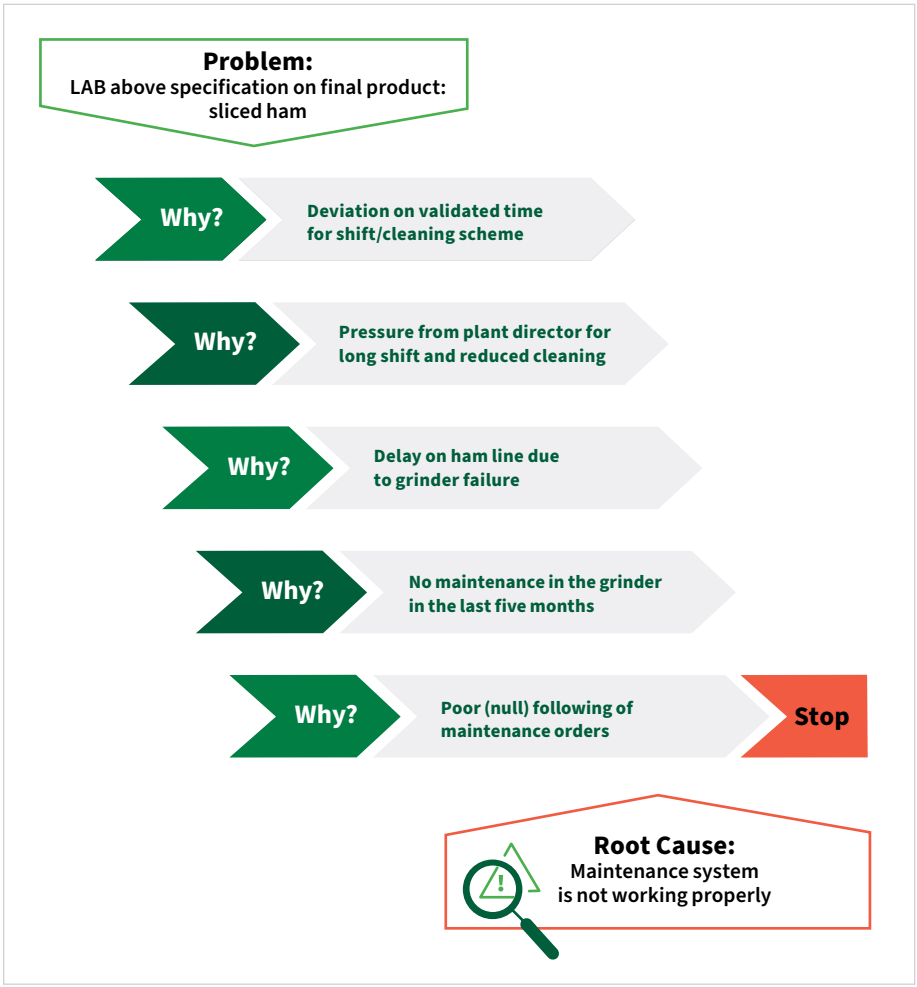
Process flow: Utilizing a process flow diagram (Figure 6), potential causes of the problem can be added and the entire process can be viewed as the potential cause of the problem. An advantage of the process flow method is discovering additional problems not considered at the beginning of the analysis. This analysis also fosters familiarization with the process. At the same time, those familiar with the process may omit causes due to the normalcy bias. Therefore, this method is difficult to use for very complex processes and many potential causes can be identified repeatedly.

Figure 6. Example of process flow diagram



Stratification or enumeration of causes: Stratification is based on the construction of an Ishikawa diagram by directly considering potential causes and grouping them by similarity (Figure 7). Some authors consider brainstorming and the Five Whys (one of the most-used strategies) to be part of the stratification strategy. Its advantages are grouping causes by class, focusing on the process and ease of construction. In disadvantages, it requires significant knowledge of the product or process and potential causes.

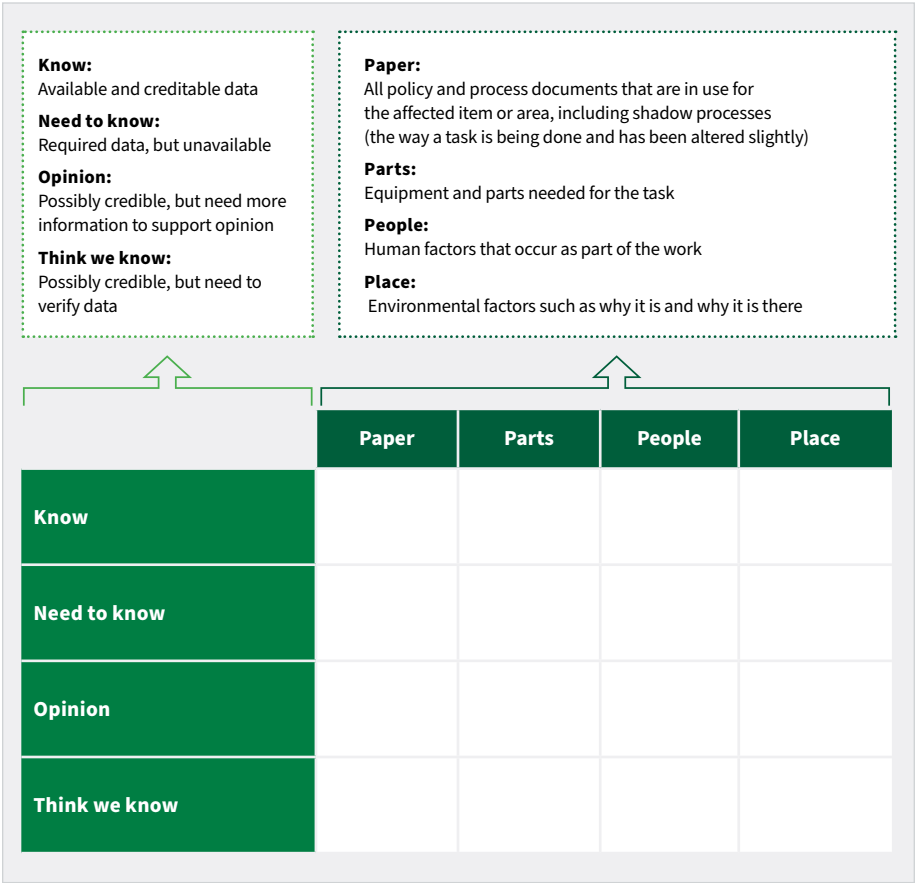
Figure 7. Example of Five Why process



Brainstorming: Participants should create written lists of possible causes, then similar ideas should be grouped and weighted by importance. The major advantages of this approach are eliciting divergent ideas and combining team members' ideas to discover causes. The disadvantage is that the team members who are very sensitive may feel judged. Using this method requires an experienced facilitator who can conduct a non-judgmental and productive work session then process and communicate the session's results.

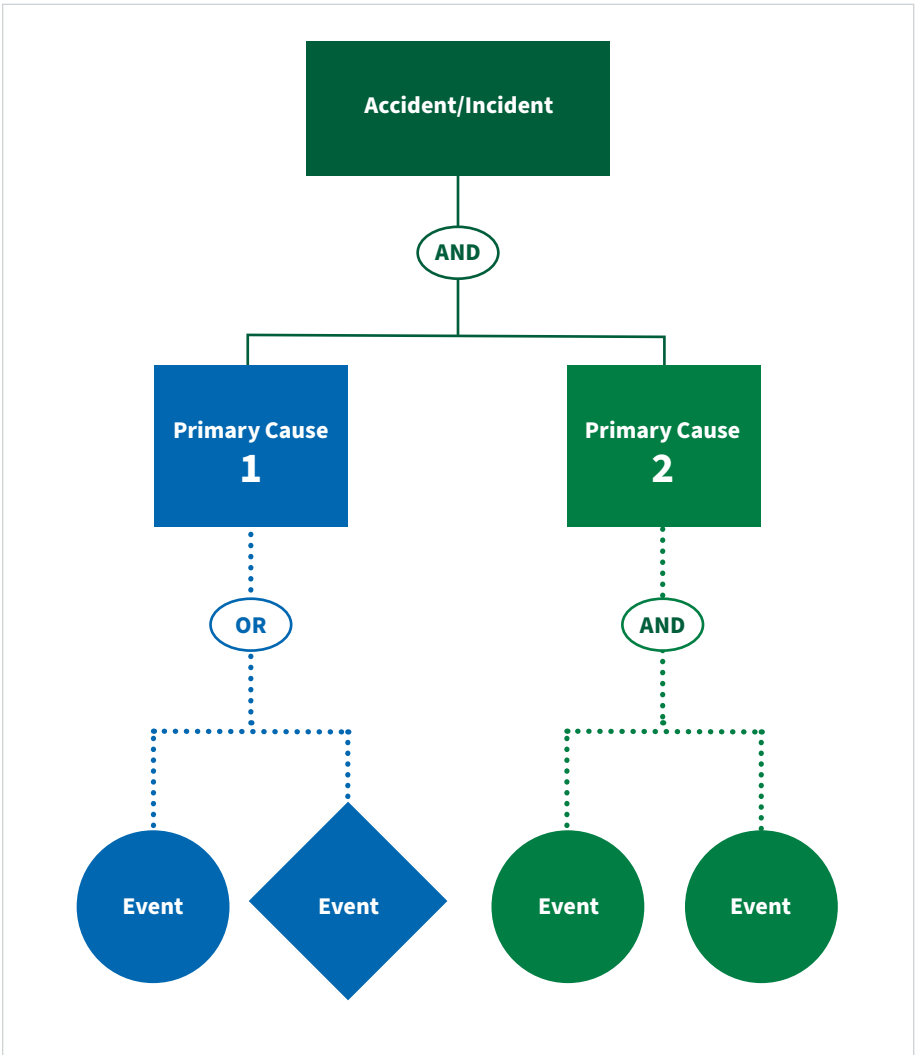
KNOT & 4 P's analysis: This method allows a team to record all evidence and data obtained during an investigation then categorize it into a matrix (Figure 8). This is useful because the information will drive decision-making during the investigation. However, the method requires verification of all the available information and exclusion of irrelevant information, which can be time-consuming.

Figure 8. Example matrix



Fault tree analysis: This method translates a physical system into a structured logic diagram (a fault tree) (Figure 9). Causes are related to one event of interest at the top of the diagram. Events are connected using the terms "AND" and "OR." The analysis can show an incident may be caused by one event or by multiple simultaneous events. (The analysis might also be used to show that events, causes and incidents are not related.) This method is used for risk assessment by safety and aerospace companies. Its deductive nature enables an in-depth investigation of problems, promoting a methodical examination of the ways lower-level incidents can contribute to larger-scale failures.

Figure 9. Example of Fault tree analysis[4]



9.3.4 Prioritization of possible root causes

Next, root causes should be prioritized to identify those most imperative to address. Various prioritization approaches are available, including:

- **Possibilistic:** assumes all potential root causes to have equal possibility of occurring
- **Probabilistic:** considers likelihood of each potential root cause; first consideration to the most likely cause
- **Prognostic:** considers potential severity of consequences for each root potential cause; first consideration to the most serious causes
- **Pragmatic:** focuses on potential root causes most likely to be effectively addressed through available treatments or interventions.

Several sources of information may be reviewed including bibliographic analysis, internal knowledge, and external consultants, if applicable.

The team may conduct a bibliographic analysis, looking for information previously obtained by other companies or from scientific studies. Finding information about how to solve food safety problems can be difficult due to the inherent discretion companies want to exercise in these cases. Scientific articles and food safety guides or recommendations may help support decisions that are made.

Utilizing internal knowledge, the team can be empowered to prioritize potential root causes. Diversity of expertise among team members will help validate and support the feasibility of selected corrective actions and their relative priorities. Microbiologists, quality engineers, maintenance technicians, process specialists, and personnel who work directly on the production line will provide different viewpoints.

Voting and ranking can help prioritize root causes to be addressed. Start by assigning each cause a score of 5, 3, 1 and 0 for strong correlation, moderate correlation, weak correlation, and no correlation with the problem. All the scores obtained for each of the causes should be added then selected in order of priority. Another option is to contrast each measure against another to give priority to each one over the rest.

Classic error detected: Describing a root cause while working from an office, without observing and evaluating the process from the production floor.

9.4 Implement and verify

9.4.1 **Step 5.** Develop countermeasures

Root causes may be addressed using comprehensive corrective and preventive actions (known as CAPA). Such actions can be immediate, corrective or preventive:

- Immediate actions are taken to quickly correct or contain an issue but the actions are considered reactive and likely will not fully address an issue so it will remain.
- Corrective actions are taken to eliminate the cause, if the issue has been detected.
- Preventive actions are taken to prevent an issue from reoccurring.

For each root cause, immediate, corrective and preventive actions (or countermeasures) can be assigned but some causes will not require all three types of actions. In addition, some causes may require multiple actions while the same actions may be directed at several causes. As the complexity and significance of the root cause increases, the greater number of actions that may be identified.

Countermeasures can range from basic changes in personnel practices, re-validation of cleaning processes changes in pre-requisites or processes, structural changes to equipment or facilities, to re-validation of the entire safety management system.

As a good practice, countermeasures contain actionable items and have an assigned individual responsible for conducting or supervising them. Some companies prefer to assign tasks directly to individuals, rather than creating committees or teams for activities, since planned follow-up may be lost.

Each action includes a deadline (depending on its priority and feasibility) indicating when the countermeasure should be implemented.

For sources of information about selection of countermeasures, a company may again use scientific literature or guides, rely on internal experience and/or follow recommendations from industry experts.

Classic error detected: Focusing on containment and detection, without addressing the cause.

9.4.2 **Step 6.** See corrective actions in operation

Many actions will have immediate effects while others, such as major changes in facilities, equipment, and systems, may take days or weeks to implement and take effect.

For some actions to show effects, it may be necessary to have a plant in operation. In doing so, steps should be taken, such as implementing stringent release criteria, to reduce the probability of releasing end-product under uncertain conditions or before corrective actions take effect.

As noted earlier, positive changes in one area can have challenging or negative effects in other departments or areas within a company. During the CAPA process, various parameters may change, and it may be prudent for the team to be alert and aware of unexpected changes occurring in other areas. If they occur, it may be necessary to return to step 4.

Classic error detected: Dreaming and planning, but not acting.

9.4.3 **Step 7.** Monitor processes and results

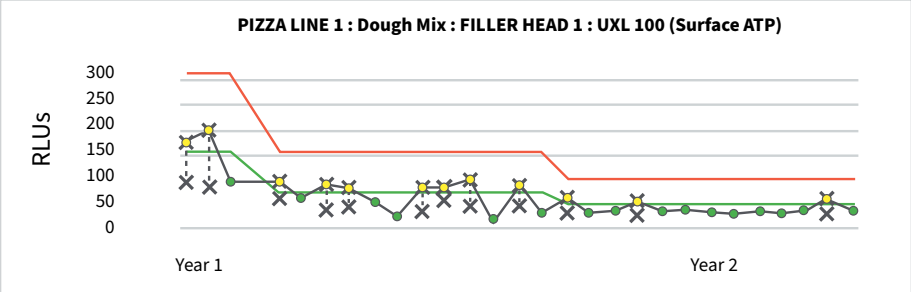
When actions have immediate effects, this may quickly be evident in both the product and the process. However, some actions will generate slow changes in a product and/or process. Therefore, the supervisory team should be aware of any process changes or findings and be prepared for the consequences. This approach can result in a good change-management process.

In some cases, a tightened sampling plan (with more frequent sampling and/or more stringent requirements) or an investigational sampling plan (which can detect the cause of a problem) may be utilized, to increase detection of defective units, find a problem's cause and or re-validate a process. Guides for conducting tightened sampling are available (ICMSF 7).

Some changes or improvements will be implemented in stages. Fulfilling the objective of initial changes may be an indication to begin the next stage and implement changes which increase stringency.

For example, improvements in a cleaning process may have been selected as a preventive measure for a microbiological issue where ATP (measured as RLU) in food contact surfaces will be used as an indicator of cleanliness. Achieving reduction will take time and additional effort. Figure 10 illustrates a decrease in RLU at acceptance levels of 250 to 150 RLU in two phases. In the first phase, the company will try to decrease results to 200 RLU. In the second phase (when applicable), additional effort will be required to refine the newly implemented process and decrease variability to achieve 150 RLU.

Figure 10. Adoption of more stringent pass/fail levels for continuous improvement of hygiene control



Depending on the actions taken, changes may or may not be observed in some process indicators such as mesophilic aerobic bacteria, lactic acid bacteria, *Enterobacteriaceae*, and *Listeria* spp., as well as in ATP levels or the presences of allergens.

To evaluate the effects, companies will have to utilize data before and after the actions were undertaken. Graphs that may be useful include boxplots, presence of findings by date, levels of the target found in the batch or environment, and others. Results should be shared with the team to show the success of implemented activities.

The best way to evaluate the success of a root cause analysis and CAPA process is to observe whether the implemented changes have had the desired effects. For example, if a plan to control *Salmonella* in environments included eight countermeasures, success would be defined as having sufficient periods to determine the plant has come under control (e.g. as supported by reduced frequency of *Enterobacteriaceae* or *Salmonella* positive test results) due to the new conditions or implemented changes, bearing in mind the seasonal effect of the situation.

Classic error detected: Execute corrective actions without measuring effectiveness or conducting follow-ups.

9.5 Step 8. Standardize successful processes

Many problems are seasonal so they are expected to return periodically. However, there is no guarantee the team that handled a current problem will manage future problems. Therefore, it is important to keep records of the findings and solutions utilized, including those actions that were not effective. Good record-keeping can provide an organization and its future teams with tools and a pathway to get pursue and achieve similar solutions.

A company's record-keeping system should describe the activities conducted, findings and actions taken, and monitoring and measurements of the solution's effectiveness. Software systems designed to manage root cause analysis can support companies, especially in monitoring and documenting processes.

Each organization should have Standardized Operating Procedures (SOPs) to use in conducting root cause analysis for deviations related to production environment and environmental monitoring.

These SOPs may include important information such as:

- Conditions for starting a formal RCA
- Designations of RCA team members
- RCA procedures the organization will follow
- Guidance for vector swabbing
- Vector swabbing details, including sampling times, number of swabs, items to swab, and analytes
- Required disassembly of equipment
- Holding and testing end-product and requirements for product release
- Stopping the production line, including authority for that decision
- Reprocessing, diverting or destroying positive product lots
- Duration of enhanced sampling after a positive result is found
- Conditions requiring subtyping of findings
- Conditions for resuming production

Classic error detected: Relying only on verbal descriptions of processes. If a process is not written, it does not exist.

Learn more about environmental monitoring

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CHAPTER 10

Data management and utilization in environmental monitoring

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10.1 Importance of data management and utilization

Food and beverage companies invest time and money implementing and maintaining their environmental monitoring programs. A well-designed data management system can enable them to get the most value out of that investment. Environmental monitoring data is valuable and can help a company understand food safety risks and production issues, while decreasing the likelihood of future mistakes. This has clear implications for public health, but there can be additional positive outcomes and business benefits associated with food safety, quality, productivity, and output.[1] Analysis and utilization of environmental monitoring data can support the identification and mitigation of risk; aid preventive maintenance and decision making related to capital investments; and enhance business opportunities with risk-averse customers. Data management can also directly support access to global markets, for example, a company receiving an audit against GFSI-benchmarked schemes must provide documentation of their environmental monitoring program (EMP).

In summary, there are many benefits to robust data management efforts, as they can:

- Assist in generating a formal record of key food safety programs.
- Enable analysis and re-use of valuable environmental monitoring data.
- Increase return-on-investment (ROI) from environmental monitoring activities.

10.2 Common barriers to successful data management systems

Professionals in the food industry have limited resources and many demands on their time, so it must be acknowledged that the establishment of a data management system can require a significant investment of both time and effort. Common barriers include management support, time and effort, and level of expertise.

10.2.1 Management support

Despite the benefits of EMP data collection and analysis, management at some companies may struggle to see the value of these programs. They may lack understanding about the impact of the food processing environment on product quality and safety, have poor appreciation of the value of EMP data, or lack a food safety culture within their organization. For example, if management believes their facility doesn't (or couldn't) have any food safety issues, they will always struggle to see the value in continued investment in those programs. Or worse, management may not want to know if problems in environmental hygiene exist, using the logic that if they're not aware of a problem now, why go looking for trouble? These thought processes can lead to continued under-valuing of food safety programs and

requires food safety and quality personnel to explain and justify the importance of an EMP data management system. More information on understanding the current state of your company's risk culture can be found in Chapter 11 of this handbook.

Furthermore, a compelling and effective presentation of meaningful data can help overcome this barrier. *See the section on "telling a story with data" later in this chapter.*

10.2.2 Time and effort

Traditionally, poorly documented data has taken less time to collect, organize, and store than well-documented data. For example, results scribbled hastily on a form or printed documents, shoved haphazardly into a binder takes less effort to maintain than a digitized, standardized database that is routinely analyzed to identify trends. Selecting personnel with the capacity to do this work, and implementing regular check-ins on their progress, is necessary to ensure an EMP data management system is effective. Particularly during the early stages of implementation, it will take more effort for a company to collect data in an organized manner than it would take to collect data as quickly as possible. However, well-developed data management systems may ultimately save time and company resources by automating processes related to data capture, cleaning, storage, and analysis.

10.2.3 Expertise

Food safety and quality staff vary widely in their experience and confidence in using digital systems. For example, a staff member who typically spends 80% of their time on the production floor, has no laboratory responsibilities, and only uses a computer for emails and occasional word processing may find the task of developing a data management system daunting if not impossible. By contrast, a staff member who primarily works at their computer, has experience with laboratory or analytical equipment that generate data, and accesses data across many platforms within the company may be more comfortable conceptualizing and implementing data management systems. However, both staff members would benefit from the existence of a strong EMP data management system at their company. Commercially available software and tools can decrease the barrier of adoption by reducing reliance on staff experience and labor.

Different companies may find these potential barriers more or less difficult to overcome depending on their available resources. Most of the difficulty lies in the initial efforts to bring EMP data management systems online and develop practices for analysis and application. Once in place (or, for example, after a successful use-case has been completed), ongoing maintenance and improvement can become much less difficult. However, ongoing maintenance costs vary depending on where a company is on the "build-it versus buy-it" continuum for their analytics system.

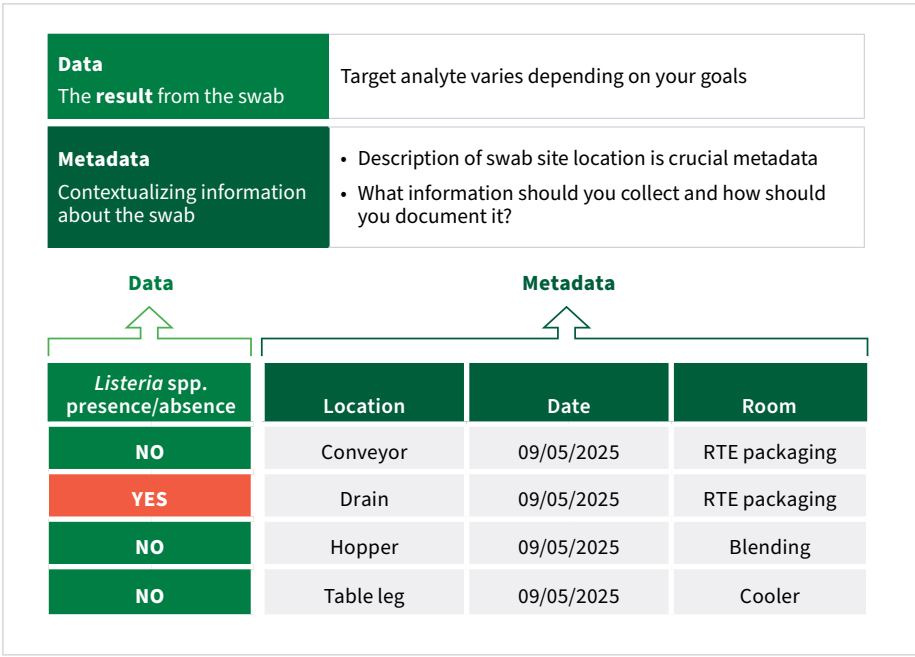
10.3 Structure and components of environmental monitoring data

Food businesses generate many different types of data: quantitative and qualitative values recorded in production; the results from analyses, including data visualizations such as graphs; and information or interpretive findings (e.g., fishbone diagrams or investigation reports). Being specific about the type of data that one intends to generate and the form it will take is essential. Simplistic statements about "needing more data" are overly broad and lead to confusion among team members. In contrast, being precise with the language used to describe data and analysis can build interest and consensus among team members.

An opportunity to build specificity into a data collection system is by defining the data and metadata one intends to gather through an environmental monitoring program. "Data" is essentially the result of a test that was conducted. For example, if a company is swabbing surfaces to detect *Listeria* spp., then the positive or negative result from that test is data. If a company is swabbing surfaces to quantify *Enterobacteriaceae* levels, then the number of colony forming units is the data. If a company is conducting ATP tests, the pass/fail result or RLU level is the data. Selecting the target analyte (i.e., for which one wants to collect data) depends on a number of factors described in the other chapters of this handbook. Generally, however, results of tests are data.

What is metadata? Metadata is any information that adds context to a result.[2] Obviously, this includes information about the location from which a sample was collected (e.g., conveyor leg). Other metadata variables could include the collection date and time, the product being produced during sampling, visual observations about the location, and the test or method of analysis used to collect the data. Companies differ in the metadata variables they collect, but careful consideration of information that describes where, when, and how samples are collected is required. An example of data and metadata gathered in an environmental monitoring program is shown in Figure 1.

Figure 1. Example of environmental monitoring program metadata



10.4 Focus on metadata

The importance of collecting useful metadata cannot be overstated. Good metadata is complete and thorough, machine readable, and can be standardized and anatomized, as follows:

10.4.1 Complete and thorough

Valuable metadata is complete and thorough enough to provide sufficient context for the result. For instance, if a company does not collect metadata on a variable of interest (such as, *which* forklift was swabbed) they will not be able to conduct an effective root cause analysis.[3] This concern also can be found when examining historical facility data to identify important trends. If metadata on a key variable of interest (season, person conducting swabbing, product being manufactured) was not collected at a given time, then no analysis on that variable of interest can be performed.

10.4.2 Machine readability

Good metadata is machine readable, meaning that it can be analyzed within a software program. Of course, machine readable data will be in a digital form and not simply recorded as paper records.

Digital formats should also support analysis. For example, data recorded in a spreadsheet can be sorted and analyzed while data in a scanned or electronic document cannot.

10.4.3 Standardized and anatomized

Useful metadata should be standardized and anatomized, as shown in Figure 1. Standardization means the same term is always used to describe an object rather than using multiple terms for that object. For example, a company should not use the terms *drain, floor drain, and drain basket* interchangeably to describe the same item.

Anatomization means the data and metadata are organized in small, discrete units. In Figure 1, each column of metadata data is one discrete variable. This is preferable to having multiple facets of collected data in a single, open response field *(e.g., conveyor on line 2 collected on 2 May at 3 pm)*. Combined, unstructured responses increase the likelihood that a data collector will miss documenting key information. It also makes downstream data analysis more tedious as data must be "cleaned" before analysis can be performed.

Other changes relating to ways data is generated (e.g. sampling device or test method used) should also be clearly noted.

10.5 Data governance in environmental monitoring programs

Having a clear plan for data collection and storage is essential to success. This includes procedures across the data life cycle, from planning data collection and analysis, to storage and re-use, to managing long-term access.[4] Mature data management systems address each of these steps. However, data management systems used in the food and beverage industry have often focused only on the collection and storage aspects of this life cycle.

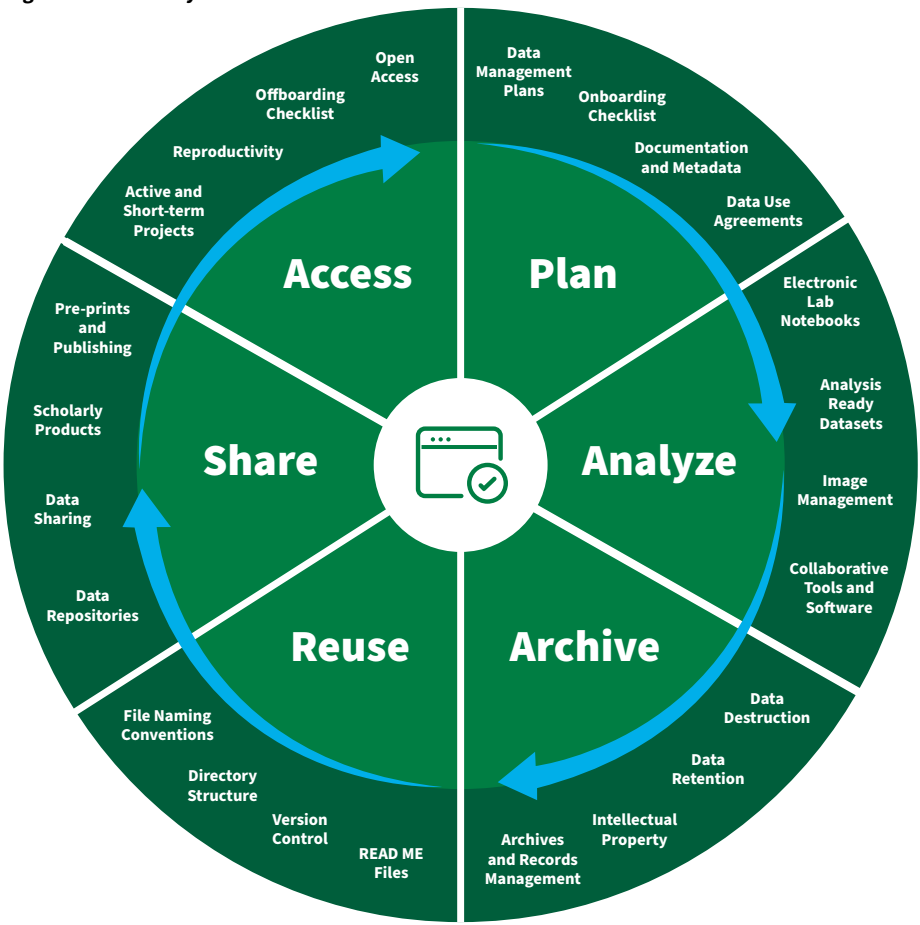
EMP data should be easy to communicate to stakeholders within the business. While a spreadsheet may be an improvement over paper records, it still requires the manual sending of an email with a link or attachment to enable others to access and use the data. It also assumes that the recipient is adept at sorting, filtering and pivoting data within that format. Instead, a database-driven data management system can enable the combination of metadata and results data to be formatted and can automatically trigger communication to specific individuals when access to data and the ability to take action are essential.

Companies often put significant efforts into generating large quantities of data and storing it in filing cabinets or on servers.

This approach to data management may support functions such as audit compliance. However, it limits the ability of a company to analyze and re-use the data to obtain further insights and gain much greater return on investment (ROI).

Therefore, increasing the maturity of a data governance system (Figure 2) can help increase ROI. Data governance simply refers to everything the company does (including programs, policies, practices) to ensure that data is stored securely, is accurate, and is usable and accessible later. For example, a very basic system of data governance is simple collection and storage of environmental monitoring results in a binder. A company receives a lab report and adds it to a binder stored in the office of the QA manager. In this system, data is retained and can be shown during audits or inspections to prove that environmental monitoring has been performed. However, the company is unlikely to get more ROI from this data because it is difficult to access and analyze. The company's ability to identify patterns and trends in this data is largely dependent on the memory and insight of the

Figure 2. Data lifecycle



QA manager who collects the results. The employee may remember that a particular cracked concrete slab has tested positive for *Listeria* spp. multiple times. But if they leave the company, that institutional knowledge is also gone. And the new person in that role is unlikely to spend hours reading back over those historical reports. Generally, this system is a relatively immature form of data governance and primarily supports one-off corrective actions; the company gets the results, makes a response, and moves on.

A more mature form of data governance would involve aggregating data into a software program that structures the data and metadata as described above (data is digitized, complete, standardized, anatomized). This digital log can be shown to auditors and inspectors but also enables analyses. Sites can track and trend results, easily quantifying their failure rates and seeing how their results change over time.

Also commercial software programs can be used to construct databases and some offer additional support features. For example, a program may prompt users to enter very specific types of metadata and users can often create graphs and tables from the company's data with very little effort. This is in sharp contrast to a company that uses a binder of printed lab reports from which no downstream analysis or visualization of data is possible.

10.6 Data analysis in environmental monitoring programs

Companies should also identify the types of downstream data analyses they plan to perform after they have captured high-quality, digitized data and metadata. Planning for future analyses can help ensure that necessary data attributes are being collected. Several approaches to downstream data analysis are discussed here, including summary statistics, visualization (by location, time, and type), and complex statistical models.

10.6.1 Summary statistics

Summary statistics are relatively simple calculations that track and trend results over time. To get the most value from summary statistics, a schedule to capture results and review them as a team should be established. For example, a company may schedule a quarterly meeting to view the percentage of environmental monitoring swabs that were positive for *Listeria*. During an annual program review, a company may identify their average ATP level on food contact surfaces and determine if the level is increasing or decreasing over time. With digitized data and metadata, calculating these types of results is simple while summary statistics support a more data-driven conversation (Figure 3).

Figure 3. Summary statistics

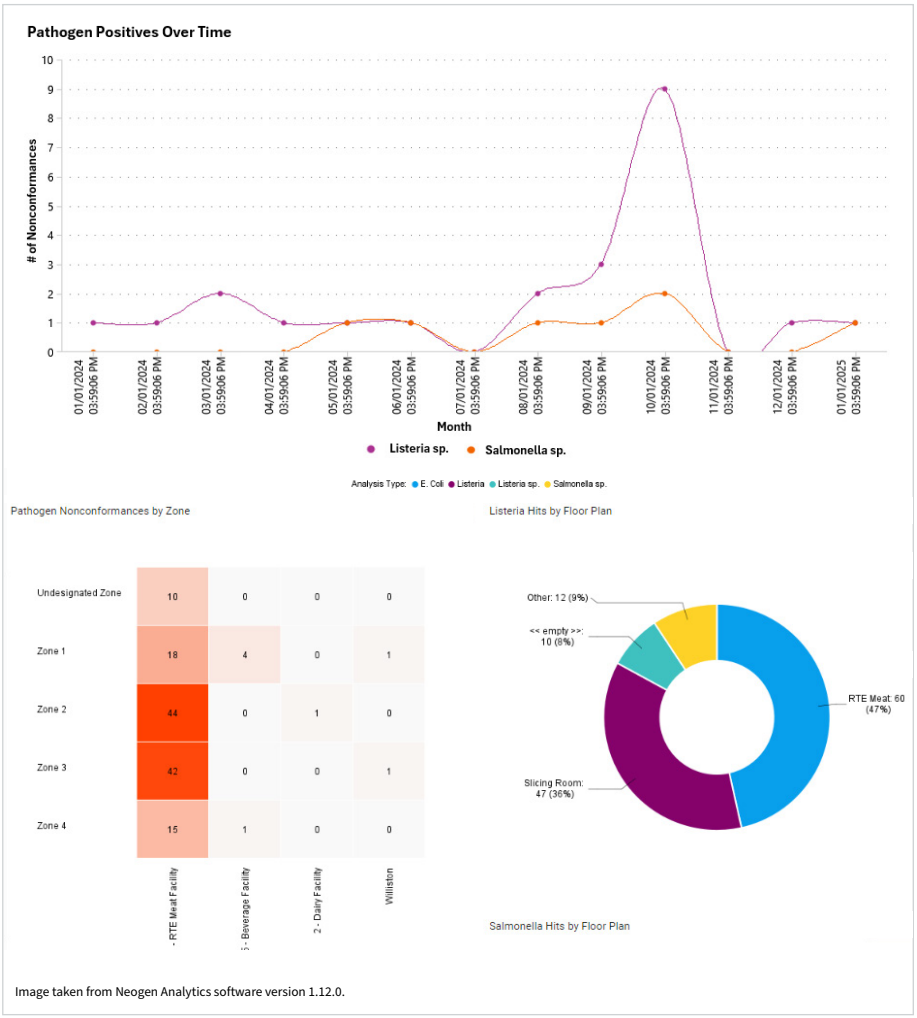


Image taken from Neogen Analytics software version 1.12.0.

10.6.2 Visualizations

Often, data is analyzed by visualization. Many companies use this approach (i.e., graphs, figures, schematics) as their primary output from data analysis to help identify trends and patterns. Visualizations can be based on a variety of features, if the company has good metadata describing those features. For example, data can be visualized or stratified by location, time or type. Figures 4–8 illustrate a series of visualizations and highlight the types of metadata necessary to construct these figures.

10.6.2.1 Visualization by location

Data can be stratified by location. For example, the bar graph in Figure 4 shows yeast and mold counts collected from air sampling plates at various places in the food manufacturing facility. Results are displayed based on the sampling location. In Figure 5, a heat map identifies sites of positive tests and shows them on a floor plan. Companies can use this method to identify problematic locations and help team members see trouble spots.

Figure 4. Example of environmental monitoring data visualization: Bar graph

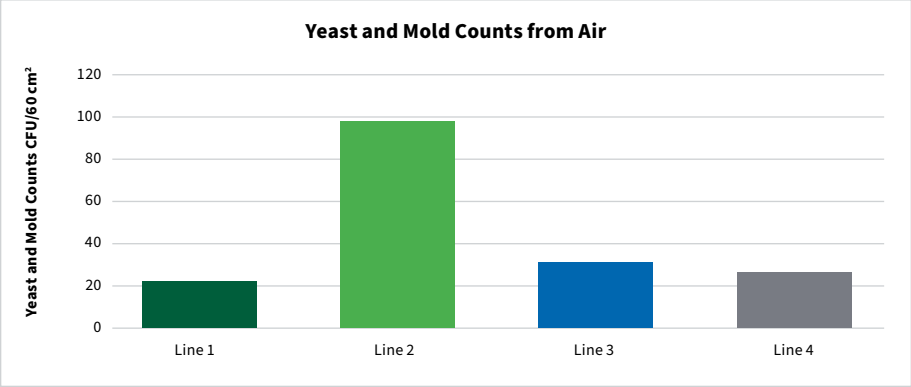


Figure 5. Example of environmental monitoring data visualization: Heat map

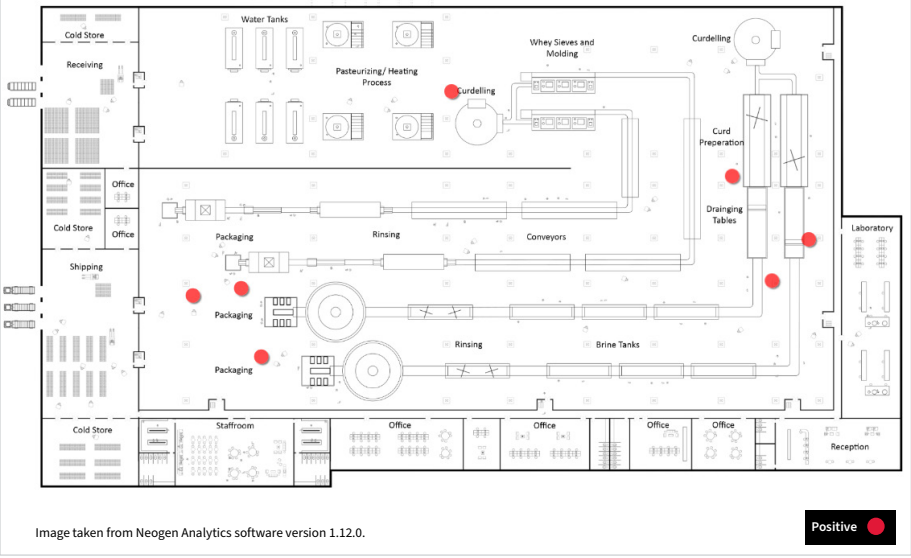
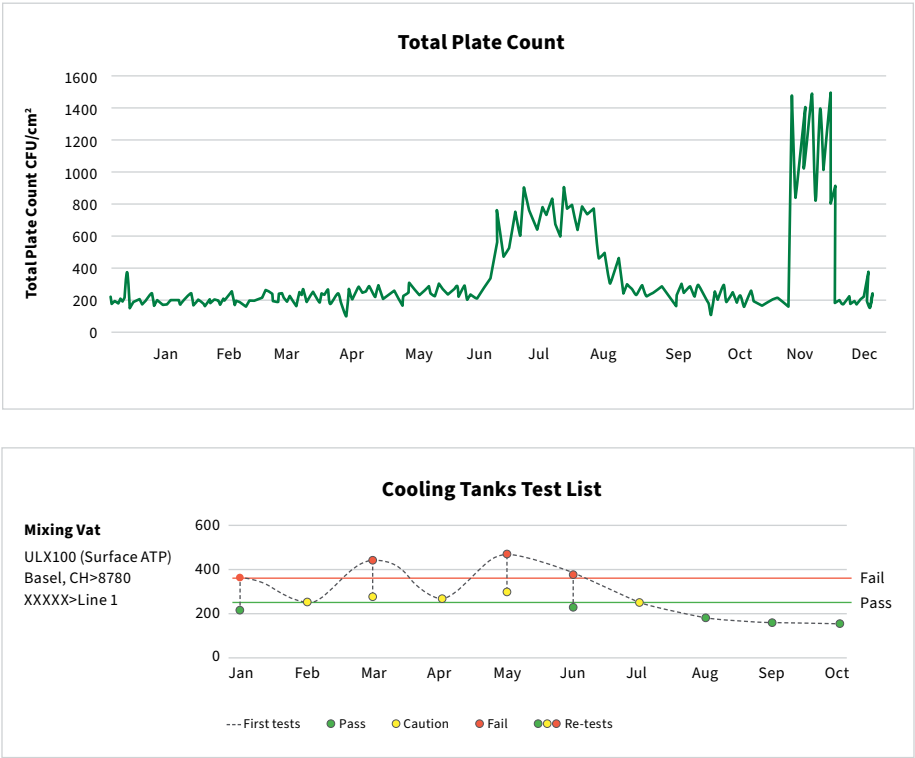


Image taken from Neogen Analytics software version 1.12.0.

10.6.2.2 Visualization over time

Time is another variable that can be used to visualize results. If metadata describes when samples were collected, then these data visualizations can be easily constructed. The first graph in Figure 6 shows Total Plate Count results over time, illustrating clear seasonal differences and highlighting when results increased above baseline Total Plate Count levels. The second graph in Figure 6 shows ATP results. Several different data lines are overlaid on this figure to highlight various trends (e.g., test results, results from re-tests following failures, thresholds for pass/fail). Using a multi-component figure can help team members quickly understand multiple aspects of a problem.

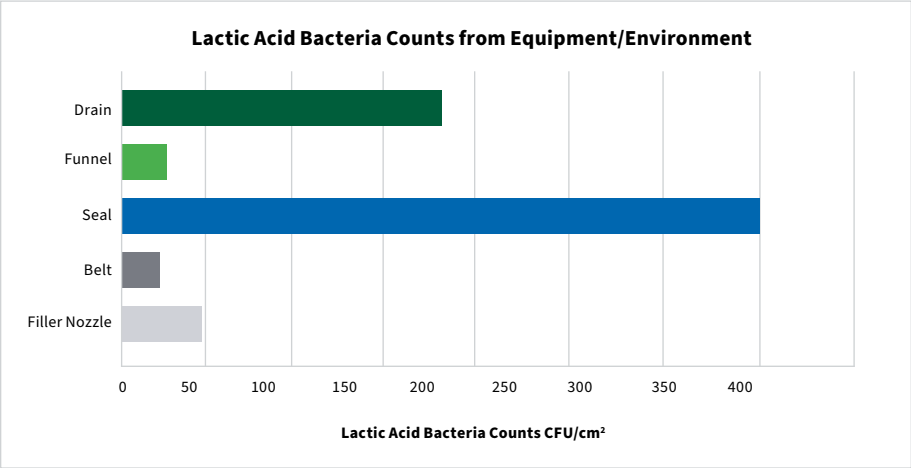
Figure 6. Example of environmental monitoring data visualization: over time



10.6.2.3 Visualization by type

Results can also be visualized based on their type or category. For example, attributes of the sample location may be of interest. Figure 7 shows the results for lactic acid bacteria counts grouped by the type of surface that was swabbed (e.g., drain, seal, nozzle, etc.). This type of analysis may help a company understand if a particular program (drain SSOP's, preventive maintenance for replacing equipment seals, captive shoe program) is effective or contributing to problematic environmental monitoring results.

Figure 7. Environmental monitoring data visualization: by location



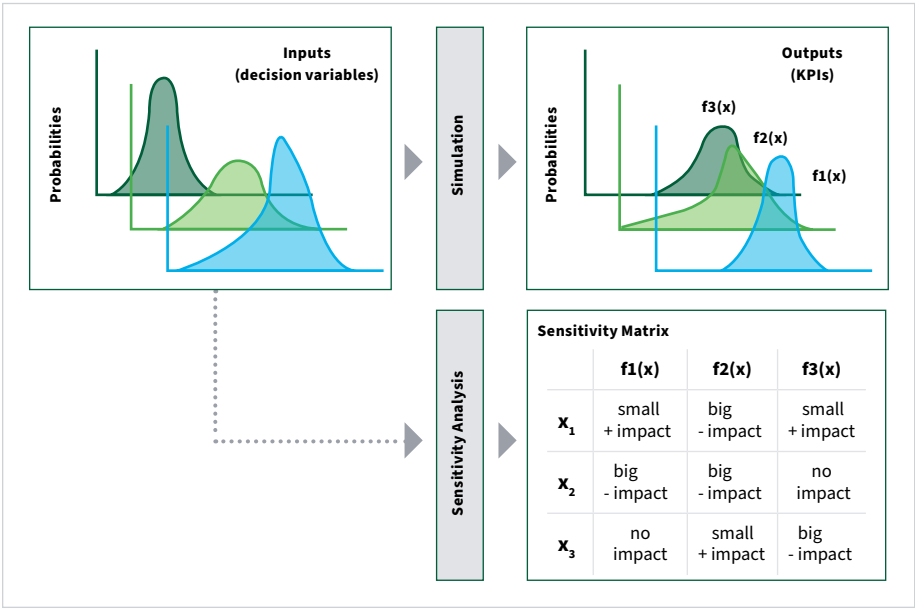
10.6.3 Complex statistical models

Another approach to downstream data analysis is complex statistical modeling (Figure 8). This tends to be employed by larger companies or in collaborations between academia and industry. These activities generally involve using historical data to build a predictive model that helps forecast future outcomes or assess the likelihood of future events.[5]

Examples include:

- Predicting microbial growth.
- Simulating microbial transmission patterns throughout a food facility.
- Identifying facility operations that have greatest impact on safety or quality.
- Quantifying the risk of product spoilage or scale of pathogen contamination.

Figure 8. Example of complex statistical modeling



10.7 Telling a story with data

Data management and analysis programs, no matter how capable, cannot interpret results and define company responses. The staff at a company must perform these tasks. As data collection and analysis become increasingly automated and results can be generated with a click of a mouse button, it will be even more important for companies to invest in the interpretative skills and communication work required to act on those results.

The food safety and quality staff primarily tasked with data collection and analysis are the first line to interpret results. These staff will take on some "mental load" in identifying the key learnings from an analysis of data. In contrast, a common mistake is assuming the data will "speak for itself." If the main takeaway message cannot be defined by the staff who collected the data and conducted the analysis, it is very unlikely that management will arrive at a meaningful conclusion when shown the results. Therefore, staff should avoid presenting an excessive number of figures simply because they are easy to generate. So-called "data dumping" can obscure the main message and, although staff may feel it shows how busy they have been generating figures and reports, it often has the opposite effect as it can appear disorganized and unproductive.

After the main learnings from the data have been identified, it is important to use good communication strategies to share the findings, define responses, and move people to act. Again, it cannot be assumed that simply by showing the data, the audience will understand and be motivated. Communication and storytelling techniques can be used to achieve these goals. For example, data communicators can make an emotional appeal by connecting the importance of their results to human experiences around foodborne illness. Or a data communicator could provide context for data that is difficult to understand by reporting in a way the audience can meaningfully interpret.

10.8 Getting started

Companies may start from different places in development of their data management systems and external experts are also resources for companies to consider. But regardless of their current status, they should work to identify small, clear steps that can improve their approaches to data collection, management, analysis, and communication.

Finally, development of good data management systems is an ongoing process. It is not the case that a company either has every aspect of highly developed programs in place or has nothing.

Learn more about environmental monitoring

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Connect with a Food Safety expert

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CHAPTER 11

From compliance to continuous improvement: balancing culture, FSMS, and HACCP for safer food

By

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The journey to achieving exceptional food safety involves more than regulatory compliance; it requires embedding food safety into an organization's culture, systems, and behaviors. This chapter explores the critical balance between food safety culture, Food Safety Management Systems (FSMS), and the principles of HACCP, illustrating how these elements work together to foster safer food production. By shifting from a reactive approach to a proactive mindset, organizations can move beyond compliance to achieve continuous improvement, reduce risks, and enhance consumer trust.

This chapter outlines the progression from basic compliance to advanced maturity in risk management, highlighting leadership behaviors, organizational practices, and technical tools at each stage. Through practical examples, case studies, and proven methodologies like Dr. John Butts' "Seek and Destroy" approach, readers can gain actionable insights into transforming their food safety practices to achieve operational resilience and industry leadership.

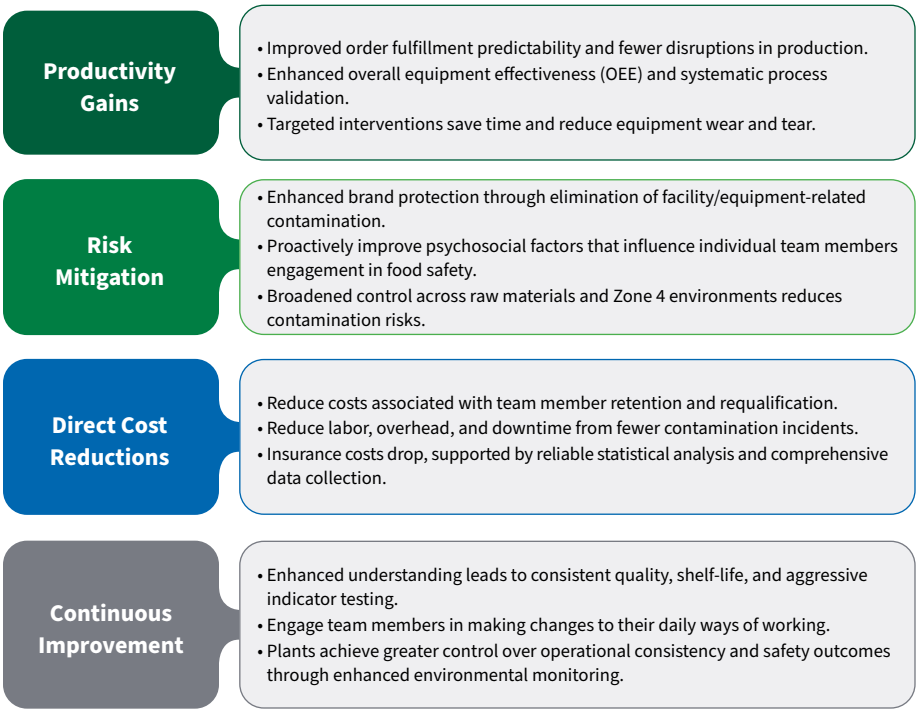
11.1 The case for balancing culture and systems in food safety

Achieving and maintaining hazard control is fundamental to forward-thinking food safety management. This chapter highlights how integrating biological, chemical, and physical hazard control with risk culture and environmental monitoring within a Food Safety Management framework can drive operational success. By proactively managing risks, organizations can go beyond compliance to foster continuous improvement, enhance productivity, and reduce costs.

The relationship between effective environmental monitoring programs and an organization's culture is more significant than most food safety practitioners and business leaders realize. Much angst can spread throughout a food company when positive results are detected through verification activities, especially in low-mature cultures at the Doubt and Awareness stages (Section 11.3) where food safety activities are largely completed by food safety professionals. Food safety in these stages is crisis-management-driven, with leaders stressing the importance of "doing things right" while conducting investigations that fail to get to root causes. The development of such effect-driven behaviors that wait for a crisis to engage operation professionals can be harmful to consumers, brands, and overall company financial performance.

Linking environmental monitoring programs to organizational and food safety culture is critical to connecting the organizational mission, strategy, values, and behaviors by teams as well as individuals.

Figure 1. Benefits from balancing culture and systems



The Global Food Safety Initiative (GFSI) has defined food safety culture as "A company's shared values, norms, and beliefs that affect mindsets and behaviors toward food safety in, across, and throughout the company".[1] The validated Cultivate Maturity Model breaks this definition into five dimensions and five stages of maturity.[2] Looking at the descriptors for the Internalized stage (the highest and ultimate stage of maturity) shows a reliance on, among other parameters, Zone 4 swabbing and process reliability to eradicate and control organisms. In other words, this is a culture that believes in keeping organisms far away from food products and has a mindset that investing in re-designing equipment and infrastructure is an important and ongoing activity.

No organization can get to the Internalized stage without understanding the multi-dimensional aspect of a culture of food safety. To illustrate this, Table 1 shows a set of system elements and behaviors relating to environmental monitoring (EM) that may assist in connecting systems and culture as expressed through leader and team member behaviors. It is important to note that no two cultures are the same and that, unlike the scientific expertise that many rely on to design effective environmental programs, experts may have to be engaged to help build a plan specific to an organization.

Table 1. Cultural dimensions, system elements, and environmental monitoring behaviors

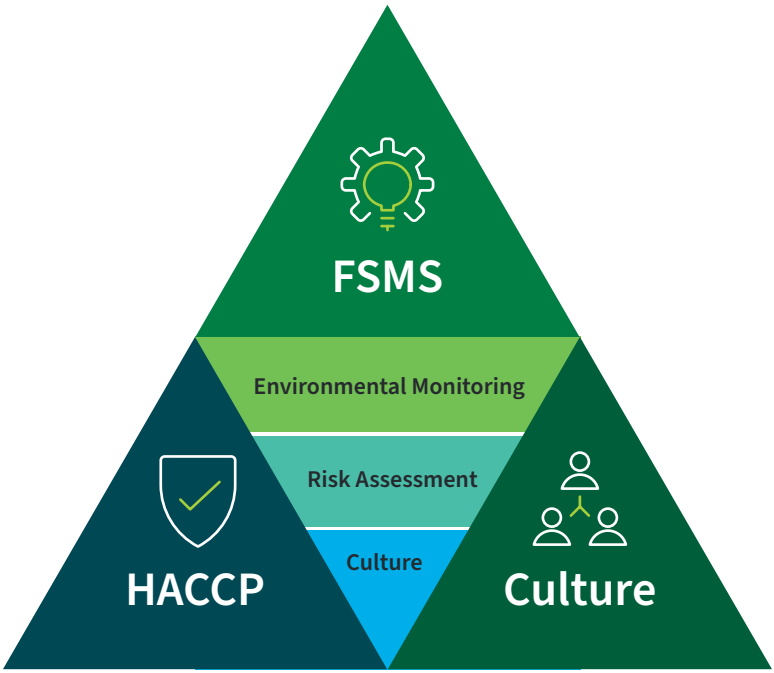
Cultural Dimension[3]	EM System Elements (examples)	EM Behaviors (examples)
Values and Mission Focus areas: • Trust • Leadership messaging • Resources	• Integrate EM into company/ plant/business strategic and operational cycle • Enable all leaders to message EM	Leaders of all functions actively ask questions about food safety and EM in strategy and budget discussions. Leaders of all functions integrate food safety and EM messages in their ongoing communications.
People Focus areas: • Recognize and Motivate • Effective Communication • Competency	• Food safety education for everyone: "Put a swab in everybody's hands…" • Multidisciplinary team	All employees are expected to take company food safety education as part of their role-specific competencies. All EM insights — good and bad — are investigated by teams from multiple functions.
Adaptability Focus areas: • Problem Solving • Change Management • Innovation	• Carrot vs. the stick • Root causes • Process and product design	Team leaders use indicator sites and positive consequences (e.g., reward findings), resulting in problem prevention and continuous improvement, which builds trust in the food safety process.
Consistency Focus areas: • Feedback • Integration • Accountability	• Communication rhythm • Insights driven by EM data • Roles and responsibilities	Leaders design food safety and EM into the company rhythm (i.e., board discussions, leadership meetings, plant huddles, and frontline team discussions). EM data are integrated into the company's business intelligence (BI) solution and insights discussed from board-room to frontline.
Risks Focus areas: • Application • Sensemaking • Physical conditions	• EM pictures and stories • HACCP team narratives • Facility and equipment design	Technical team members generate ongoing messages and stories for others to use in team member onboarding and engagement.

11.2 Building a comprehensive food safety framework: integrating risk culture, FSMS, and environmental monitoring

Food safety management has transitioned from narrowly addressing microbial process control to adopting a more comprehensive approach. This new holistic approach integrates risk assessment, environmental monitoring, and culture (Figure 2) into Food Safety Management Systems (FSMS) — which includes but is not limited to HACCP. In this context, risk culture refers to the values, beliefs, and behaviors that **shape how an organization identifies and responds to risks**. FSMS and environmental monitoring work most effectively when they are part of an organizational culture that actively engages all levels in understanding and managing food safety risks.

In addition, this evolution emphasizes proactive strategies to manage food safety risks.

Figure 2. Key elements that must be balanced and integrated to optimize food safety performance



The following describes the three elements in more detail:

Characteristics of a culture that delivers safer food

A mature risk culture is a cornerstone of modern food safety management. It reflects the values, beliefs, and behaviors within an organization that drive the identification, assessment, and management of risks. Embedding risk culture throughout the organization **fosters collective responsibility and continuous improvement**, ensuring food safety practices are sustainable and dynamic. A **forward-thinking risk culture transforms compliance from a reactive task into a proactive mindset**, empowering organizations to anticipate and mitigate potential risks before they manifest.

FSMS and Environmental monitoring

Environmental monitoring is essential for effective risk assessment and hazard control, serving as a **foundation for identification and management of microbial and chemical risks**. By continuously monitoring critical areas for pathogens, allergens, and other microbial and chemical residues, organizations **can use comprehensive data to assess the effectiveness of their control measures** and ensure the safety of final products. Environmental monitoring supports the principles of HACCP (Hazard Analysis and Critical Control Points) as discussed in the following paragraph.

HACCP and Risk Assessment

Integrating environmental monitoring into a robust risk assessment framework strengthens food safety by providing **data-driven insights**. Regular risk assessments informed by environmental monitoring help organizations **anticipate potential hazards, establish critical limits based on real-world data, and evaluate the effectiveness of preventive measures**. This proactive approach not only addresses current risks but also prepares the organization for **emerging challenges** in food safety.

By fostering a mature food safety and risk culture, employing rigorous risk assessments, and maintaining robust environmental monitoring programs, organizations can effectively:

1. Improve hazard identification.
2. Enhance hazard control.
3. Improve their ability to predict and prevent contamination events.
4. Engage all levels of the organization in the shared responsibility for food safety.

Together, these elements promote a resilient and effective food safety system and ultimately can provide consumers with safer foods.

11.3 Evaluating an organization's cultural maturity

As discussed in the Risk Culture Whitepaper from Cultivate SA, food safety success relates to technical compliance and fostering a proactive risk culture.[4] FSMS and environmental monitoring work most effectively when they are balanced with organizational culture. A mature culture actively engages all levels in understanding and managing food safety risks.

- **Engagement at all levels:** A mature risk culture fosters accountability and shared responsibility across the organization, embedding food safety into daily operations and leaders standard work.
- **Sustainable Food Safety Practices:** This integrated framework encourages continuous improvement and resilience in addressing both known and emerging risks.

By integrating culture, FSMS and Environmental monitoring and environmental monitoring with HACCP and Risk Assessments an organization can better anticipate and manage complex food safety risks, fostering trust among members of the organization, and ensuring long-term success in delivering safe high-quality food products.

The effectiveness of Food Safety Management Systems (FSMS) depends on progress in an organization through five stages of cultural and operational maturity. As an organization progresses through the following stages, namely Doubt, React, Know, Predict, and Internalize, it can build stronger connections between FSMS and proactive risk management, improving their capacity to proactively manage risks.

Stage 1 — Doubt

At this initial stage, an organization primarily views risk management activities, such as environmental monitoring and FSMS implementation, as regulatory compliance obligations rather than valuable safety tools. Risks or associated costs may be underestimated or dismissed, and FSMS practices, such as hazard analysis or corrective actions, may be limited or not fully integrated, reflecting a lack of appreciation for the broader benefits of proactive risk management.

Stage 2 — React

With growing awareness, environmental monitoring begins to reveal recurring hazards, and systematic risk assessments take shape. Data highlights vulnerabilities in an organization's processes, making risk assessments more meaningful by exposing the true extent of risks. However, the absence of a strong risk culture often leads to reactive responses rather than proactive measures. Leadership starts to recognize the need for FSMS tools, such as documented procedures and preliminary action plans, but these efforts tend to address issues only after they arise rather than preventing them before they occur.

Stage 3 — Know

As an organization matures, environmental monitoring and risk management become integral components of its operations. Strategies align to identify specific risk factors, such as contamination sources or growth niches, and systematically address pathways. FSMS provides structure through routine audits and training programs, supporting the implementation of targeted preventive measures. A developing risk culture fosters prevention-oriented thinking, encouraging thoughtful and strategic approaches to managing risks before they escalate, laying the groundwork for long-term improvements.

Stage 4 — Predict

With a more mature risk culture, FSMS drives proactive measures, such as robust preventive controls and employee engagement across all levels. Environmental monitoring and FSMS principles work together seamlessly to prevent contamination, sustained by clear communication, timely corrective actions, and consistent management reviews.

At this stage, environmental monitoring data is actively monitored. A fully embedded and robust risk management principles work cohesively to prevent contamination and other hazards before they occur. A mature risk culture ensures employees at all levels actively participate in risk assessments, corrective actions, and continuous improvement, with all stakeholders — from leadership efforts. Preventive thinking becomes ingrained in daily operations, reducing the likelihood of incidents.

Stage 5 — Internalize

At the highest level of maturity, FSMS integrates advanced tools such as statistical analysis and predictive modeling to identify and mitigate risks before they escalate. Environmental monitoring data and robust risk management principles work cohesively to prevent contamination and other hazards. A fully developed risk culture ensures that employees at all levels — from leadership to line workers — actively participate in risk assessments, corrective actions, and continuous improvement efforts. Preventive thinking becomes embedded in daily operations, transitioning the organization from a reactive to a predictive approach. This proactive mindset enables the organization to adapt to emerging challenges while maintaining a resilient and sustainable food safety system.

11.4 Leadership and behavior: driving food safety at every level

Building on the structured framework of food safety evolution discussed in the previous sections, this section delves further into the human element that plays a critical role in embedding advanced risk management systems into daily operations. The focus lies in aligning individual and collective behaviors with cultural maturity stages to drive lasting progress toward food safety excellence.

By examining the interplay between organizational culture, leadership, and process control at each stage, this section highlights the importance of fostering a proactive workforce and provides a roadmap for organizations striving to achieve food safety excellence through continuous improvement and frontline ownership of risk management practices.

Stage 1 — Doubt: Basic awareness and compliance

At this foundational stage, organizations are beginning their journey toward effective risk management. The focus is primarily on meeting regulatory requirements, with little understanding of the ways a risk culture can influence food safety. Leaders and employees may view food safety as a cost rather than a critical part of organizational success. This stage lays the groundwork for future progress by introducing the concept of food safety protocols and compliance as essential to operational integrity.

Norm	Little to no knowledge about the connection between organizational culture and food safety. Focus in on meeting basic regulatory requirements.
CEO Behavior	Begins to learn about the importance of food safety and microbial control, emphasizing the need for regulatory compliance.
Frontline Supervisor Behavior	Starts to communicate the importance of following established food safety protocols.
Food Safety Manager Behavior	Assesses the current state of food safety knowledge within the organization and develops a plan for improvement.
Impact on Process Control	**Limited impact**, as the focus is primarily on meeting basic regulatory requirements.

Stage 2 — React: Strengthening technical knowledge

In the second stage, an organization begins to strengthen its technical knowledge of food safety practices. However, this knowledge has not yet been fully embraced or embedded into leadership and frontline behaviors. Progress is made as employees and leaders recognize the importance of food safety, but the organization remains reactive, addressing issues as they arise rather than preventing them proactively.

Norm	Strong technical knowledge in food safety practices, but not yet fully embedded in CEO and supervisory behaviors.
CEO Behavior	Develops a deeper understanding of the role of organizational culture in promoting food safety, highlighting its importance in company-wide communications.
Frontline Supervisor Behavior	Implements food safety best practices within teams, encouraging collaboration to identify and address potential issues.
Food Safety Manager Behavior	Ensures that training programs reflect current industry knowledge and regulatory requirements, delivers comprehensive training to employees at all levels.
Impact on Process Control	**Moderate impact**, as stronger technical knowledge leads to better risk identification and implementation of best practices. However, process control may still be primarily driven by regulatory requirements.

Stage 3 — Know: Cross-functional integration

This stage marks a significant shift as organizations embrace cross-functional collaboration and deep knowledge-sharing. Risk management practices are integrated across all levels, fostering alignment between food safety initiatives and overall business objectives. Leaders actively promote a culture of food safety, and employees begin to take ownership of their roles in maintaining and improving food safety processes.

Norm	Deep knowledge of food safety practices is shared and integrated across all roles within the organization.
CEO Behavior	Actively promotes a culture of food safety through consistent communication and engagement with employees at all levels.
Frontline Supervisor Behavior	Supports employee development by fostering a learning environment and facilitating access to training resources, ensuring continuous improvement.
Food Safety Manager Behavior	Collaborates with leaders across the organization to align food safety practices with overall business objectives.
Impact on Process Control	**Significant impact**, as deep knowledge is integrated across all roles, leading to a more cohesive approach to process control and continuous improvement.

Stage 4 — Predict: Continuous improvement

At this stage, organizations prioritize continuous improvement, embedding proactive risk management into their culture. Leaders and employees work together to enhance food safety measures, leveraging data, innovation, and collaboration. This stage represents a shift from reactive and preventive approaches to predictive strategies that anticipate risks and mitigate them before they occur.

Norm	The organization prioritizes a culture of continuous improvement in food safety practices.
CEO Behavior	Leads by example in fostering an environment of ongoing learning and improvement, encouraging innovative ideas to enhance food safety measures.
Frontline Supervisor Behavior	Actively engages with employees to identify areas for improvement, sharing best practices and promoting a proactive approach to risk management.
Food Safety Manager Behavior	Develops and implements comprehensive risk management plans, incorporating regular assessments, corrective actions, and ongoing monitoring to ensure continuous risk mitigation.
Impact on Process Control	**High impact**, as the organization prioritizes continuous improvement in food safety practices, leading to better risk management and proactive process control strategies.

Stage 5 — Internalize: Frontline ownership

The final stage represents the pinnacle of risk culture maturity, where frontline employees take ownership of food safety practices. Leadership drives innovation and fosters industry-wide collaboration, positioning the organization as a leader in food safety. Employees at all levels actively contribute to continuous improvement, ensuring that food safety is not only maintained but advanced through cutting-edge practices and shared accountability.

Norm	Frontline employees take ownership of food safety practices, contributing to industry leadership and innovation.
CEO Behavior	Drives a culture of innovation and knowledge-sharing, positioning the organization as an industry leader in food safety practices.
Frontline Supervisor Behavior	Encourages innovation within teams, empowering employees to take ownership of food safety initiatives and share best practices with other industry professionals.
Food Safety Manager Behavior	Engages with industry networks, actively contributing to the development of new food safety practices and ensuring the organization remains at the forefront of industry innovation.
Impact on Process Control	**Highest impact**, as frontline employees take ownership of food safety practices, driving innovation and contributing to industry leadership in process control strategies.

11.5 "Balance and Integration in Action: The Seek and Destroy Method" told by Dr. John Butts

Dr. John Butts' Seek and Destroy method, published in the Journal of Food Protection and illustrated in Chapter 8, is a strategic approach aimed at preventing contamination in food production facilities.[5] This method emphasizes the importance of aggressive environmental testing and control through a systematic process. The Seek and Destroy method consists of three primary stages: seek, destroy, and verify.

Seek: In this phase, rigorous and aggressive environmental testing is conducted to identify and locate potential sources of contamination, such as harborage sites for pathogens. The process involves a thorough examination of the facility, with a focus on areas that may contribute to food safety risks. This stage highlights the significance of attention to detail and meticulousness in detecting potential hazards.

Destroy: Once the potential sources of contamination have been identified, targeted sanitation and cleaning processes are employed to eliminate harborage sites. This phase accentuates the necessity of adopting effective sanitation practices and customized strategies to combat specific contamination issues. By eradicating these sites, the facility can significantly reduce the risk of food safety incidents.

Verify: The final stage of the process involves validating the effectiveness of the implemented sanitation measures through follow-up testing and monitoring. This step ensures that the cleaning processes have successfully eliminated the identified contamination sources, thereby maintaining the facility's dedication to continuous improvement in food safety practices.

At the core of the Seek and Destroy method lies a cross-functional approach that encourages collaboration among different departments within a food production facility. This collaborative mindset fosters a more unified and efficient response to potential food safety risks, promoting a mature food safety culture. By working together, teams can share knowledge and resources to ensure the facility remains a safe and hygienic environment for food production.

Despite its benefits, Dr. Butts recognizes that implementing the Seek and Destroy method may present certain challenges for organizations. These challenges can include limited resources, resistance to change, and a lack of understanding of the significance of environmental testing. To overcome these obstacles, Dr. Butts offers practical tips and advice:

Leadership commitment: Gaining the support of senior management is essential in prioritizing food safety initiatives and allocating the necessary resources for their implementation. Management should actively partake in promoting food safety practices and serve as role models for other employees. Through their leadership, they can create a culture that values and prioritizes food safety.

Learning and development: It is crucial that all employees, from entry-level workers to senior management, comprehend the importance of food safety practices and the role of environmental testing in preventing contamination. Comprehensive training programs and ongoing education can help instill a shared

commitment to food safety across the organization. By promoting awareness, employees become more vigilant in identifying potential risks and contribute to maintaining a safe working environment.

Effective communication: Encouraging open communication among employees and departments can cultivate an environment where information is readily shared, and best practices are adopted. This collaborative atmosphere can help organizations tackle food safety challenges and drive continuous improvement. When team members feel comfortable sharing their concerns and ideas, they contribute to the overall success of the facility's food safety efforts.

By adopting the Seek and Destroy method and following Dr. Butts' practical tips, organizations can effectively promote cross-functionality and cultivate a mature food safety culture. This approach can ultimately result in the production of safe, high-quality food products and reduce the risk of foodborne illness outbreaks. In an industry where consumer trust is paramount, prioritizing food safety is not only an ethical responsibility but also a key factor in ensuring long-term success.

11.6 Taking immediate action: a roadmap to improvement

1. Adopt digital tools for real-time monitoring and trend analysis.
2. Conduct regular learning and development events for everyone, including senior leaders.
3. Start a pilot environmental monitoring program focusing on high-risk areas.
4. Develop cross-functional teams to review and refine HACCP plans, including psychosocial factors.
5. Leverage the tools in this chapter to evaluate where your organization stands in its food safety and risk culture maturity journey.

CASE STUDY

Transforming food safety practices

A medium-sized food manufacturer faced persistent contamination events despite adhering to basic HACCP and pre-requisite procedures. The company realized that its approach was reactive and heavily focused on compliance rather than prevention. To address this, the company's leadership decided to adopt a more proactive food safety strategy by implementing a robust environmental monitoring program and investing in the development of a mature risk culture.

Steps taken

1	**Culture Development** The organization focused on training programs for all employees, fostering a culture where food safety was prioritized over production speed. Leadership actively participated in audits and promoted open communication across departments.
2	**Environmental Monitoring** The organization identified high-risk zones (e.g., Zone 1 and transfer areas) and began systematic swabbing and testing for microbial pathogens and allergen residues.
3	**HACCP Refinement** The data collected through environmental monitoring informed updates to the company's HACCP plans, enabling better identification and management of critical control points.

Outcome

Within a year, contamination events dropped by 80%, and the company gained recognition as an industry leader in food safety. The transformation demonstrated the power of integrating environmental monitoring, HACCP, and risk culture maturity. By engaging all levels of the organization, the company achieved long-term improvements in food safety and operational resilience.

This story is a testament to the principles outlined by Dr. John Butts (2018[6], 2022[7]) in his Seek and Destroy methodology and aligns with Jespersen's (2023)[4] insights on cultivating a proactive risk culture. By combining these frameworks, the manufacturer not only resolved immediate issues but also positioned itself for sustained success in food safety management.

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Scott Egan is the technical services manager for Neogen Asia Pacific region. In this role, he keeps abreast of local regulations, emerging trends and industry best practices to help support the local industry while also providing expert support for Neogen's portfolio of food safety products. His undergraduate degree in biomedical science was received from the University of Western Sydney, and he has previously worked in pathology and industrial microbiology laboratories and held quality control, research and development and process improvement roles for the manufacture of diagnostic assays.

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Michele Fontanot is a professional consultant services manager for Latin America at Neogen. Based in Peru, Fontanot leverages her expertise on food safety to assist customers in the region working to implement Neogen products and technologies in ways that effectively meet their processing and manufacturing needs aligned to international regulations and standards. She has earned a bachelor's degree from Cayetano Heredia University in Lima and a Master of Science from the University of Chile in Santiago.

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Gabriela Lopez Velasco, Ph.D.

Neogen

Dr. Gabriela Lopez-Velasco, based in St. Paul, Minn., has been part of Neogen since 2015. She works with the research and development team as a Senior Microbiology Research Lead, where she works in the development of diagnostics methods for food testing with expertise in pathogen detection. Before her current position, she was part of the global application engineer team where she served as subject matter expert in microbiology and allergen testing platforms providing technical expertise and education in food safety diagnostics.

Gabriela has a degree in Food Chemistry and a masters degree in Biochemistry from the National Autonomous University of Mexico. She obtained a doctoral degree in Food Science and Technology from Virginia Polytechnic Institute and State University. And worked at the postharvest research center in UC Davis as a postdoctoral researcher.

Ana Lozano

Neogen

Ana Lozano is the Technical Services and Specialist Manager for Neogen's Food Safety Division in Canada. She has over 20 years of experience across various food industry sectors. Her extensive background in quality control, quality assurance, and laboratory supervision allows her to understand professionals' challenges. Ana's customer-focused approach strengthens client relationships and fosters team collaboration, ensuring continued growth and success.

She actively shares her knowledge as a guest speaker at industry events and holds an MSc in Food Safety Quality Assurance and a BSc in Microbiology. Ana's combination of technical expertise and dedication to customer success distinguishes her as a leader in the food safety industry.

Louise Roberts

Alimenti Food Sciences Ltd

Louise Roberts is the founder of Alimenti Food Sciences, a trusted authority in food safety, compliance, and sustainability. With extensive experience in the food manufacturing sector, including work for Tesco and Sodexo, amongst others. Louise has made it her mission to empower businesses to implement and maintain the highest standards of quality and safety. Through her work at Alimenti Food Sciences and her blog at, Alimenti.co.uk, Louise continues to advocate for a more sustainable future in food production, bridging the gap between compliance and environmental responsibility.

Abigail Snyder, Ph.D.

Cornell University

Dr. Abby Snyder is an associate professor of microbial food safety at Cornell University. She received her BS from The Ohio State University and her PhD from Cornell University. She directs a research lab which investigates how foodborne microbes persist under hostile environmental stresses. This work falls into three major themes: 1) microbial survival on inanimate surfaces, 2) pathogen persistence in low moisture food systems, and 3) microbial extreme tolerance. One of the longterm goals of this work is reduce the risk of cross-contamination from surfaces in food manufacturing environments into food. As part of these efforts, she has ongoing research projects in sanitation tool development and novel approaches to environmental monitoring.

Kelly Stevens, Ph.D.

General Mills

Dr. Kelly Stevens is the senior manager for the Global Operations Food Safety and Regulatory team at General Mills. She is accountable for global food safety and regulatory compliance, is a FSPCA Trainer of Trainers and served as General Mills' FSMA implementation team lead. Stevens joined General Mills in 2004 and has held varying roles in the quality organization, including three field assignments: quality engineering and quality manager, manager of microbiology and quality manager of premiums, licensing and sampling activities. She received M.S. and Ph.D. degrees in food science from North Carolina State University.

Alec Teagarden

Neogen

Alec Teagarden is highly skilled in microbiology, product development and the application of cutting-edge methods for food testing. He received a Bachelor of Science in Food Science Technology from Minnesota State University, Mankato. As a product owner, Alec plays a pivotal role in developing and implementing Petrifilm laboratory practices, automation, and data solutions. His work is instrumental in simplifying and enhancing food testing processes, ultimately contributing to safer and higher-quality food products in the market.

Aljoša Trmčić

Cornell University

Dr. Aljoša (Al) Trmčić is a Senior Extension Associate in the Dairy Foods Extension Program at Cornell University. He earned a joint Ph.D. in Food Science and Biotechnology from the University of Ljubljana and AgroParisTech. With a strong background in dairy microbiology, Al works closely with the food industry, providing expertise in environmental monitoring programs and food safety. His research focuses on controlling dairy-associated spoilage microorganisms and pathogens, integrating environmental monitoring strategies and predictive modeling tools to enhance food safety and quality.

Martin Wiedmann, Dr. Med. Vet, Ph.D.

Cornell University

Dr. Martin Wiedmann is the Gellert Family Professor in Food Safety at Cornell University's College of Agriculture and Life Sciences. With training as both a veterinarian and food scientist, Wiedmann's academic programs emphasize a comprehensive and interdisciplinary farm-to-table approach to food safety. His research focuses on the transmission and systems biology of bacterial foodborne pathogens and spoilage organisms, and involves the application of a variety of disciplines and collaboration across multiple institutions. He received his veterinary degree and doctorate in veterinary medicine from the Ludwig Maximilian University of Munich, and his Ph.D. in food science from Cornell University.

Randy Worobo, Ph.D.

Cornell University

Dr. Randy Worobo is a professor of food microbiology at the Cornell University College of Agriculture and Life Sciences. The primary objective of his research is alternative approaches to enhance the safety and quality of food, and includes microbial spoilage of foods and beverages, non-thermal processing alternatives for juice and beverages, as well as pathogen transmission and survival on fruits and vegetables. Worobo also engages with the industry through his extensive outreach program, which is accomplished through workshops, conferences and direct contact with food processors around the world. He received his Ph.D. from the University of Alberta.

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