Proceedings of 2019 IFSH/FRI Joint Symposium: Managing Microbiological Testing as a Preventive Control Verification

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SUMMARY

In 2015, the U.S. Food and Drug Administration (FDA) published the final rule for Current Good Manufacturing Practice, Hazard Analysis, and Risk-based Preventive Controls for Human Food (PCHF). Food manufacturers are now required to comply with this regulation and others related to the Food Safety Modernization Act (FSMA). In the transition from the former "reactive" stance to the more "proactive" approach to food safety that FSMA has prescribed, how should food manufacturers use microbiological testing within food safety programs? The flexibility built into the preventive controls regulations shifts responsibility from regulators to manufacturers, who must decide when, where, and how to utilize microbiological testing. Although numerous helpful resources are available, simple, cookbook-style instructions for conducting food safety-related microbiological testing do not exist, challenging manufacturers and sometimes regulators. Among the organizations from which food manufacturers have sought assistance are the Institute for Food Safety and Health (IFSH) and the Food Research Institute (FRI) of the University of Wisconsin-Madison. Recognizing an unmet need, these groups co-hosted a meeting on October 24, 2019, at which food manufacturers, regulators, scientists, and others shared knowledge and resources while discussing the best uses of microbiological testing in managing food safety under the FSMA.

OVERVIEW

Robert Brackett (Director of IFSH) provided background for the meeting in his introductory remarks. Among the most confusing elements of the preventive controls (PC) rule for food manufacturers are the validation and verification requirements, especially when microbiological testing is required. Ready-to-eat (RTE) foods, in particular, may require microbiological testing to be part of their preventive controls verification strategies, because such foods will not receive a subsequent "kill step" (e.g., cooking) by the consumer before the foods are eaten. The goal of the meeting, as explained by Brackett, was to discuss how microbiological testing could most effectively be used to verify preventive controls.

The need for microbiological testing under the preventive controls rule

Fittingly, the first main presenter of the day was the technical lead for the FDA's PCHF rule. Jenny Scott *(Fig. 1)* reminded the audience that the underlying premise for both HACCP and FDA's preventive controls rule is that prevention of hazards is more effective than pathogen testing for ensuring food safety. Microbiological testing is inherently "probabilistic" in nature: you cannot prove that a pathogen is not present unless you test the entire lot of food. As discussed by Scott and echoed by numerous other speakers throughout the day, testing of finished product is therefore of limited usefulness in proving lot or batch safety; you cannot test the entire lot and still have product left to sell.

Figure 1. Meeting speakers Jenny Scott and Robert Buchanan.

Microbiological testing is still important, however, even if the built-in flexibility of FSMA's rules means that no particular tests are explicitly required. Under the PCHF rule, a facility that has identified hazards requiring a preventive control must verify that the preventive controls (PCs) are effective at significantly minimizing or preventing a hazard. If that hazard is a microbial pathogen, microbiological testing of finished product may be used initially to validate a preventive control.

Microbiological testing can also be used by the manufacturer to demonstrate that a particular preventive control is being implemented effectively and consistently on an ongoing basis.

Different types of preventive controls (and related FSMA requirements) can be verified by microbiological testing. Microbiological testing can provide evidence that an antimicrobial process such as high-pressure processing has been correctly performed (a process preventive control). It is also important for allowing customers (food manufacturers or retail buyers) to know that the ingredient or finished product they are purchasing meets certain specifications that fulfill their supply chain preventive controls and verification requirements. It is also critical for verifying sanitation controls and useful for showing that current Good Manufacturing Practices have been achieved.

A key but subtle distinction between "testing to verify that a preventive control is working" versus "testing to establish lot acceptability" lies in the rationale for the microbiological testing. Are you testing to ensure that your systems to control microbiological hazards are working correctly, or to ensure that there are no pathogens present in a small, presumably representative, sample of the final product? Because this distinction is often missed, considerable confusion exists among food manufacturers and others. To help clarify these issues for food manufacturers and regulators, Scott presented a preliminary list of basic principles to help guide the use of microbiological testing when manufacturing an RTE food *(Fig. 2)*. Guided by these draft principles, Scott also discussed draft criteria that RTE food manufacturers can use to help them decide whether, or how often, they should perform microbiological testing *(Fig. 3)*.

Jenny Scott described how these preliminary principles and criteria could be used by food manufacturers to design microbiological testing strategies for different types of RTE foods. Some of these principles were used in the group exercise discussed later. When faced with the final question as to whether or not a firm should test finished products, Scott said, "It depends." Microbiological testing may be necessary at the ingredient stage or for the end product (where such testing can demonstrate the successful application of controls). There is not always a right or a wrong answer, as different facilities making the same product may come up with different answers about whether or not to test (and both could be correct).

Verification vs. validation, one more time

Robert Buchanan reviewed the regulatory definition of verification found in the preventive controls for human foods rule that pertains directly to microbiological testing *(Fig. 4)*.

1. Microbiological testing should be risk-based.
2. Microbiological testing for verification of process control is different from microbiological testing for lot acceptance.
3. Microbiological testing is most useful when
   - ingredients in a food have the potential to contain pathogens and there is no (or a marginal) kill step in the manufacture of the finished product, and/or
   - finished products have the potential to be contaminated from the environment.
4. Microbiological testing should be increased when information indicates that the operation is not under control.
5. A facility should consider the nature and extent of supplier control programs for ingredients and environmental monitoring programs in the facility in determining the role of finished product testing to verify control measures in the facility.
6. Sampling small amounts of product more frequently provides better information about process control than taking a larger sample equivalent in weight to the sum of the weight of the smaller samples.

Figure 2. Suggested principles for determining the need for microbiological testing when manufacturing RTE foods.

- Have pathogens been associated with the food or its ingredients? Has the food been associated with illnesses?
- Are the ingredients likely to be contaminated?
- Are there robust processing control procedures such as a kill step or other reduction methods/controls? If so, is the reduction a result of the formulation or the application of a process control measure, and what is the magnitude of the reduction?
- Is there a potential for recontamination from handling or the environment?
- Does the product support survival or growth of pathogens?
- What is the shelf life of the product?
- Is this product meant specifically for a higher risk population?
- Is consumer handling and use likely to increase or decrease risk?

Figure 3. Suggested questions for decisions on performing microbiological testing when manufacturing RTE foods.

As noted by several speakers, some of the confusion related to the use of microbiological testing for verification of preventive controls stems from misunderstanding the difference between "verification" and "validation." Pamela Wilger (Cargill) reminded the audience that validation asks, "Will it work?" while verification asks, "Is it working?" While "validations" are required only initially and when major changes occur to ensure that controls within a manufacturing process actually minimize or prevent a hazard, "verifications" are repeated on a frequent, regular basis to ensure that the process is being consistently performed in a way that will minimize or prevent that hazard.

Wilger emphasized that microbiological testing also plays a critical role in validation if a preventive control seeks to minimize a microbial hazard, and microbiological methods used in verification activities must be validated for the sample type and sample size being tested.

Microbiological testing for verification activities

Microbiological testing is important in verification of several types of preventive controls: to ensure that process controls are working; to verify that sanitation controls are effective; and to enable manufacturers and suppliers to share information regarding the microbiological status of food products or ingredients (supplier controls).

Microbiological testing for verification of process controls

Microbiological testing can be used to verify that a process used to control a pathogen is working properly on a day-to-day basis. For example, after washing lettuce, a producer may periodically test for an indicator organism in the washed lettuce at defined intervals during production to make sure that their (previously validated) lettuce washing method is consistently effective in preventing cross-contamination.

End product testing may be useful to verify the effectiveness of the overall manufacturing process in controlling microbial hazards, but it should not be relied upon exclusively to ensure safety or quality of a lot. For example, a hummus manufacturer may schedule regular *Enterobacteriaceae* testing of product to verify that a high-pressure processing (HPP) step consistently reduces microbial levels. This testing verifies a processing control and isn't being used solely to determine whether that lot of hummus is acceptable for release.

Microbiological testing for environmental monitoring and verification of effective sanitation

Environmental monitoring is a form of microbiological testing that can be used to verify that sanitation controls in a manufacturing facility are working consistently and effectively. As is true for all types of microbiological testing used for verification, the goal is to actually find the pathogen or indicator organism if it is present, not to fear such results. Deann Akins-Lewenthal (Conagra Brands) told the audience they should actively seek out positive results, because knowing where pathogens are is the only way to eliminate them from a manufacturing facility. A company's sampling plan should follow this ethos.

Although it is important to focus on sampling areas where the highest risks occur (such as food contact surfaces in RTE manufacturing facilities), Akins-Lewenthal also recommended monitoring low-risk areas to identify microbiological problems within a facility that could potentially migrate into high risk areas. Sampling should cover all production days and shifts. Environmental monitoring data need to be tracked in a way that allows trends to be identified, whether through the use of software designed for this purpose or by simply mapping positive results over time onto a diagram of the facility. Having a new set of eyes periodically conduct a mass swabbing is a good way to ensure that certain locations are not being routinely missed during testing.

Microbiological testing for supplier verification

Under the preventive controls rule and the Foreign Supplier Verification Program rule, manufacturers are required to perform supplier verification activities when hazard analysis has identified a hazard requiring a preventive control. This means that when a manufacturer receives an ingredient, it must verify that hazards associated with that ingredient are controlled. This can be done in several ways: the supplier could perform regular microbiological testing on the ingredient, and this information would be included on a certificate of analysis (COA) for each lot of the ingredient. The COA then would be reviewed by the manufacturer before use. The manufacturer should perform other steps to ensure that the testing performed (or directed by the supplier) was performed appropriately. Supplier verification of a control for a microbiological hazard could also involve

21 CFR §117.165 Verification of implementation and effectiveness.

(a)(1). Verification activities. You must verify that the preventive controls are consistently implemented and are effectively and significantly minimizing or preventing the hazards. To do so you must conduct activities that include the following, as appropriate to the facility, the food, and the nature of the preventive control and its role in the facility's food safety system:

(a)(2). Product testing, for a pathogen (or appropriate indicator organism) or other hazard;

(a)(3). 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.

Figure 4. Verification related to microbiological testing as required by the preventive controls rule.

TABLE 1. Different types of microorganisms used for different types of testing

Type of Microbiological Testing	Surrogate Organism	Indicator Organism	Pathogen
Validation of a process preventive control	+ (in manufacturing facility)	+	+ (in laboratory)
Verification of a supply chain preventive control	-	+	+
Verification of a process preventive controls	-	+	+
Verification of sanitation controls	-	+	+

the manufacturer (or a third party) performing periodic microbiological testing of an ingredient.

It is also possible that the microbial hazard will be controlled by subsequent validated processing steps performed by the manufacturer (e.g., a cook step). It is not necessary to test an ingredient if a subsequent step will eliminate a pathogen or if pathogens are never found in an ingredient (such as an oil or sweetener). For example, it is not necessary to use microbiological testing to verify that tahini used to manufacture hummus is free of *Salmonella* if the hummus is HPP-treated after packaging.

Supply verification requires communication and coordination between the supplier and purchaser. Fabien Robert (Nestlé) discussed how a large global food company (Nestlé) partners with its suppliers to help them supply products that meet Nestlé's microbiological specifications. Nestlé uses several strategies to build food safety awareness with its partners across the supply chain. The company freely disseminates documents describing best practices (e.g., "Guidance to minimize microbiological risk — washing produce") to its suppliers and others. Prevention plans for Nestlé's suppliers are developed through continuous reviews, vulnerability assessments, and mitigation plans. By engaging upstream (starting in the field, if necessary) with its partners (its suppliers), microbiological risks can be better managed.

HOW TO CONDUCT MICROBIOLOGICAL TESTING

What organism(s) should be tested for?

When developing a food safety plan, the hazard analysis should identify what pathogens might be hazards associated with a particular food product or ingredient. Hazard analysis, according to Wilger, should not be based on customer requirements (although some companies do share their risk assessments with their customers) and needs to be revisited when changes occur or new information arises. Not all hazards are immediately apparent. Both intended and unintended uses of products need to be considered. For example, consumers may not follow storage directions. Hazards during transportation need to be considered. Separate hazard analyses should be conducted for a product if it is manufactured at different facilities, even if the same process is used to make the same product, as subtle differences that can alter risk may be present.

Appendix 1 "Potential Hazards for Foods and Processes" to the FDA Draft Guidance for Industry: Hazard Analysis and Risk-Based Preventive Controls for Human Food (2016) may be helpful in determining the key hazards, although Jenny Scott acknowledged that the FDA "created a lot of confusion" with this guide (which is being revised). She cautioned that just because something is listed in the guide doesn't mean you are required to test for it; it is merely a guide. In many cases, it makes sense to look for other microorganisms instead of or in addition to the pathogen. The choice of which microorganism to test for depends on the reason for the microbiological testing *(Table 1)*.

Pamela Wilger discussed the use of surrogate organisms rather than the pathogen of interest when conducting validation testing. She defined surrogates as non-pathogenic organisms that behave similarly to target pathogens within a particular food matrix and for a particular process (such as thermal treatments). A surrogate has to be as robust as the target pathogen in terms of its ability to withstand an intervention in the specific food product. Surrogate microorganisms are of particular use in process validation studies when the validation work is being performed in the actual food manufacturing facility.

Indicator organisms, which are commonly used to signal the potential presence of food safety-related pathogens in an environment, ingredient, or product, are generally more commonly encountered and are non-pathogenic. Although sampling may miss pathogens themselves (because their presence is infrequent and not uniformly distributed), it may find indicator organisms. Wilger explained that because they are more likely to be found, indicator organisms can be quantified and used to monitor trends indicating possible control problems. In choosing an indicator organism, it is important to consider what potential pathogens might be present and which indicator organisms are appropriate signals for the possible presence of those pathogens. She illustrated with a Venn diagram *(Fig. 5)* how testing for total *Enterobacteriaceae*

levels (commonly used in Europe and gaining use in the U.S.) can be more telling than testing for coliform or generic *E. coli*. Wilger noted that although all these tests are good indicators of hygiene, *Enterobacteriaceae* testing illuminates the potential for *Salmonella* spp. to be present more than do other indicators.

Meeting attendees were interested in whether companies tested for *Listeria* spp. or *Listeria monocytogenes* when conducting environmental monitoring in their manufacturing facilities that are at risk for *Listeria monocytogenes*. The FDA

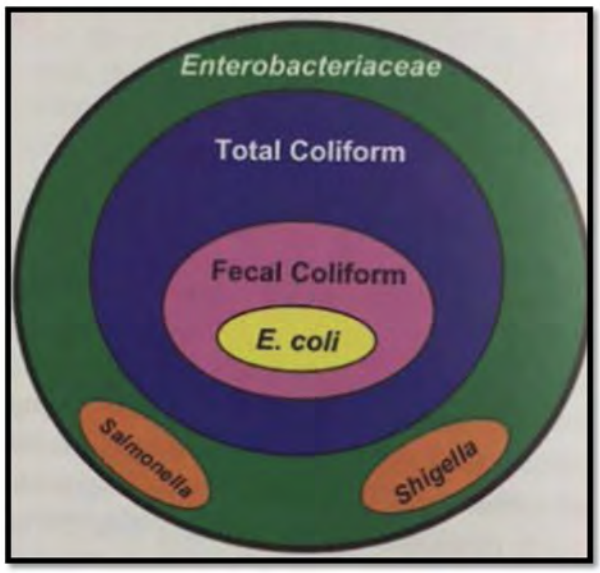

Figure 5. *Enterobacteriaceae* testing provides information about enteric pathogens that coliform testing does not (reprinted from *Environmental Microbiology*, 3rd Edition; Gerber, C. P.; Indicator Organisms; Figure 23.1; Copyright 2015; with permission from Elsevier).

draft guidance "Control of *Listeria monocytogenes* in ready-to-eat foods" recommends testing for *Listeria* spp. because such testing will find *L. monocytogenes* as well as other, more common, non-pathogenic species of *Listeria*. If *Listeria* species are found, it indicates the potential for *L. monocytogenes* to be present because conditions are suitable for its survival and/or growth. However, one meeting participant indicated that their company policy is to test for *Listeria monocytogenes* when testing food contact surfaces to facilitate rapid response actions if a positive is found.

What microbiological test should you use?

When conducting microbiological testing for verification of PCs, an appropriate, validated method should be used, according to meeting speakers Akins-Lewenthal and Thomas Hammack (FDA). Just because an AOAC method exists doesn't mean it will work in all food matrices, cautioned Hammack. The analytical method used should be fit for purpose, which means it is validated for the sample size and for the microorganism within the food or other matrix that is being tested. It is also important to verify that the method works in the hands of qualified analysts.

Hammack discussed validation requirements for microbiological methods that might be used as part of verification activities. Method validation is required for new methods and whenever a significant modification to an existing method (such as a new food matrix or larger sample size) is made. If an assay is modified without proper validation, a negative result can be questioned. For example, if an assay was previously validated to detect *L. monocytogenes* in milk but is then used (without additional validation) to detect the same organism in yogurt, there may be a difference between the milk and yogurt matrices that prevents *L. monocytogenes* from being detected in the yogurt. If a negative result is obtained in yogurt, it is not clear whether *L. monocytogenes* is truly absent or something present in the yogurt interfered with the assay. Validation is done to ensure that negative results truly are negative and that new methods are equivalent to previously validated reference methods, to demonstrate that a method is truly fit for purpose, and to comply with regulatory requirements. In contrast, verification of microbiological methods involves demonstration that a laboratory can properly perform validated methods and achieve adequate performance through training records, proficiency testing, etc.

How should you perform sampling?

Jenny Scott commented that the more hazardous a pathogen and the more likely that it may grow in an RTE food, the more samples are needed for a testing program and the more important a positive result becomes. The sampling process in a microbiological testing program is therefore critical. However, in contrast to analytical detection methods, which are well studied, sampling methods are underappreciated and poorly understood. Nevertheless, according to consultant and meeting speaker Nancy Thiex, better sampling is often more effective at reducing overall error than a more expensive analytical instrument.

Robert Buchanan, professor emeritus at the University of Maryland, provided a primer on microbiological sampling plans. Two general classes of sampling plans exist: attribute (which categorize the test result into "presence" or "absence" or within defined numerical ranges) and variables (which use quantitative data directly). Both classes of sampling plans can be used with either culture-based or "omics"-based testing.

Buchanan also explained the concept of defect rate, and illustrated how defect rates below 2% mean more samples need to be tested to avoid missing the defect. Lower defect rates decrease the cost effectiveness of testing programs and increase the likelihood that a contaminated lot will be accepted. To circumvent low limits of detection, it may be necessary to increase the size of the analytical unit, increase the number of samples, or enrich/concentrate samples. Regardless, the defect rates in many foods are well below the practical limits of sampling. That does not mean that microbiological testing is not

useful; it is still an important tool for verifying the effectiveness of a food safety system (for example, by testing for indicator organisms). However, no sampling plan can guarantee that a product is pathogen-free.

The effectiveness of a microbiological sampling is also dependent on distribution of the contaminant within a lot. Pathogens often are not randomly distributed. Buchanan gave an example in which pre-harvest sampling of a produce field used field irrigation patterns (a likely contamination source) to illustrate how knowledge of a food production or field environment and its potential contamination sources can improve sampling strategies.

Thiex reminded the audience that sampling also occurs in the analytical laboratory; lab workers will sample only part of the material they receive, which will introduce sampling errors. Sampling error is typically "huge" compared to the error associated with analytical methods, which are usually smaller and have been well characterized during the method validation. Thiex discussed three tools for taking and testing "GOOD" samples: (1) establishing Sample Quality Criteria, (2) characterizing the material properties and (3) applying the Theory of Sampling developed by the late Pierre Gy to mitigate sampling error through collection of sufficient mass and sufficient number of increments. Thiex advocated increased training on this set of tools, which is the basis for a systematic approach to representative sampling of food products detailed in two recent publications: (1) *Guidance on Obtaining Defensible Samples (GOOD Samples)* and (2) *Guidance on Obtaining Defensible Test Portions (GOOD Test Portions)* (see "Resources and References"). Thiex emphasized that validation of protocols and incorporation of quality control are as important for sampling as for analytical methods.

How to set specifications

Katherine M. J. Swanson (retired consultant), discussed important factors to consider when setting specifications and action limits for microbiological test results. Specifications are often part of a purchasing agreement between a buyer and the supplier of an ingredient or food. They are also internal limits used to monitor manufacturing processes. As explained by Swanson, specifications may be mandatory or advisory and usually cover much more than just food safety (e.g., most specifications cover parameters affecting quality). Swanson discussed the "anatomy" of a microbiological specification. Testing methods should be specified and describe the number, type, and size of samples and the frequency of testing. For every tested organism (whether an indicator or pathogen), there should be an action limit and an actionable outcome (rejection, process adjustment, recall, etc.) if that limit is exceeded. As discussed by several speakers, it may be more useful and informative for a specification to include test results for indicator organisms to identify trends rather than simply looking for the presence or absence of pathogens. Indicator organism testing is not always useful, however. Swanson used coliforms as an example: They are useful in assessing milk but not in evaluating fresh produce, on which coliforms are always naturally present.

How to set action limits. What actions should be taken when you get a positive?

Swanson stated that credible sources need to be used to set action limits for specifications and cited various helpful resources (see "Resources and References"). In some cases, regulatory standards may exist. Trade association guidelines and international standards such as the Commission Regulation (EC) No 2073/2005 on microbiological criteria for food standards and Codex Alimentarius Commission's Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods (CAC/GL 21–1997) may also be useful tools in identifying appropriate action limits. The 2011 ICMSF book *Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance* is another useful resource for setting specifications. Although FSMA requires FDA to establish risk-based, technologically-feasible performance standards which could be useful for setting specifications, such standards have not yet been released. When performing microbiological testing to verify a preventive control, a "positive" result (one that does not meet your specification) requires a corrective action. That action should be part of a corrective action plan that is developed before problems arise. If the positive result occurs when performing environmental monitoring, Akins-Lewenthal recommended verifying the impact of the corrective action by obtaining at least three passing swabbing events in a row within the affected area.

Attorney Maile Hermida of Hogan Lovells discussed the legal dilemma that food manufacturers face: "Should we conduct microbiological testing, knowing that some false positives will occur that could be a liability for my company? Or should we avoid testing even if that makes our company negligent?"

Ignoring or minimizing microbiological problems ("it's an outlier," "the finished product tested negative," "no one will get hurt," etc.) does not make them disappear. False positives are the exception, not the norm. It's important to identify potential contamination early and respond to it appropriately, including identifying the root cause. Criminal investigations associated with foodborne disease outbreaks have often focused on ignored warning signals. Hermida discussed the statutory and regulatory framework surrounding testing and pointed out that FSMA significantly expands FDA's access to records during inspections. Legal privilege cannot be used to hide testing results, and merely copying a lawyer on testing documents does not protect them from disclosure.

FDA and state regulators are now routinely conducting "swab-a-thons" in RTE facilities. During an inspection, inspectors may swab 200 to 300 strategic sites within the facility. New technology such as whole-genome sequencing can allow regulators to precisely match patient isolates with product samples or environmental samples (sometimes collected years

earlier), opening up more potential for liability. To mitigate risk, Hermida recommended being proactive by implementing strong and effective preventive programs for microbiological pathogens and taking (and documenting) aggressive corrective actions when pathogens are found.

Break-out group exercises

After the formal talks, the symposium participants were divided into groups to participate in exercises. The goal of these exercises was to apply what they had learned during the day by discussing microbiological testing programs that could be developed and used as part of a FSMA food safety plan for an RTE food product. Each group was assigned a different type of RTE food product (frozen cheesecake, fresh-cut melon, dry dairy powders, deli salad) and instructed to perform a hazard analysis for biological hazards, discuss preventive controls, and then consider criteria for a microbiological testing program.

An example of the conclusions reached by one of the groups (facilitated by Michelle Danyluk) is shown in *(Fig. 6)*.

Beyond their conclusions (such as those shown in *(Fig. 6)* the group exercises were informative in other ways:

- Conducting a hazard analysis and identifying preventive controls is not trivial, and even experts require significant time and background resources to complete these activities.
- There can be many different "right" answers for how microbiological testing can be useful in a food safety plan, and even those with considerable experience with a particular product type might come up with different strategies.
- The cost, time, and practicality of preventive controls and microbiological testing strategies are important factors that might be ignored in a theoretical exercise but must be considered in real-life.

RTE fresh-cut fruits (e.g., cut melon, sectioned grapefruit, sliced pineapple)

Question 1. What principles and criteria should a company apply in determining the need for and in designing an effective microbial testing program to verify that processes are effectively controlling microbial pathogens?

a. **What pathogens are associated with the food or ingredients?**
   There is variability in the pathogens (hazard) and prominence (likelihood of occurrence) that is dependent on a number of factors, including commodity, farming system, region and other variable events (such as season or weather); poor worker hygiene practices can contribute to viral or parasitic risk.

b. **Are the ingredients likely to be contaminated?**
   Yes, again depending on commodity type and its attributes (i.e., pH, water content, rind, etc.)

c. **Are there robust processing control procedures such as a kill step or other reduction methods/controls?**
   No

d. **Is there a potential for recontamination from the handling or the environment?**
   Yes

e. **Does the product support survival or growth?**
   Depends on the pH of the product

f. **Is this product meant for higher risk population?**
   Product is made for the general population, but high-risk populations may purchase or be served in hospital or nursing home facilities.

g. **What is the shelf life of the product?**
   The typical shelf life of the product is 1 to 2 weeks.

Question 2. Are there situations in which testing other than for pathogens or indicator organisms (e.g., enzymes), would be an appropriate verification activity?

Yes, monitoring of cold chain for fresh-cut produce for which time and temperature have been identified as needed for food safety and verification and monitoring of wash systems.

Question 3. Are there situations where (microbial) verification testing would not be necessary if there is evidence that the appropriate treatment was, in fact, applied?

Not likely, since there is no "kill step applied." Supplier verification may include pre-harvest testing or testing at receipt for presence or absence of pathogens (as per commodity type and other considerations).

Question 4. When microbial testing is an appropriate verification activity (for finished product), what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)? What are appropriate indicator microorganisms for verifying that processes adequately control pathogens?

Finished product testing (lot control and/or process control) may be appropriate if there is a change in supplier, if there is concern regarding an emerging issue, if environmental monitoring data demonstrate a trend or if other seasonality considerations for the fruit/vegetable source change the risk profile of the starting material.

Question 5. What principles and criteria should a company apply in determining the frequency of testing finished product to determine if the company's food safety system for that product is effective?

For criteria, refer to National Advisory Committee on Microbiological Criteria for Foods (2018) Response to Questions Posed by the Department of Defense Regarding Microbiological Criteria as Indicators of Process Control or Insanitary Conditions. *Journal of Food Protection*: January 2018, Vol. 81, No. 1, pp. 115–141.

Question 6: Are there situations in which testing at sites other than the end of the process can achieve the goal of verifying the adequacy of control of microbial hazards?

Pre-harvest testing or testing at receipt at the processing facility may be considered, depending on commodity and/or other risk assessment parameters. Activities associated with supplier verification, assays and/or electronic monitoring of wash water systems must account for varying acidity; microbial susceptibility and spoilage; comingled lots; cross-contamination from slicing. If we can conduct field or pre-harvest testing, that would be one situation. There should be a direct correlation, assuming that the environmental monitoring is demonstrating a trend and/or change that demonstrates an increase in risk of contamination during the processing; verification activities could include zone 1 testing and/or process control type of testing application.

Question 7: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

For end products, microbiological testing is not considered a primary means of routinely assessing product safety and stability. Assessment of safety is best carried out through the environment, the processing line. Microbiological testing can provide a supporting role here, to verify washing step process control, and can be reduced based on results demonstrating that the process is well under control. If significant changes are introduced or if there is a failure in the process control, then testing can be intensified temporarily, to verify that the process returns to being under control.

Figure 6. Criteria for design of a microbiological testing program for fresh-cut fruit.

CONCLUSIONS

When a hazard analysis identifies a hazard that requires a preventive control, microbiological testing can help a food manufacturer be confident that their manufacturing process is capable of controlling pathogens (validation) and is actually doing so (verification). It also can be used to verify sanitation of the environment and for supply chain verification activities. Microbiological testing for verification of controls for hazards identified as requiring a preventive control is particularly important for RTE foods, which will not be cooked before consumption.

Should your firm test finished products for pathogens? "It depends" and "it's complicated." Although testing of finished products can inform you that one of your preventive controls is not working, it is not recommended for determining final lot acceptance. Because the defect rate (i.e., the rate at which pathogens are present in samples) is likely to be very low, it is not feasible to sample enough of the lot to ensure that it is free of pathogens.

It is not always the best strategy (and in some cases not advisable) to test for your target pathogen. The use of surrogate organisms in the validation of preventive controls and the use of indicator organisms to verify the effectiveness of preventive and sanitation controls will likely provide more useful information than pathogen testing. Use of a surrogate organism allows validation work to be completed on the same equipment and in the same facility in which manufacturing occurs. In addition, indicator testing is often quantitative and can help identify trends that inform process control more quickly.

The goal of environmental monitoring for pathogens is to seek and find them, not to fear positive samples. The only way to eliminate contamination is to find it. New technology (next-generation sequencing that can link illnesses to manufacturing facilities) and regulatory changes (increased enforcement actions) together provide strong incentives for a food manufacturer to identify and fix environmental problems as soon as possible.

TABLE 2. Resources for developing microbiological testing programs

General Topic	Document	Link
Preventive Controls	FDA Draft Guidance for Industry: Hazard Analysis and Risk-based Preventive Controls for Human Food (2016) *(more chapters are in development)*	https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-hazard-analysis-and-risk-based-preventive-controls-human-food
	FDA Appendix 1: Potential Hazards for Foods and Processes *(appendix to the guidance document above; currently being revised)*	https://www.fda.gov/media/99581/download
	National Advisory Committee on Microbiological Criteria for Foods: Response to Questions Posed by the Department of Defense Regarding Microbiological Criteria as Indicators of Process Control or Insanitary Condition (2018)	https://jfoodprotection.org/doi/pdf/10.4315/0362-028X.JFP-17-294
Setting Microbiological Specifications	Commission Regulation (European Commission) No. 2073/2005 on microbiological criteria for food standards	https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02005R2073-20140601
	Codex Alimentarius Commission's CAC/GL 21 –1997 (last modified 2013) Principles and guidelines for the establishment and application of microbiological criteria related to foods	http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B21-1997%252FCXG_021e.pdf
	International Commission on Microbiological Specifications for Foods (ICMSF). 2011. Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance. New York, NY: Springer.	https://www.springer.com/gp/book/9781441993731
Environmental Monitoring	FDA's Control of *Listeria monocytogenes* in Ready-to-Eat Foods: Draft Guidance for Industry (2017)	https://www.fda.gov/files/food/published/Draft-Guidance-for-Industry--Control-of-Listeria-monocytogenes-in-Ready-To-Eat-Foods-%28PDF%29.pdf
	USDA FSIS Compliance Guideline: Controlling *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat meat and poultry product (2014)	https://www.fsis.usda.gov/wps/wcm/connect/d3373299-50e6-47d6-a577-e74a1e549fde/Controlling-Lm-RTE-Guideline.pdf?MOD=AJPERES
	American Frozen Food Institute's Environmental Monitoring Unit of Its *Listeria* Control Program	https://affifoodsafety.org/lcp/environmental-monitoring/
Method Validation	FDA's Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds (2015) *(currently in revision)*	https://www.fda.gov/media/83812/download
	Appendix J: AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (2012)	http://www.eoma.aoac.org/app_j.pdf

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TABLE 2. Resources for developing microbiological testing programs (cont.)

General Topic	Document	Link
Method Validation	ISO 16140-1:2016–Microbiology of the Food chain–Method validation–Part 1: Vocabulary	https://www.iso.org/news/2016/06/Ref2093.html
	ISO 16140-2:2016–Microbiology of the Food chain–Method validation–Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method	https://www.iso.org/news/2016/06/Ref2093.html
	ISO 17468:2016–Microbiology of the Food chain–Technical requirements and guidance on establishment or revision of a standardized reference method	https://www.iso.org/standard/59858.html
	ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories	https://www.iso.org/standard/66912.html
Standard Microbiological Methods	Bacteriological Analytical Manual (BAM)	https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam
	FDA's Compendium of Analytical Laboratory Methods for Food and Feed Safety	https://www.fda.gov/food/laboratory-methods-food/compendium-analytical-laboratory-methods-food-and-feed-safety
	USDA Microbiology Laboratory Guidebook	https://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook
	EPA Clean Water Act Analytical Methods	https://www.epa.gov/cwa-methods
Trade Association Documents	American Spice Trade Association's Clean, Safe, Spices Guidance Document (2017)	https://www.astaspice.org/food-safety/clean-safe-spices-guidance-document/
	American Frozen Food Institute Listeria Control Program *(a collection of resources for environmental monitoring, sanitation, and more, many of which have general applicability to non-frozen foods)*	https://affifoodsafety.org/lcp/advanced-search/
	Grocery Manufacturers Association's Control of *Salmonella* in Low-moisture Foods Guidance Document (2009)	https://www.gmaonline.org/downloads/technical-guidance-and-tools/SalmonellaControlGuidance.pdf
Surrogates	Hu, M., and J. B. Gurtler. 2017. Selection of Surrogate Bacteria for Use in Food Safety Challenge Studies: A Review. *J. Food Prot.* 80:1506.	https://jfoodprotection.org/action/showCitFormats?doi=10.4315%2F0362-028X.JFP-16-536
Sampling	Xu, A., and R. L. Buchanan. 2019. Evaluation of sampling methods for the detection of pathogenic bacteria on pre-harvest leafy greens. *Food Microbiol.* 77:137.	https://www.sciencedirect.com/science/article/pii/S0740002017303258
	International Commission on Microbiological Specifications for Foods (ICMSF). 2018. Microorganisms in Foods 7. Microbiological testing in food safety management. Second edition. Springer International Publishing.	https://www.springer.com/gp/book/9783319684581
	Special issue (March/April 2015) of *Journal of AOAC International* that contains a series of reviews on representative sampling of food and feed materials	https://www.ingentaconnect.com/content/aoac/jaoac/2015/00000098/00000002

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TABLE 2. Resources for developing microbiological testing programs (cont.)

General Topic	Document	Link
Sampling	Association of American Feed Control Officials' GOOD Samples: Guidance on Obtaining Defensible Samples (2015)	https://www.aafco.org/Portals/0/SiteContent/Publications/GOODSamples.pdf?v2
	Association of American Feed Control Officials' GOOD Test Portions: Guidance on Obtaining Defensible Test Portions (2018)	https://www.aafco.org/Portals/0/SiteContent/Publications/GoodTP_final_web.pdf?v3
	Gy, P. 1998. *Sampling for Analytical Purposes,* Wiley.	https://www.wiley.com/en-us/Sampling+for+Analytical+Purposes-p-9780471979562
	Pitard, F. F. 1993. *Pierre Gy's Sampling Theory and Sampling Practice: Heterogeneity, Sampling Correctness, and Statistical Process Control, 2nd Edition.* Boca Raton, FL: CRC Press.	https://books.google.com/books/about/Pierre_Gy_s_Sampling_Theory_and_Sampling.html?id=PsjcZfwrZR8C
Educating Suppliers	Best practices educational documents for suppliers to minimize microbiological risks and contamination	https://www.nestle.com/aboutus/suppliers
General References	Buchanan, R. L., and D. Schaffner. 2015. FSMA: testing as a tool for verifying preventive controls. *Food Prot. Trends* 35:228.	http://www.foodprotection.org/files/food-protection-trends/May-Jun-15-buchanan.pdf
	Marcel H. Zwietering, L. Jacxsens, J-M Membré, M. Nauta, and M. Peterz. 2016. Relevance of microbial finished product testing in food safety management *Food Control* 80:31	https://www.sciencedirect.com/science/article/pii/S0956713515300918
	Thippareddi, H., E. A. E. Boyle, and D. E. Burson. 2005. Chapter 30 – Monitoring, validating and verifying the effectiveness of HACCP systems. *In* Improving the Safety of Fresh Meat, Edited by John N. Sofos: Woodhead Publishing. *(This older reference illustrates some of the same issues that arose during the implementation of HACCP)*	https://www.sciencedirect.com/science/article/pii/B9781855739550500303

The flexibility given food manufacturers by FSMA means companies have many options to consider when developing their microbiological test strategies. It also means that companies need to have personnel or consultants who fully understand foodborne disease risks, food microbiology, sampling, testing, food processing and engineering. We all can benefit from the experience of others, as evidenced by the fact that even FDA is seeking advice from experts on the utility and necessity of microbiological testing of RTE foods.

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RESOURCES AND REFERENCES

Many of the speakers highlighted documents and resources that they found helpful in developing microbiological testing plans for foods *(Table 2)*.

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