

FSMA: Testing as a Tool for Verifying Preventive Controls

Robert L. Buchanan^{1*} and Donald Schaffner²

¹Center for Food Safety and Security Systems, College of Agriculture and Natural Resources, University of Maryland, Symons Hall, Room O119, College Park, MD 20742, USA;

²Dept. of Food Science, Rutgers University, New Brunswick, NJ 08901, USA

ABSTRACT

In 2013, we were approached by the PEW Charitable Trusts (PEW) to develop a scientific “white paper” describing the principles, concepts, and potential applications of microbiological testing to verify preventive controls implemented as part of food safety system that combined both HACCP and Good Hygienic Practices. This was undertaken with the knowledge that it would be included as an independently developed appendix to Pew’s comments to FDA regarding microbiological testing aspects of the FSMA preventive controls proposal. [For the sake of full disclosure, both of the authors received an honorarium from PEW for undertaking this project.] On Feb 26, 2014, the Pew submitted its comments to the open FSMA docket (FDA-2011-N-0920-1257), including our document, Appendix B.

The appendix reviewed some of the key aspects of verification testing, laying out how it differs from traditional “batch testing” of food products. This included describing the key characteristics needed in implementing verification testing as part of an integrated food safety system. This included a series of recommendations to FDA regarding inclusion of verification testing in FSMA. Since its initial posting, we have been encouraged by multiple individuals to publish our comments so that they could be more widely shared among food safety professionals. While Federal dockets are accessible by the public, it is not the easiest way of sharing information. We also wanted to use this as an opportunity to encourage other scientists to consider submitting scientific comments to future regulatory proposals, thereby helping ensure an active debate of the scientific foundation upon which sound food safety policies must be built.

We are presenting the document as originally presented when placed in the “preventative controls docket” for the proposed regulation. The only changes that were made to the original document were to correct a few typos that were discovered after transmission to the FDA.

INTRODUCTION

At the request of The Pew Charitable Trusts, we were asked to summarize scientific concepts and practices related to the testing of food to verify preventive food safety controls that are pertinent to the finalization of FDA regulations associated with the FDA Food Safety Modernization Act. The comments that follow are focused on the role of microbiological testing for verification; however, many of the principles and parameters discussed are also pertinent to other physical and chemical hazards. It is important to note that while we were requested to prepare this report by Pew, the comments, interpretations, and recommendations in this report are based on our personal experiences and evaluations of the scientific underpinnings of microbiological testing of foods and practices associated with its utilization by both industry and governments.

In considering the role of microbiological testing in food safety systems it is important to note that there are multiple forms of microbiological testing (e.g., process

verification, lot release, investigational), each with its own protocols and underlying mathematics. Most food safety systems generally include some form of microbiological testing, but it is critical that the right form of testing is used for the application being considered. It is also important to emphasize that it is highly unlikely that a single microbiological test will be adequate for all foods and all applications. As will be discussed later in this document, while a general framework can be articulated, flexibility in specific applications and targets will be needed for broad application of microbiological verification testing programs.

Testing within a food safety systems based on preventive controls

It is important to clearly define verification and distinguish it from “monitoring” before getting into specific issues and recommendations related to microbiological verification. Monitoring can be defined as the measurements and observations that are taken in real-time that reflect the

*Corresponding Author: Phone: +1 301.405.1209; E-mail: rbuchana@umd.edu

proper functioning of key components of the food safety system that ensure food safety. Such measurements are typically associated with critical control points (CCPs) and key activities associated with good hygienic practices (GHPs). They are the attributes upon which decisions related to the safety of a food are based. Conversely, verification is designed to assess whether the system is continuing to function as intended, i.e., has the system or the hazards associated with the food changed so that safety cannot be ensured. As an example, consider the production of a food that relies on achieving a 5-log thermal inactivation of *Salmonella* based on surveys that indicate that the level of *Salmonella* in the raw material is <1 CFU/100 g. Such a process would be monitored by determining the time and temperature achieved during the thermal process. In this case verification could consist of periodically examining finished product samples for a fecal indicator microorganism or for *Salmonella*. If CCP monitoring indicated the process was functioning properly but verification testing indicated that a microbiological indicator or pathogen was present at an unacceptable level, these results would indicate one of several possibilities. The alternatives include: (a) the incoming raw materials had significantly increased levels of contamination; (b) a new source of contamination had arisen after the thermal treatment; or (c) in fact the thermal process was not functioning properly, despite indications to the contrary. Verification testing is typically an activity carried out periodically and often involves assays that do not provide results in real time.

There is often substantial confusion about the types and purposes of microbiological testing conducted as part of food safety systems. The form of testing that has been traditionally used is lot-batch testing. The purpose of lot testing is to examine a product lot for which you have no information (e.g., at a port of entry). It typically involves analysis of a large number of samples acquired on a random basis from the entire volume of food under consideration. In a well-designed and managed food safety system, this type of testing should not be necessary since specific controls should have been put into place and monitored to ensure that the preventive controls have been applied to the entire volume of food. When traditional lot testing is implemented within a food safety system, as in “hold-and-release” testing, the function of such testing is as a preventive control and not as a verification tool. The strengths and limitations of this type of testing are well established (5), and if there is a high rate of contamination (i.e., a high percentage of servings are contaminated) this type of testing can effectively prevent contaminated food from entering commerce. The limitations of traditional lot testing as a preventive control are twofold. First, its effectiveness decreases substantially when the “defect rate” (percentage of servings containing the contaminant) drops below 2 – 3% because of the large number of samples that have to be examined and the cost associated with such

testing. Second, it is not a real-time activity with limited utility for fresh foods that have a limited shelf life. In such circumstances, food manufacturers generally invest in better process design. However, improvements in process design often require substantial capital investments, which may be a major economic burden for small to medium-sized food manufacturers. FDA may employ the equivalent of lot testing during plant investigations; however, this is restricted to a small number of lots due to the low frequency of FDA inspections. Major increases in this type of testing by FDA would be dependent on major increases in inspectional personnel, laboratory facilities, and laboratory personnel.

Microbiological testing for verification serves a different purpose and typically involves a category of testing that is referred to as process control verification testing. As introduced above, the purpose of verification testing is not to prove that a lot of food is safe, but instead, its focus is on demonstrating that the preventive controls are functioning as intended. This is achieved by “cross-lot” testing, with a limited number of tests being conducted across lots over time instead of extensive testing of each lot. It is effectively easier (and less expensive) to determine that a process is no longer under control compared to proving a specific lot of food is safe. It is worth reiterating that in such a system, the safety of the food is achieved through the performance and monitoring of the CCPs (and GHPs) and not the microbiological testing. The characteristics of such process control-based testing are discussed in more detail later in this document.

A third type of microbiological testing commonly employed by food manufacturers and regulatory agencies is environmental testing. This can involve the testing of both non-food contact surfaces and food-contact surfaces, though the interpretation and significance of the findings are substantially different. This is typically a form of verification testing where samples are periodically taken to assess the effectiveness of a facility’s sanitation activities and other related prerequisite programs. There are instances where environmental testing is used as a “sanitation control point” and as such equivalent to a monitoring activity. For example, the examination of pre-startup equipment by examining swabs for levels of the chemical ATP is a sanitation monitoring activity instead of a sanitation verification activity. ATP is Adenosine Triphosphate, the primary chemical used for storing and using energy in biological systems. Environmental testing will be discussed further later in the document.

The advantages of statistical process control approaches to verifying food safety systems based on preventive controls has been a major component of two major food safety regulatory initiatives in the United States. The first was the USDA Food Safety and Inspection Service (FSIS) “Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems,” which was proposed in 1995, finalized in 1996, and fully implemented in 2000. Both the proposal

and final rule included a system of process verification for raw meat and poultry based on microbiological process control verification testing. The proposal focused on testing for generic *Escherichia coli* over time against commodity-specific microbiological criteria based on baseline studies of the industry that established realistic technological capability. The final rule modified this approach, keeping the *E. coli* testing requirement for industry but also adding testing for *Salmonella enterica* that was conducted by FSIS. A “moving window” approach was selected as the decision criterion, which consists of having fewer than a specified number of positive samples within a specified time frame (i.e., the window). As a hypothetical example of a moving window approach to microbiological testing, consider *Salmonella* testing of a commodity once per day using a simple presence or absence test. Let us further assume that, based on a statistically derived microbiological criterion, if there is more than 1 positive sample in a 7-day period, this would be indicate that the food safety system’s preventive controls were no longer operating as originally designed. As each day’s sample is analyzed, our 7-day “window” moves forward by one day, thereby continuously updating the verification of the degree of process control. As the window moves, any time the current window includes more than 1 positive, the process would be considered out of control and require appropriate corrective actions. While there have been changes in the specifics associated with sampling for verification requirements for different raw meat and poultry products since the regulation was implemented, the general approach of testing for process control has been maintained.

It is important to note that *S. enterica* was not used as a “pathogen removal” criterion as would be the case for ready-to-eat foods, but instead was used as an indicator organism, i.e., *S. enterica* was not considered an adulterant. As will be discuss later in the document, the designation of *S. enterica* as an indicator was critical to the effectiveness of this program, and has served as a model for HACCP verification programs. The one area that we would consider unsuccessful was the use of generic *E. coli* testing. The FSIS’s focus on using the *Salmonella* testing for the basis for regulatory decisions has resulted in FSIS largely ignoring the *E. coli* data being collected daily by the meat and poultry industry. This effectively limits the utility of the data to single establishments, i.e., there is no consolidation of the industry data. Such data could be instrumental to both the industry and USDA in assessing the state and variability among the industry control programs, demonstrating continuing improvement, and identifying additional risk factors.

The second application of statistical process control-based microbiological testing was a targeted application within the FDA “Hazard Analysis and Critical Control Point (HACCP); Procedures for the Safe and Sanitary Processing and Importing of Juice” regulation, which was proposed in 1998, finalized in 2001, and fully implemented in 2004.

Within this regulation, all juices are required to be treated with a 5-D inactivation step (i.e., a 5-log reduction, which is equivalent to a 99.999% reduction) after the juice is expressed. An exception was intact citrus fruit, which could rely on an equivalent treatment of the fruits’ surface prior to juice expression. This exception was based on extensive baseline data provide by the citrus juice industry. However, to ensure that such treatments remained effective, FDA required that a juice manufacturer relying on an alternative treatment verify its process by microbiological testing. Tests of two 10-ml samples per 1000 gallons of juice produced for generic *E. coli* were used as an indicator of fecal contamination. Again, a “moving window” approach to testing was employed with two positive assays in a moving window of seven consecutive samples considered to be indicative of a loss of control, requiring corrective actions to be taken by the manufacturer. This regulatory program is considered to be responsible for the dramatic decrease in outbreaks associated with juices in the United States, including fresh-squeezed juices that receive surface treatments only, while minimizing test costs, and providing flexibility to juice manufacturers (7).

These are two examples of government and industry adapting microbiological testing to address the unique requirements for verifying the ongoing effectiveness of preventive controls. The advantages of this approach is that it takes full advantage of the wealth of knowledge available concerning the manufacturing of a food, minimizes the cost and burden of testing, and provides an objective quantitative measure of the continuing effectiveness of the manufacturer’s food safety system. Thus, the first conclusion of this report is that *“Focused microbiological testing programs based on the application of process control testing sampling plans are desirable for many foods, by simultaneously verifying the effectiveness of preventive controls and minimizing the cost of such programs that will be ultimately borne by the consumer.”*

An underlying assumption that we have made is that any final program adopted by FDA will likely focus on verification testing conducted by the industry. While FDA could do testing in a manner similar to what is done by USDA/FSIS for meat and poultry, relying solely on testing by FDA may be unrealistic considering the approximately 300,000 food manufacturing facilities for which they have inspectional responsibility. Increased testing by FDA in conjunction with its inspectional activity would be desirable; however, due to the relatively low frequency of such inspections, the agency should focus on verifying the verification testing performed by industry.

What are the microorganisms for which industry should test?

The specific microorganisms that should be considered in a microbiological-based verification testing program is dependent on the pathogens of concern, the food in question, and the conditions of processing, storage, distribution and

use. In an ideal situation, the microorganism is one that occurs reasonably often at low levels so that its presence over time can be evaluated for significant changes. While direct determination for specific pathogens can be used, this is typically non-optimal due to the low frequency of detection. Furthermore, with ready-to-eat foods, the detection of most infectious bacteria would result in the product being considered adulterated, which would eliminate the greatest benefit of process control verification testing, i.e., being able to take appropriate corrective actions before a threshold for non-compliance is exceeded. As an analogy, this would be like driving a car that had a speedometer that only registered a value when the car reached or exceeded 90 mph. While we certainly would want to know whether the car reached that speed, it would be critical to learn its speed before it reaches a dangerous level. Thus, the ideal targets for such testing are indicator microorganisms that are indicative of conditions or inadequate controls that would result in an increased risk of pathogens being present. This is part of the reason why the *Salmonella* testing program used by USDA/FSIS for verifying process control for raw meat and poultry uses *Salmonella* as an indicator microorganism and does not extend to ready-to-eat products.

Based on the CDC estimates for the annual episodes of foodborne diseases, the top 10 foodborne pathogens in rank order are: norovirus, *Salmonella enterica*, *Clostridium perfringens*, *Campylobacter* spp., *Staphylococcus aureus*, Shiga toxin-producing *Escherichia coli*, *Shigella* spp., *Yersinia (enterocolitica and pseudotuberculosis)*, *Toxoplasma gondii*, and *Giardia intestinalis* (11). In its Preliminary Regulatory Impact Analysis, FDA provided illness, outbreak, and illness dollar burden data for illnesses attributed to foods under the scope of the proposed rule. Generally, no matter which metric was used, *Salmonella* spp. ranked first, and *Listeria monocytogenes* ranked second, and *Mycobacterium bovis* ranked third. Where allergens were included in the ranking, allergens ranked third, and *M. bovis* ranked fourth. *M. bovis* would not be considered a target for process control verification testing programs due to its low burden of disease impact and the difficulties associated with its detection, particularly at low levels.

Potential conditions or process failures that are typically associated with a loss of process control and subsequent increased risks of foodborne disease include fecal contamination from multiple sources, excess handling by food workers, inadequate refrigeration, inadequate cooling rates, inadequate thermal processing, inadequate cleaning and sanitation, and contamination and recontamination of dry products. Examples of indicator microorganisms that have been used with varying degrees of success as a means of verifying the adequacy of these control factors are listed below.

- Fecal contamination: *Escherichia coli*, coliforms, thermotolerant coliforms (historically called fecal coliforms), *Enterobacteriaceae*, *Enterococcus* spp., *Clostridium perfringens*, *Salmonella enterica* (raw animal

products only). Male-specific (F+) coliphages have been used to indicate the effectiveness to control fecal contamination leading to an increased risk of enteric viruses in water and shellfish.

- Excess handling of foods: *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus* spp.
- Adequacy of cold chain: *Escherichia coli* and thermotolerant coliforms,
- Adequacy of cooling rates: *Clostridium perfringens*
- Adequacy of thermal processing (refrigerated ready-to-eat foods): *Enterobacteriaceae*, *Listeria* spp.
- Cleaning and sanitation: *Enterobacteriaceae*, total aerobic mesophilic plate counts, and bacterial ATP (equivalent to total aerobic mesophilic plate counts)
- Contamination/Re-contamination of dry products: *Enterobacteriaceae*, *Bacillus cereus* group, sulfite-reducing clostridia

In addition to these indicator tests, additional specialized indicator tests for specific classes of foods or pathogens may be required. For example, foods that are packaged using modified atmosphere technologies may require specialized incubation conditions that will support the growth of microaerophilic pathogens or indicator microorganisms. Another example is the testing for virulent strains of *Vibrio parahaemolyticus*. While it has been established for over a decade that only a small percentage of *V. parahaemolyticus* are pathogenic, the analysis of seafood or estuarine waters uses the detection of the species in general as an indicator microorganism. In this instance the decision criterion is based on the presence of *V. parahaemolyticus* exceeding a specified level. Similar approaches have historically been used with *S. aureus*, where low levels are used as indicators of excess handling by humans and elevated levels being indicative of a direct foodborne disease threat.

In those instances where foods are examined for specific pathogens, the two most common targets for FDA-regulated foods are *Salmonella enterica* and *Listeria monocytogenes*. This is consistent with illness or outbreak data, as well as information from the FDA Reportable Food Registry. However, it is important to distinguish pathogen-oriented testing that is used for process verification from testing that is effectively one of the critical control points that are used to control the presence of the microorganism. If such testing is employed for each lot being produced, it is generally considered a monitoring activity or critical control point, and not as a form of verification testing.

Similar considerations are pertinent to the use of environmental testing as a means of verifying the effectiveness of GHP-related activities. The effectiveness of such testing programs is dependent on the degree to which the food processing system is “sealed,” and thus not likely to be contaminated by the surrounding environment. Testing of environmental surfaces is best done using indicator organisms, if feasible. However, if there is no correlation

between the presence of the indicator microorganism and conditions that are likely to lead to an increased risk of the pathogen being introduced from environmental sources, then direct testing for a target pathogen may be necessary. For example, in nut processing, where there is no observed correlation between *Salmonella* and other common microbial indicators, then *Salmonella* would be the microorganism of choice. Common indicator microorganisms that have been used to assess the rigor of sanitation programs include aerobic plate counts, *Enterobacteriaceae*, coliforms, thermotolerant coliforms, *E. coli*, enterococci, *S. aureus*, yeast/mold counts and *Listeria* spp. Alternative technologies, such as the levels of bacterial ATP, are also used extensively to verify sanitation programs. The specific indicator microorganism that should be used is dependent on both the food/ingredients being manufactured and the processes used. For example, *Enterobacteriaceae*, spore-forming bacteria and yeast/mold counts are widely used to verify sanitation programs related to dry food products, whereas *Listeria* spp. is a better indicator microorganism for the production of refrigerated ready-to-eat foods.

As apparent from the discussion above, the selection of appropriate targets for microbiological testing programs is a complex decision that often requires expert advice. Accordingly, we recommend that *“FDA should develop guidance documents that provide practical advice on the selection of target microorganisms for microbiological testing for verification programs for end product, in-line, and environmental sampling, including guidance on the underlying assumptions and relative performance of such indicators. Where there is uncertainty concerning the relation between proposed indicator microorganisms and pathogens of concern, FDA should commission research to determine the effectiveness of proposed target microorganisms.”*

Where along the food chain should industry test?

Microbiological contamination flows along the food chain from the initial point of contamination, through the various steps in manufacturing, distribution, marketing, preparation, and ultimately consumption. As the contamination moves along the food chain, the extent and degree of contamination increases, decreases, or remains unchanged depending on the nature of the microbiological hazard and the processes and conditions that the ingredients and the food are subjected to along the food chain. Thus, the results of microbiological testing reflect the integrated impact of the conditions and processes to which the food or ingredients has been subjected to prior to the point at which a sample is taken. In other words, microbiological testing of raw ingredients at receipt provides the status of the ingredients prior to receipt. Testing of finished products at the time they enter commerce reflect the integration of the microbiological status of raw ingredients with the effectiveness of the preventive controls during manufacturing. Finally testing at retail reflects the

integration of the impacts of raw material and manufacturing with the conditions and sources of contamination associated with distribution and marketing.

Traditionally, microbial testing of foods has been associated with “end product” sampling, reflecting its effective integration of all the steps in the formulation and manufacturing of the food prior to release into commerce. This is equally true for both testing for process verification and for traditional “hold-and-release” testing programs. Microbiological sampling of end-products for many foods is the most efficient and informative verification testing for both industry and government agencies. However, there are situations where alternative sites along the food chain may be more pertinent for the overall goal of verifying the adequacy of the food safety systems. For example, consider perishable foods that rely on the adequacy of the refrigeration chain from manufacturing through marketing. A prudent approach to verifying the adequacy of controls for such products would be to either periodically map the temperature of the product along the distribution chain, or to periodically take microbiological samples to assess the levels of key indicator microorganisms.

A second alternate location for sampling is key raw materials for products that do not undergo a substantial degree of processing before the product reaches the consumer. The prime example in this instance is the USDA/FSIS meat and poultry HACCP regulations, which as mentioned above, focuses the verification testing program on raw products. An underlying assumption in this instance is that a significant portion of the meat and poultry is cooked or handled by consumers in a manner that would not ensure the elimination or avoidance of pathogens, and thus ensuring product safety requires minimization of pathogen levels in the raw product. On the other hand, verification testing of raw materials for pathogens adds little to assessing food safety controls for foods subjected to an overwhelming thermal treatment (e.g., thermal processing) unless the manufacturer or FDA can articulate conditions that would lead to the levels of specific microbiological hazards exceeding the capability of the thermal processing to eliminate.

Similar considerations regarding where to sample are pertinent for the environmental testing of food facilities. Sites for environmental testing can be separated into three classes: (a) food contact surfaces; (b) non-food contact surfaces that have a reasonable likelihood of serving as a source a pathogenic microorganism as a result of cross contamination; and (c) non-food contact surfaces that are unlikely to serve as a source of cross contamination. The latter two categories can vary substantially among food facilities depending on the degree to which the ingredients and foods are handled by sealed systems that serve as a barrier to cross contamination. Such testing programs will typically involve two types of sampling locations: (a) targeted sites that have a high risk of serving as a reservoir for foodborne pathogens if sanitation

programs are inadequate; and (b) randomly selected sites that could verify the overall adequacy sanitation programs.

It is evident that flexibility must be provided in selecting sampling points so that verification performed at locations that optimize the effectiveness of testing program in relations to the foods and facilities are being evaluated. While end-product testing is likely to play a prominent role in microbiological verification testing, other sampling sites can be equally important for specific foods and/or food manufacturing technologies. The selection of specific sampling sites should reflect the verification needs of specific food industry sectors and commodities, and should be an integral part of verification portion of their preventive controls plan. This is potentially a significant challenge for small- to medium-sized food producers and processors, who often lack the scientific expertise to make informed decisions related to sampling site selection. Thus, a specific recommendation is that *“FDA should develop guidance documents that provide practical advice on the selection of sampling sites in relation to end product, in-line, and environmental sampling, including guidance on the underlying assumptions for various selection sites and potential interpretation of data that are likely to be encountered.”*

How often should industry test?

In determining the frequency of testing for verification, it is important to once again emphasize that the goal is not to determine the safety of a specific lot, but instead such testing is conducted to determine if the food safety system is still functioning as intended. Further, it is important to emphasize that there is no “one size fits all” in regard to frequency in testing. However, there are underlying principles and techniques associated with process control samples that provide a sound statistical basis for setting the frequency of testing decisions. Two key factors upon which decisions related to testing rates are based are the frequency at which a testing criterion will be exceeded and the response time that is needed in declaring a system out of control. Both are typically determined as part of a “process control study” where the performance of a food safety system in relation to the selected target microorganism(s) is evaluated during a period when the system is known to be under tight control. Such control studies can either be done on an industry-wide basis or can be performed for individual food facilities. The latter is important for individual food facilities regardless of whether a national baseline study has been done. It is worth noting that prior use of process control testing as part of USDA and FDA HACCP regulation national or regional baseline studies were done as part of a national process control study.

The FDA assumed for purposes of its cost estimates that raw materials and ingredients that are tested would have 5 representative samples tested on a quarterly basis. Such testing might be sufficient if the levels of the target microorganism were reasonably high so that differences

in concentrations could be analyzed to see if there were significant changes. However, this would not be desirable or appropriate in relation to the response time. For example, consider that the decision criterion was that an indicator microorganism in 1 out of the 5 samples exceeded a specified level at any one sampling time but, if this occurred twice in a year, it would indicate that the system was marginally out of control. In this example, it could take up to 9 months to interpret the data as demonstrating that the results of the verification testing indicated a loss of control. Such a lag time is too long for effective verification.

In a second example, consider that FDA required weekly testing using a specified sampling plan to determine the presence of generic *E. coli* in a refrigerated processed food. Depending on the number of samples examined per week and the baseline level of generic *E. coli* expected, it could be several weeks before the system is deemed out of control if the mean log concentration of *E. coli* was marginally above the sensitivity of the sampling plan. Since this ready-to-eat food would be considered to be non-compliant regardless of whether the critical control points were in compliance, the product would be considered as being produced under process deviation during the period that the “window” had an excess number of positive samples. An optimized sampling plan for verification considers both the expected level of “failures” when the system is under control and the response time when control is lost. In general, an increased number of small samples taken more often provide greater discriminatory power than a reduced number of large samples taken less often (5, 9, 12). For example, taking one sample per day each day of the month is much more effective for verification than taking 30 samples on one day per month (5, 9, 12). In relation to response time it is much more realistic in terms of sampling frequency to sample at least once per day in most mid-sized company and multiple times per day in large facilities. Very small facilities may only warrant sampling once per week depending on the volume of food produced, as is the case in USDA and FDA HACCP regulations.

Consideration should also be given to what the FDA response will be when a facility “fails” the verification testing program multiple times. Assuming that the testing is designed to achieve a reasonable rate of statistical confidence, three separate incidences of failure within a specified timeframe would usually be considered sound statistical grounds for mandating a re-validation of a facility’s food safety system.

A second issue in process control-based sampling is what to do after the results of the testing exceed the established criterion. The best situation is to increase the rate of sampling when the test results indicate a loss of control. This has two effects. The first is that the intensive sampling will assist in the root-cause analysis conducted on why excessive positive results are being observed, despite the CCP monitoring attributes indicating that the system is in control. Second, because process control testing takes as long to indicate that

a system has returned to an “in-control” state as it did to indicate that control was lost, the increased rate of sampling would allow the companies to return to an “in-control” state more rapidly after the root cause had been identified and corrected.

Based on the comments and approaches discussed in the proposed regulation, it is recommended that *“FDA should seek specific advice from either the National Advisory Committee on Microbiological Criteria for Foods or the FDA Food Advisory Committee on testing for process control, ensuring that the committee is suitably augmented with expertise in process control statistics.”*

What are the corrective actions that should be required for verification results that indicate loss of control?

As important as the specific microbiological tests used to verify effectiveness of preventive controls-based food safety is how the results of the testing would be employed to improve performance. The corrective actions taken should be consistent with the purpose of the testing, i.e., to verify that the food safety system is functioning as intended. Since the purpose of verification testing is distinctly different from the purpose of testing to monitor critical control point performance, the corrective actions to be taken when verification results exceed decision points should be clearly articulated in a facility’s preventive controls plan. The focus of a company’s response to verification testing results that exceed an established criterion should be used to investigate the root cause of the aberrant findings with the goal of identifying the underlying cause of the deviation and returning the system to the performance level the system was designed to achieve.

A tiered response to incidents of testing results that exceed the established criterion is appropriate. This, in part, reflects the fact that in any microbiological testing program designed to verify performance of food safety system there will be occasional “positive” results despite the system actually being in control. When designing a verification testing program, there is a need to establish the frequency of non-compliant values that can be expected over time when the food safety system is functioning as intended before initiating the program. As discussed above, this is determined using process control studies that establish performance when the food safety system is working under control. In such instances, an investigation of the verification deviation will not identify a root cause and no corrective action may be warranted. However, this does not mean that a non-compliant result can be ignored. An effective review of the system after an initial verification deviation could indicate true changes in the food production system, thereby allowing early system modifications/corrections that will prevent unexpected future system failures.

If there are continuing incidents of verification deviation, the response should increase in stringency. Multiple

verification failures within a specified timeframe (see below) should be viewed as statistically-based proof that the food facility has inadequately identified the factors after the microbiological performance of its food safety system, particularly if the monitoring of critical control points continue to indicate the system is functioning as intended. For example, even if the confidence level for a single verification failure were 80%, three such incidents would indicate that the likelihood that these verification failures represent a true loss of control would be $\geq 99\%$. It is recommended that *“FDA should require that if a food facility has more than three (3) verification deviations within a specific period of time (based on the sampling regime) that they must reexamine and revalidate their preventive controls plan.”*

What is the role of environmental sampling in process verification testing programs?

As FDA has indicated in the proposed rule, the purpose of environmental sampling (also called environmental monitoring) is to verify that the food processing plant sanitation program is actually effective at controlling the pathogen(s) of concern. As FDA notes, peer-reviewed research has shown that environmental monitoring can identify the presence of situations that can lead to contamination of food (8, 10). Thus, we recommend that *“FDA should require some form of environmental sampling of the food processing environment for an appropriate indicator microorganism or if more appropriate specific pathogens. The goal of this testing should be to provide a statistically based verification that the sanitation programs are achieving the appropriate level of microbiological control of the environment of the food facility.”* A food facility’s environmental verification program should be part of the facility’s preventive controls plan. The programs should be consistent with the guidance provided above in regard to selection of target microorganisms, frequency of sampling, and sampling sites.

As indicated elsewhere in this document, two of the foodborne pathogens of concern in relation to environmental sampling are *Salmonella* and *L. monocytogenes*. *Salmonella* is a concern for low-moisture foods that are typically processed in a “dry” manufacturing environment and includes such products as cereal, peanuts, nuts, nut butters (including peanut butter), spices, dried herbs, milk powder, chocolate, etc., while *L. monocytogenes* is of concern in “wet” food processing environments. As with testing for indicator microorganisms in foods, environmental indicator microorganisms are the primary means of verifying control of environmental contamination in the processing environment. The typical indicator microorganisms used for *Salmonella* are the *Enterobacteriaceae*, while the typical indicator used for *L. monocytogenes* is *Listeria* spp. (3).

As FDA notes in the proposed rule, *Salmonella* is member of the family *Enterobacteriaceae*, and thus at least on the face of it, *Enterobacteriaceae* would appear to be a possible indicator microorganism. The European Food Safety Agency (4), the International Commission on the Microbiological Specifications for Foods (6), and the FAO/WHO (2) concluded that *Enterobacteriaceae* are not a suitable environmental index microorganisms for predicting the levels of *Salmonella* in manufacture of powdered infant formula (PIF), but are useful for evaluating the degree of sanitation in PIF facilities. It is recommended that “*FDA should consider the use of environmental testing for Enterobacteriaceae in food facilities where this indicator microorganism provides a useful measure of the effectiveness of sanitation programs.*”

FDA also notes that it is generally recognized that *Listeria* spp. are “indicators” of the potential presence of *L. monocytogenes*, and that the agency’s current thinking is that *Listeria* spp. is an appropriate indicator microorganism for conditions that are likely to increase the risk that *L. monocytogenes* is present in the food processing environment. As mentioned above, *Listeria* spp. is not appropriate as an index microorganism (1) but is generally considered a good indicator microorganism. It is recommended that “*FDA should consider the use of environmental testing for Listeria spp. in food facilities where this indicator microorganism provides a useful measure of the effectiveness of sanitation programs.*”

When verification testing for the two indicator microorganisms above indicates that environmental sanitation programs are not achieving the level of control for which they are designed, the appropriate response should be to both increase the rate of testing for the indicator microorganism(s) and initiate testing for specific pathogens, i.e., *Salmonella* and/or *L. monocytogenes*, particularly on food-contact surfaces. This should continue until the level of the indicator microorganism returns to its baseline value. During the period of increased sampling, the identification of a target pathogen on food-contact surface should trigger the increased testing of products for the pathogen. Thus it is recommended “*FDA should develop a tiered response that leads to more intense testing when microbiological verification testing of the environment indicates that sanitation programs are not achieving the appropriate level of control.*”

Need for FDA to articulate performance standards that objectively articulate level of control required

In many ways this last issue is the most important one, as performance standards that objectively articulate a level of control allow more definitive answers to questions like “how often should foods be tested?” and “how many samples should be taken?”

Modern microbiological risk management systems like those proposed by Codex Alimentarius often speak of the Appropriate Level of Protection (ALOP) for microbial

hazards. ALOP is typically expressed in terms relevant to public health, such as a number of cases per 100,000 individuals. Using a risk-based approach of working backwards from an ALOP, considering different food products and their rate of consumption in the population, and given dose-response functions for given foodborne pathogens, it is possible to define a food safety objective (FSO). The FSO represents a prevalence and concentration of a pathogen in a food at the time of consumption that should be achieved to meet public health goals. While an FSO is a useful derivative of an ALOP, it still does not make it perfectly clear what a food processor needs to achieve to meet the ALOP.

Two related terms have been proposed to further assist food processors to do their part in meeting an ALOP (5). Those terms are the Performance Objective (PO) and Performance Criterion (PC). The PO is like a FSO, except at a mid-point in the food production process, i.e. it represents a prevalence and concentration of a pathogen at a point in the process. When a PO is regulatory in nature, it is a Performance Standard (PS). As a side note, the PC is defined as “the effect of one or more control measures needed to meet or contribute to meeting a PO,” and when it is regulatory in nature, it is sometimes called a Process Standard.

This ability to relate the stringency of a food safety system to these metrics is dramatically changing the process by which microbiological criteria are being established and interpreted. Instead of selecting criteria values arbitrarily or based on historic values, risk-based calculations are being used to relate testing requirements to PO values (5, 6, 12, 13). With a reasonable risk assessment, it is possible to calculate the microbiological performance that is needed to achieve a specific level of control and then relate that to specific sampling plans. Similarly, it is possible to start with a microbiological criterion and determine the PO value that is going to be achieved. This can be done for both traditional within-lot testing and between-lot process control verification testing. Thus, for complete transparency, the sampling plan associated with modern microbiological criteria should provide two performance metrics. First, a target level of control that is required (e.g., 99.9% of the servings have less than a specified level of a specified target pathogen or indicator microorganism) and second, the degree of confidence required of the sampling plan that this level of performance is being achieved (e.g., 95% statistical confidence). This type of information is required to demonstrate that microbiological testing requirements are transparent, risk-based, and science-based.

It is worth noting that FDA scientists, statisticians and applied mathematicians have played a key role in the development of these types of risk-based microbiological sampling concepts and techniques. It was surprising that such expertise was not reflected in the proposed regulation. It is also worth noting that this type of scientific information must be brought to bear if FDA implements microbiological testing programs that are subsequently challenged either nationally or internationally.

Based on the current state of the science of microbiological sampling it is recommended that *“FDA should articulate performance standards that clearly indicate the level of control and characteristics of microbiological sampling plans used in conjunction with food and environmental testing programs.”* While the current document focuses on testing for process verification, any use of lot testing required or recommended by the FDA should completely disclose the performance characteristics of the sampling regime. It is worth noting that the need to establish Performance Standards was one of the clear mandates articulated by Congress when it developed and passed the FDA Food Safety Modernization Act.

The need for flexibility and an approach for providing it

We conclude that the best way to address the issue of testing in a preventive controls system is by establishing a general framework for determining which facility-specific verification testing programs should be incorporated into a plant’s preventive controls plan (e.g., HACCP plan). A facility should articulate the types of microbiological testing they will perform and the rationale underlying these selections. Such programs should consider key GHP- and HACCP-based controls.

Such requirements should be developed by the food facility after consulting guidance from FDA, academia, and the industry. As an example of how this might be approached we offer a simple hypothetical decision tree that could be used by manufacturers to determine the focus of their testing programs.

Question 1: Is the microbiological testing being conducted being used for “hold-and-release” of a final product?

- a. Yes. Typically this is not testing for process verification but instead is a control measure that is integral to the food safety system, i.e. this is a monitoring activity, not verification. Advice should be sought regarding the characteristics and sampling plans for lot testing. Such testing may be for indicator microorganism or pathogens but needs to be appropriate for the “defect rate” that is likely to be encountered for the food of concern. This testing can be used for raw materials and in-line samples but is most often associated with end-product testing.
- b. No. Proceed to Question 2.

Question 2: Are the sources and frequency of contamination and process failures that lead to increased risk of the presence of foodborne pathogens known?

- a. Yes. Review list of potential microbiological hazards and indicator microorganisms and proceed to Question 3.
- b. No. Conduct a hazard review and conduct appropriate process control or baseline studies and then proceed to Question 2 again.

Question 3: Is this a food where microbiological testing for verification is feasible (i.e., the methods required can be undertaken by a majority of food microbiology testing labs)

and practical (i.e., requires the number of samples per day to be processed to be within the capability of the majority of food microbiology testing labs at a cost that is realistic to the value of the food)?

- a. Yes. Proceed to Question 4a.
- b. No. This is often associated with food where microbiological verification is not appropriate due to either the number of samples that would have to be tested or there are alternate approaches that are more effective. For example, microbiological testing of low-acid canned foods is generally not considered a viable approach to verifying process effectiveness. Instead, this is typically done by an evaluation of the thermal process and container integrity.

Question 4a: Is there one or more indicator microorganism that could be used effectively to measure the loss of control by the food safety system?

- a. Yes. Proceed to Question 5.
- b. No. Proceed to Question 4b.

Question 4b: Is there a pathogen that could be used to verify when the food safety system has lost control?

- a. Yes. Proceed to Question 5.
- b. No. A more detailed examination of the factors affecting the microbiological safety of the food is required. This may also be indicative of the need to identify an appropriate indicator microorganism or develop new detection technologies. For example, routine testing of food for noroviruses is currently considered impractical and potential surrogate indicators are of limited utility. However, targeted research in detection methodologies or common fecal indicators could change this situation rapidly. After review, if alternate approaches are discovered, return to Question 1. This may also require consideration of additional preventive controls being introduced if the hazard evaluation indicates that this is a significant public health risk.

Question 5: Has FDA or the industry provided guidance on the levels/frequency of the selected indicator microorganism or pathogen that is likely to occur for the food being produced when a food safety is operating “in control?”

- a. Yes. Use these values as a means for initially setting the parameters for a microbiological testing program for verification. Move toward implementation.
- b. No. Conduct a process control study. Typically, such studies last for 30 to 60 days to obtain sufficient data to determine the variability of the food production/processing system.

Once these questions have been successfully answered and the test microorganism(s) selected, the specifics of the testing program in terms of sampling plans, frequency of testing, and actions to be taken can be developed.

This is only one example of the types of decision trees and related tools that could be developed for food manufacturers to assist them in making informed decisions regarding the microbiological verification programs. Many related approaches can assist in maximizing effective manufacturing controls that protect public health while minimizing costs that will have to ultimately be borne by the consumer. It is recommended that *“FDA should work with industry and academia to develop appropriate guidance document that will assist manufacturers in designing and optimizing their verification testing programs.”*

SUMMARY

Microbiological testing has long been an integral part of ensuring the safety of foods in the United States. While the degree of sophistication in ensuring the safety of the food supply has changed the types of testing that is needed, it has not lessened the importance of appropriate testing programs. Considering the changes in the approach that will be implemented in the final regulation we believe that FDA should:

- Require microbiological verification testing programs for most foods,
- Such testing should be conducted by food facility operators,
- FDA should have ready access to the results of the testing and corrective actions taken when established criteria are not met, and should review such records during routine inspections,

- The specific indicator microorganisms or pathogens that will be the target of a food facility’s verification testing program should be articulated and justified by food companies as they develop their preventive controls plan,
- The FDA must recognize that flexibility is needed in the selection and approach to microbiological testing if such programs address the extreme diversity among foods and processing approaches for which it has regulatory responsibility,
- Where feasible, testing programs should focus on appropriate indicator organisms when possible to allow corrective actions to be taken before rejection criteria are exceeded,
- The testing programs should be consistent with sound principles of process control,
- The FDA should provide appropriate guidance documents on the concepts, principles, and methods for process control verification testing and process control statistics,
- The FDA should seek baseline microbiological data either from industry or by commissioning appropriate surveys, and
- The FDA should articulate performance standards and the statistical performance characteristics of all required sampling regimes.

It is our opinion that these attributes provide the required foundation needed if the FDA is going to be able to claim that science-based and risk-based microbiological testing for verification has been implemented as part of their new regulatory requirements.

REFERENCES

1. Alali, W. Q., and D.W. Schaffner. 2013. Relationship between *Listeria monocytogenes* and *Listeria* spp. in seafood processing plants. *J. Food Prot.* 76:1279–1282.
2. Buchanan, R. L., and R. Oni. 2012. Use of microbiological indicators for assessing hygiene controls for the manufacture of powdered infant formula. *J. Food Prot.* 75:989–997.
3. Cordier, J.-L. and ICMSF (International Commission on Microbiological Specifications for Foods). 2013. Microbiological criteria and indicator microorganisms. P. 81–90. In *Food Microbiology: Fundamentals and Frontiers*, 4th ed. M. P. Doyle and R. L. Buchanan, eds. ASM Press, Washington D.C.
4. EFSA (European Food Safety Authority). 2004. Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the microbiological risks in infant formulae and follow-on formulae. *The EFSA Journal* 113:1–35.
5. ICMSF (International Commission on Microbiological Specifications for Foods). 2002. “Microorganisms in Foods 7: Microbiological Testing in Food Safety Management.” Kluwer Academic Plenum Publishers. New York.
6. ICMSF (International Commission on Microbiological Specifications for Foods). 2011. “Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance.” Springer Science+Business Media, New York.
7. Jain, S., S. A. Bidol, J. L. Austin, E. Berl, F. Elson, M. LeMaile-Williams, M. Deasy III, M. E. Moll, V. Rea, J. D. Vojdani, P. A. Yu, R. M. Hoekstra, C. R. Braden, and M. F. Lynch. 2009. Multistate outbreak of *Salmonella* Typhimurium and Saintpaul infections associated with unpasteurized orange juice – United States, 2005. *Clin. Infect. Dis.* 48:1065–1071.
8. Jarl, D. L., and E. A. Arnold. 1982. Influence of drying plant environment on salmonellae contamination of dry milk products. *J. Food Prot.* 45:16.
9. Jogenburger, I., M. W. Reij, E. P. J. Boer, M. H. Zweitering, and L. G. M. Gorris. 2012. Modelling homogeneous and heterogeneous microbial contaminations in a powdered food product. *Intl. J. Food Microbiol.* 157:35–44.
10. Pritchard, T. J., K. J. Flanders, and C. W. Donnelly. 1995. Comparison of the incidence of *Listeria* on equipment versus environmental sites within dairy processing plants. *Intl. J. Food Microbiol.* 26:375–384.
11. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. -A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States – major pathogens. *Emerg. Infect. Dis.* 17:7–15.
12. van Schothorst, M., Zwietering, M. H., Ross, T., Buchanan, R. L., Cole, M. B., and International Commission on Microbiological Specifications for Foods. 2009. Relating microbiological criteria to food safety objectives and performance objectives. *Food Cont.* 20:967–979.
13. Whiting, R. C., A. Rainosek, R. L. Buchanan, M. Miliotis, D. LaBarre, W. Long, A. Ruple, and S. Schaub. 2006. Determining the microbiological criteria for lot rejection from the performance objective or food safety objective. *Intl. J. Food Microbiol.* 110:263–267.