Microbiological Testing by Industry of Ready-to-Eat Foods Under FDA's Jurisdiction for Pathogens (or Appropriate Indicator Organisms): Verification of Preventive Controls

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2018-2020 NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS

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EXECUTIVE SUMMARY

FDA's final rule "Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" (the CGMP & PC rule) *(46)* requires a facility that has identified hazards requiring preventive controls to verify that the preventive controls are consistently implemented and are effectively and significantly minimizing or preventing the hazard. Verification activities for preventive controls for microbial hazards include, as appropriate to the facility, the food, and the nature of the preventive control and its role in the facility's food safety system, product testing for a pathogen (or appropriate indicator organism). FDA is seeking advice from the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) on 1) the utility and necessity of industry testing certain ready-to-eat (RTE) foods for pathogens and 2) criteria industry could apply in determining what, if any, microbiological testing is appropriate for verifying pathogen control for the RTE foods produced in a facility. As these are FDA inquiries, the scope of NACMCF's advice includes responses for dairy products, grain-based products, meals and entrees, nuts and nut/seed products, fruits and vegetables, and spices and herbs.

The intent of this document is to provide examples and advice for manufacturers/processors to establish their own microbial targets and limits to meet preventive control requirements. It offers guidance for using microbiological testing for pathogens (or appropriate indicator organisms) to verify process control for pathogens in RTE foods under FDA's jurisdiction. Advice provided by NACMCF is intended to guide decisions to be made by each firm based on their facility, ingredients used, processing, packaging, level of anticipated control, shelf life of the product, intended use, or potential storage and handling at retail or by the consumer. The NACMCF was specifically charged with offering

guidance on: 1) principles and criteria a company should apply in determining the need for and in designing an effective microbial testing program to verify that processes are effectively controlling microbial pathogens; 2) situations in which testing other than for pathogens or indicator organisms would be an appropriate verification activity for a company; 3) situations where verification testing by a company would not be necessary if there is evidence that the appropriate treatment was, in fact, applied; 4) when microbial testing is an appropriate verification activity, considerations a company should apply in selecting the test microorganisms and what are appropriate indicator microorganisms for verifying processes that adequately control pathogens; 5) principles and criteria a company should apply in determining the frequency of testing finished product to determine if the company's food safety system for that product is effective; 6) situations in which testing at sites other than at the end of the process can achieve the goal of verifying the adequacy of control of microbial hazards; 7) the impacts of environmental monitoring on frequency and extent of product testing verification activities by companies; and 8) criteria and action a company should apply in determining that microbial testing results indicate a loss of process control and 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.

BACKGROUND

In 2015, FDA published its final rule "Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" (the CGMP & PC rule) in title 21 of the *Code of Federal Regulations* (CFR) part 117 *(51)*. A facility that has identified hazards requiring preventive controls must verify that the preventive controls are consistently implemented and are effectively and significantly minimizing or preventing the hazard. As specified in 21 CFR 117.165, verification activities for preventive controls for microbial hazards include, as appropriate to the facility, the food, and the nature of the

preventive control and its role in the facility's food safety system, product testing for a pathogen (or appropriate indicator organism). FDA has indicated that such product testing is a verification activity to help assess and verify the effectiveness of a food safety plan and the facility's capability to consistently deliver against it, not to establish the acceptability of every lot or batch.

Because of the flexibility FDA provided in the rule, advice from NACMCF on 1) the utility and necessity of industry testing ready-to-eat (RTE) foods for pathogens and 2) criteria industry could apply in determining what, if any, microbiological testing is appropriate for verifying pathogen control for the RTE foods produced in a facility, would be highly beneficial for industry. Such advice should include the test microorganism(s), the sampling plan that should be used, the type of test (*e.g.,* presence/absence or enumeration), the frequency of such testing, interpretation of results, and actions to take when such testing indicates a loss of control. Advice from NACMCF should address the appropriate use of enzymatic indicators that heat-based processes have been applied (*e.g.,* alkaline phosphatase for pasteurization of milk) and whether there are situations where verification testing of products by industry would not be necessary if there is evidence that the appropriate treatment was applied.

A 2013-2015 NACMCF Subcommittee addressed a charge from the Department of Defense (DoD) on Microbiological Criteria as Indicators of Process Control or Insanitary Conditions *(35)*. That charge was to develop microbiological and other possible criteria for DoD auditors to better evaluate process control and insanitary conditions at the point of production. Some of the information developed in the final report of that Subcommittee *(35)* were considered in addressing this charge. However, the focus here is on practical advice for manufacturers/processors subject to the preventive control requirements in 21 CFR part 117 about when they should use microbiological testing for pathogens (or appropriate indicator organisms) to verify process control for pathogens in RTE foods under FDA's jurisdiction. For

this document, process control refers to the entire operation (e.g., entire food safety system/process). It is not restricted to process preventive controls. A food safety system and the manufacturing process managed by that system are in control when, within the limits of a stable and predictable process variation, all food safety hazards are controlled to an acceptable level *(29)*.

Food categories of concern include:

Dairy Products

- Butter, margarine
- Cheese, hard (e.g., Cheddars), extra hard, grating (e.g., Parmesan, Romano)
- Cheese, fresh (Queso fresco), soft, soft-ripened (Camembert), semi-soft (Edam, Gouda), veined cheeses (Roquefort, Gorgonzola)
- Cultured, pH < 4.8
- Cultured, pH > 4.8 and <5.4
- Dried products (including dairy ingredients used to make infant formula)
- Frozen desserts
- Milk and milk products (fluid)

Grain-Based Products

- RTE baked items, refrigerated or time-temperature controlled for safety (TCS)
- RTE baked items, shelf stable or non-TCS
- RTE cereals
- RTE cold-pressed bars

Meals and Entrees

- RTE deli salads
- RTE sandwiches
- "Heat and eat" meals/entrees

Nuts (including tree nuts and peanuts) and Nut/Seed Products

- RTE nuts not processed for lethality (e.g., chopped untreated tree nuts)
- RTE nuts processed for lethality (e.g., roasted tree nuts, almond milk, coconut milk)
- RTE nut/seed butters processed for lethality (e.g., peanut butter, sunflower butter)

Fruits and Vegetables

- RTE fresh-cut fruits (e.g., cut melon, sectioned grapefruit, sliced pineapple)
- RTE fresh-cut vegetables (e.g., cut celery stalks, peeled baby carrots, sliced mushrooms, shredded cabbage, chopped lettuce)
- RTE dried/dehydrated fruits (e.g., dried cranberries, raisins, dried apricots)
- Packaged uncut leafy greens (e.g., spinach leaves, baby greens leaves)

Spices and Herbs (include consideration for intrinsic properties in certain spices and herbs (e.g., cinnamon, cloves, oregano) that can interfere with test methodology and risk from added components in spice blends)

- RTE spices and spice blends, not processed for lethality
- RTE spices and spice blends, processed for lethality
- Dried, chopped herbs

CHARGE QUESTIONS TO THE COMMITTEE

1. For the food categories listed above, 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?

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

3. Are there situations where verification testing by a company would not be necessary if there is evidence that the appropriate treatment was, in fact, applied?

4. When microbial testing is an appropriate verification activity, 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 processes that adequately control pathogens?

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?

6. Generally microbial testing by a company to verify process control is conducted on "finished product." Are there situations in which testing at sites other than at the end of the process can achieve the goal of

verifying the adequacy of control of microbial hazards? Describe the situations and the testing that would be appropriate.

7. The CGMP & PC rule requires environmental monitoring for an environmental pathogen (*e.g., Listeria monocytogenes, Salmonella*) or for an appropriate indicator organism as a verification activity if contamination of an RTE food with an environmental pathogen is a hazard requiring a preventive control (such as sanitation controls). What impact does environmental monitoring have on frequency and extent of product testing verification activities by companies? Note: Committee changed "should" to "does" for responding to this charge.

8. What criteria should a company apply in determining that microbial testing results indicate a loss of process control? What actions should a company take if test results indicate a loss of process 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?

COMMITTEE'S APPROACH TO ANSWERING THE CHARGE

The Committee leveraged the expertise of the Committee members, additional experts, published literature and government documents to develop guidance for firms considering product testing (in process or finished product) as an activity to verify that their pathogen controls are effective. In addition to answering charge questions, appendices were developed for each food grouping as examples of considerations in choosing type and frequency of microbial testing. With rare exceptions noted in the tables within each appendices, microbial targets and limits are not for lot disposition. Rather, the examples provide reference points for expected microbial population limits in foods that are produced with good quality ingredients, validated lethality steps or other process controls, and rigorous

sanitation and environmental monitoring programs. Each firm should establish their own microbial targets and limits depending on the facility, ingredients used, processing, packaging, level of anticipated control, shelf life of the product, intended use, or potential storage and handling at retail or by the consumer.

INTRODUCTION

Historically, the role of HACCP was to effectively control hazards such as microbial contamination and if properly implemented, would reduce the need for finished product testing for pathogens. But, while this concept works to reduce or eliminate pathogen testing for some foods, other food products still rely on frequent finished product testing for pathogens, whereas other foods focus on testing for indicator organisms to ensure process control.

Each individual firm should consider if microbial testing of product is an appropriate verification activity, and if so, what are the target microorganisms that are appropriate for a given commodity? Should pathogens or indicators organisms be tested, or both? What is the role of environmental monitoring and can it be sufficient?

Microbial testing results can serve as an early warning that the process is drifting out of control or signal potential catastrophic failures. Data collected (e.g., enumeration of indicator organisms, positive environmental tests) should be analyzed on an ongoing basis for trends, be used to develop statistical process control, modify microbial limits as appropriate, and establish responses to results that exceed those limits.

RESPONSES

Charge Question 1. For the food categories listed above, 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?

Microbiological testing of in-process or finished product is appropriate for some, but not all, ready-to-eat (RTE) foods to verify preventive controls in a Food Safety Plan. While finished product testing is generally not effective for controlling food safety, testing can be used for process and product verification *(30, 55)*. Product testing can verify that the overall production continuum is in control as the final product reflects the adequacy of the processing system controls and the processing environment in combination. In addition, finished product testing can be useful in detecting catastrophic failures. A food processing facility can apply several criteria to determine whether microbiological testing is appropriate for in-process or RTE finished products. The following eight questions were used to determine the conditions that determine if microbiological testing is appropriate for each commodity group and their example foods. A comparison of answers to each question for the various commodities is shown in Table 1. Detailed answers to questions for each commodity are provided in Appendices A-F.

Criteria questions:

1. **Have pathogens been associated with the food or its ingredients and has the food been associated with foodborne illness?** All of the raw commodities (i.e., those without a lethality step) discussed in this document have been associated with pathogens and/or foodborne illness. Such pathogens include *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), *Campylobacter*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium*

perfringens, and *Clostridium botulinum*. Depending on the processing environment and food, a frequent concern is post-lethality contamination. Foodborne illness can result from long-term survival of low infectious dose pathogens such as *Salmonella* or growth of *L. monocytogenes* in perishable foods at refrigerated temperatures. Spore forming bacteria survive cooking and pasteurization that are designed to kill vegetative pathogens; inadequate acidification, and/or temperature control have led to growth of toxigenic bacteria and been associated with foodborne illness. Parasites such as *Cyclospora* have also been associated with some raw agricultural commodities. However, there are no reliable testing methods for *Cyclospora.*

2. **How likely are ingredients to be contaminated, given the nature of the ingredient and the robustness of the supplier programs?** The likelihood that ingredients are contaminated depends on the source of the ingredient and the potential exposure to contaminated environments (e.g., raw milk, grains, spices, plant-based materials grown in or harvested from the ground) and whether they have received a validated robust lethality process. Food ingredients that have been harvested or processed to minimize contamination (e.g., ingredient grown using good agricultural practices; use of sanitizers to reduce cross contamination between produce items) or receive some lethality step (e.g., irradiated spices, roasted peanuts) have a lower probability of being contaminated but often rely on supplier control programs to prevent post-lethality contamination.

3. **Are there robust processing control procedures such as a kill step or other reduction methods controls?** Validated lethality steps such as thermal or high-pressure treatments (milk, juices), roasting (nuts/seeds), and baking (bakery) reduce the need for final product testing as a verification of preventive controls. However, even though vegetative microorganisms may be

destroyed, control processes need to be in place to prevent growth of toxigenic organisms during production (e.g., *B. cereus* in batters, fillings) to ensure heat-stable enterotoxins are not present after cooking; hence in-process testing may be relevant in these circumstances.

Even if a kill step is used sometime during processing, products that introduce ingredients post-lethality (e.g., lettuce to a sandwich, herbs to cheese curd, icings on baked goods), particularly addition of ingredients that are raw or minimally processed, will be at higher risk for containing pathogens and may need testing of the individual component or the finish product. Products with a short shelf-life present challenges for testing. While raw produce is washed, those washes do not necessarily achieve substantial microbial reduction in the food. Suppliers of produce to be consumed without a kill step need to comply with appropriate control measures to prevent or minimize pathogen contamination (for examples of control measures, see the Produce Safety Rule 21 CFR Part 112 *(47)*).

Although thermal treatments are common microbial reduction steps, the formulation of a commodity may also reduce risk of microbiological contamination and hence the need for product testing. For example, cold-filled acidified foods, such as prepared mustards, hot-sauces, acidified cucumbers, or salad dressings made with vinegar, frequently rely on an acid-hold procedure for lethality as an alternative to thermal processing *(6, 7, 25, 33, 42)*. In other foods, the acidity alone may not be sufficient to generate an appropriate (e.g., 5-log) kill of vegetative pathogens within several hours or days, but there may be a more gradual inactivation over time. Cultured dairy products, such as yogurt and sour cream, frequently have sufficient lactic acid production (e.g., pH decreases to <4.8 within 4-18 h) to inhibit growth of pathogens during production but also to generate additional inactivation (e.g., 1-log) during refrigerated storage

(18, 19, 34). However, acid type also has an effect on lethality rate during thermal processing and for acid-hold lethality. For example, for foods acidified with citric acid, the killing may be relatively slow, whereas foods with predominantly acetic acid (such as pourable salad dressings) may result in shorter death times *(1, 9, 42)*. Hard cheeses made with unpasteurized milk rely on a combination of high-quality milk, acidity (typically lactic or propionic acid), reduced moisture (a_w), and extended aging for pathogen reduction, although there is evidence that more than 60-day aging may be required for safety *(15, 16, 49)*.

Other commodities with low a_w (dried nuts/seeds) may also undergo slow pathogen reduction *(17, 39)*. However, because the pathogen survival time may be measured in months, there likely is not enough time for sufficient reduction in pathogen numbers to exclude the need for product testing.

4. **Is there potential for microbial recontamination of product prior to packaging? Could there be pathogens due to environmental or handling contamination?** Except for foods that are hot-filled, filled within a closed system, or which receive an in-package lethality step, all commodities have the risk of contamination from handling or from the environment.

5. **Does the product formulation allow microbial growth or survival or cause death under conditions of transportation and various types of storage (refrigerated, frozen, ambient)?** Microbial survival, growth, or death may occur as a result of intrinsic properties of the food, such as pH, acid type, water activity, salt levels, or formulation with preservatives or due to extrinsic properties such as packaging environment and transportation/storage temperatures. Verification testing may be indicated where storage conditions alone (freezing or refrigeration),

rather than intrinsic properties of the foods, are the primary barrier to microbial growth, and process and environmental controls cannot ensure absence of the pathogen. For products that do not support growth of pathogens at ambient temperatures but have a history of post-lethality contamination by low-infectious dose pathogen (e.g., peanut butter, dry milk, chocolate), testing may be relevant to detect catastrophic failures (see appendices for examples).

6. **Is this product meant for higher risk (sensitive) population?** In most of the example foods (Appendices A-F), the product is being made for the general population, but may be consumed by individuals in higher risk populations. Special considerations should be given to foods that are specifically manufactured for infants, elderly, pregnant, and immunocompromised or hospitalized consumers (e.g., milk powders used for infant formula and infant cereal, foods destined for nursing homes or hospitals).

7. **What is the shelf life of the product?** Shelf life plays a role in the potential for microbial growth as well as timeframe in which testing results will need to be available before the product is distributed and consumed. The shelf lives of the example food products in this document range from several days to 1-2 years. A longer shelf life increases the time available for microbial growth, potential for temperature abuse, and the risk that a consumer may eat a contaminated food (e.g., *L. monocytogenes* on soft cheeses). While short shelf life reduces the time for microbial growth under normal storage conditions, it may be impractical to get results from pathogen testing of the food prior spoilage (e.g., being able to detect *Salmonella* in cut melon or STEC on leafy greens).

8. **Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?** Considerations should be given to the potential for abuse of the food by the consumer once it leaves the control of the manufacturer and retail chain. Does the consumer heat the food to reconstitute it or for palatability or eat it without further preparation? Is it likely that the consumer will hold a frozen food under refrigeration or hold a refrigerated food at temperatures greater than 4°C? How likely is a consumer to use a refrigerated food beyond the use-by date, particularly if the food is not grossly spoiled?

Microbiological testing for verification of process control (as part of the facility's food safety system) is different from microbiological testing for lot acceptance.

Prior to widespread use of preventive controls, traditional microbiological testing has been lot testing for acceptance or rejection of that lot (i.e., to demonstrate that the lot is appropriate for its intended use). The purpose of lot testing is to examine a product lot for which you have no information *(8)*. This testing can be useful when, for example, a government agency tests imports at the port of entry, or a food business tests an ingredient from a new supplier. Such testing should involve analysis of a large of number of samples randomly taken from the entire volume of food under consideration *(8)*. Industry also uses "hold and release" testing for certain ingredients prior to use or in response to microbiological contamination issues. Such testing is useful to detect high rates of contamination, but it is not very effective when food safety systems are under control or to detect low rates of contamination.

The purpose of microbiological testing for verification of process control is not to demonstrate that a lot of food is safe, but instead to demonstrate that control measures are functioning as intended *(8)*. Rather than testing a large number of random finished product samples from a lot, a few finished product

samples are taken from many lots on a regular basis (routine testing). Also, samples may be taken at several intervals during production of a lot in order to detect contamination that may occur sporadically during production; often these are composited into one or more test samples. The results of the tests are analyzed to look for trends and to determine whether they meet an established criterion or indicate an out-of-control process. Testing may be conducted at a relatively high frequency initially to determine process capability. Past performance could be used to reduce the amount of testing over time *(55)*.

Microbiological testing of finished product for verification of process control can provide risk reduction, since the removal of any lots testing positive for a pathogen prevents that product from reaching the consumer. In addition, if investigations into the root cause of circumstances that led to the presence of a pathogen or to exceeding a process control criterion identify the source of the problem, this can be corrected, which will lead to the production of safer food in the future.

Microbiological testing of finished product is most useful (1) if ingredients in a food have the potential to contain pathogens and there is no kill step (or a marginal kill step) in the manufacture of the finished product, and/or (2) when finished products is reasonably likely to be contaminated from the environment.

Use of microbiological testing as a verification of control measures should consider risk to the consumer. Testing is more valuable if the pathogen of concern is likely to cause serious adverse health consequences or death, e.g., *Salmonella* vs. *Staphylococcus aureus*. Where there is a low risk to consumers, microbiological testing would be infrequent or there would be no testing.

Microbiological testing should be increased when information indicates that the operation is not under control (e.g., records indicate a deviation at a critical control point, CCP, a pathogen has been detected on a food contact surface or in the finished product, a food has been involved in illnesses).

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 a facility. In determining testing of finished product, a firm should consider all programs in place to minimize the potential for the finished product to be contaminated. Having confidence that a supplier has implemented a robust program to minimize the potential for pathogens to be present in ingredients is one of the components of the food safety system being verified. Similarly, when the source of a pathogen in a finished product could be from the processing environment, having a robust sanitation and environmental monitoring program can significantly reduce the need for finished product verification testing.

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 smaller samples. For example, taking small samples (e.g., 10-25g) on a frequent basis (e.g., every half hour) throughout a process run and testing a composite (e.g., 375 g, or multiple composites) provides more information on process control than taking a sample of the same weight (e.g., 375 g) from one or more packages, because contamination is generally expected to be nonhomogeneous and it provides a better picture across the day's production *(31)*. For certain commodities, such as dry dairy products, use of autosamplers are used to take samples throughout production and composite samples analyzed for target microorganism *(43)*.

Microbial test methods must be appropriate for the intended use (e.g., for detection of the test microorganism(s) in the specific food). To ensure reliable results, test methods should be validated to show they can detect the microorganism of concern in the specific food. For example, many spices have inhibitory properties, and the method used when testing the spice must consider this fact, e.g., by dilution of the inhibitors to the extent that the organisms of concern can grow.

Microbiological testing for process control can be used to drive excellence in quality and process improvement. Testing for microorganisms that are in sufficient numbers to enumerate and then striving to reduce those numbers as low as possible can enhance product quality. Knowing the expected range of counts can identify when a change has occurred in the system (e.g., faulty practices) by detecting numbers that are outside the range; investigation as to why the numbers increased can lead to the identification of a processing failure, an increase in microbial load in an ingredient, or another aspect of the process that warrants greater control.

SUMMARY AND CONCLUSIONS

This document provides examples and advice for manufacturers/processors to establish their own microbial targets and limits to meet the preventive control requirements about using microbiological testing for pathogens (or appropriate indicator organisms) to verify process control for pathogens in RTE foods under FDA's jurisdiction. These decisions are made by each firm based on their facility, ingredients used, processing, packaging, level of anticipated control, shelf life of the product, intended use, or potential storage and handling at retail or by the consumer.

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

Naturally occurring enzymes in raw commodities are heat sensitive and are therefore suggested as an alternative to use of other temperature-time monitoring to verify that a lethality step has been applied. However, the use of enzyme-based tests to verify the adequacy of processing is limited, particularly for multi-component foods. For enzymes to have practical application to be used as verification in lieu of product testing, they should:

- Have inactivation kinetics in the processing range that are similar to those of the pathogens of concern.
- Be consistently present at high enough levels such that the absence of detectable enzymatic activity does not occur before adequate inactivation of the pathogens of concern.
- Not be reactivated within the timeframe needed for testing the food.
- Be detected using procedures that are rapid, inexpensive, and easy to perform in a food processing setting.

The inactivation kinetics of the enzyme determined in a food ingredient in which the enzyme is present may be different when the ingredient is combined with other ingredients, and thus may no longer reflect the inactivation of the pathogen of concern. Therefore, testing for indicator microorganisms may be more practical for process verification than testing for enzymes.

Several non-microbial indicators have been identified. Alkaline phosphatase is used as an indicator of milk pasteurization *(38, 45)*. Electron paramagnetic spectroscopy can be used to detect changes in cellulose in spices in response to gamma irradiation *(40)*. Peroxidase has been used for validation of blanching in vegetable products *(28)*. The peroxidases in carrots and potatoes maintained approximately 50% of their activity after heating for a minute at 85°C *(4)*; this time and temperature combination is considered to be generally sufficient to generate a 6-log reduction of *L. monocytogenes* in many food matrices *(37)*. Thermostable deoxyribonuclease (DNase) is a product of pervasive staphylococcal growth; its presence indicates possible enterotoxin contamination in cheeses and sausages *(24, 44)*. Other non-microbial testing verification activities may include monitoring of the rate of acid production (pH, titratable acidity) during production of cheese and cultured dairy products that assures adequate competition with pathogens to prevent growth during fermentation.

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

For some foods, there is little or no benefit from microbial testing if validation and monitoring affirm that the lethality process is sufficiently robust and appropriately implemented, provided there is no opportunity for recontamination; in these instances, measuring processing parameters (e.g., temperature and time) provides adequate verification that pathogens have been controlled (e.g., foods in which a lethal treatment is delivered to product in the package).

These foods include products that are processed (e.g., validated lethality process) and hot-filled or packaged under aseptic conditions in which contamination of the food after processing is prevented, or processed in the package (e.g., cook-in-bag). The use of "clean fill" technology for certain extended shelf-life foods, such as some beverages, yogurts, and desserts, can provide protection from recontamination. For aseptic and clean-fill foods, monitoring of the parameters of the process and verification activities other than finished product microbiological testing should be sufficient.

There are also products in which the formulation is validated to be lethal to the pathogens of concern (e.g., vinegar, highly acidic juices such as lemon and lime, many mayonnaise or pourable acidified dressing formulations). Verification of formulation control (e.g., measurement of pH and total acidity) can provide appropriate evidence that that pathogens have been controlled.

For raw foods that are not subjected to a lethality step, and for foods that are subjected to post-lethality handling with potential for recontamination, verification testing is appropriate. Some of these products include untreated spices, fresh fruit and vegetables, nuts, sandwiches, and deli salads.

However, for most of the foods under consideration, food safety control will involve monitoring process parameters, ingredient testing, supplier audits, enforcement of employee hygienic practices, and a robust sanitation program verified in part by environmental monitoring/testing for microbiological indicator organisms, and records review that is supplemented by verification testing of food for pathogens or, more commonly, by indicator organisms. The extent of verification testing will depend on the confidence in the process, including how much safety is built into the process, and the other programs in place.

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

A company considering conducting microbiological testing as a verification activity should include several factors related to the possible presence of microorganisms and the type of test. One fundamental question to address is whether to test for a specific pathogen or to test for another microorganism that can indicate the potential presence of the pathogen of concern or conditions that could lead to its presence. While microbiological testing for indicator organisms (e.g., aerobic plate count, Enterobacteriaceae, coliforms, or molds in product, or *Listeria* spp. or Enterobacteriaceae in the environment) does not necessarily mean that pathogens are present, trends of "out of spec" populations of these organisms indicate that investigations are warranted to determine root cause and to evaluate the impact on the safety of the food.

In situations where microbial testing is deemed an appropriate verification activity, several criteria should be considered in selecting the microorganisms:

a. Which pathogens have been associated with the specific food or ingredient based on epidemiological and historical evidence?
b. Is there a relevant indicator organism that is more likely to be present in a given commodity or processing environment than a pathogen (such as testing for *Listeria* spp. as an indicator for *Listeria monocytogenes*)?
c. What impact do process steps have on the viability of pathogens or indicator microorganisms (is a thermal process sufficient to kill STEC but allow lactic acid spoilage bacteria to survive; do spores survive the process; is there a potential for growth of microbes during extended runs)?
d. What is the potential for recontamination of the food product after treatment and what are the microorganisms involved?
e. What are the intrinsic and extrinsic characteristics of the food that may be conducive/selective for specific microorganisms to grow or survive?
f. Is the food specifically intended for those individuals with higher susceptibility for infection to the pathogens of concern (e.g., hospital meals, infant foods)?

g. What is the expected shelf-life of the food product? Is it practical to get microbiological tests before the end of shelf life and still market the product (e.g., hold-test for short shelf-life products)?

The type of test to be used will depend on the validated microbiological methods available for a given matrix, as well as regulatory requirements. Enumeration of a pathogen in a food is appropriate when the risk of illness is related to the number of organisms present (e.g., *B. cereus, C. perfringens, S. aureus*). For low-infectious dose pathogens (e.g., *Salmonella,* some strains of Shiga-toxin producing *E. coli*, *Cyclospora*), some performance standards require detecting a single colony forming unit (CFU) in 25 g or more. Because routine plating methods are typically limited to detecting a lower limit of 10 CFU per g, many pathogen testing protocols are restricted to determining the presence or absence of the pathogen within a given sample size. In the case of some pathogens, such as *Cyclospora,* enumeration methods do not currently exist. Although higher numbers of pathogens, such as *Salmonella,* reflect greater risk for consumers, enumeration is not needed to take action in response to positive findings.

When food safety systems are under control, the presence of the pathogens of concern is not likely, and when present, they are likely to be heterogeneously distributed, and may be at a low level that is difficult to detect *(31)*. Thus, testing for other non-pathogenic indicator microorganisms that are likely to be present more frequently and in greater numbers provides the advantage of being able to detect processes in which controls have not been adequately implemented or processes that are drifting out of control and thus are at increased risk of pathogens being present *(8)*. The choice of indicator organism should consider if there is sufficient scientific evidence that the microbe is relevant for the food type and pathogen of concern *(10, 14, 29, 30, 32)*.

Trend analysis of indicator organism populations should be able to detect when controls may require corrections before pathogens become a problem or may indicate how likely that pathogen contamination has occurred; presence or populations of indicator organisms that exceed the preset limits requires investigation to prevent contaminated product from entering commerce *(54)*. Depending on the results of testing the food (or environment) for indicator organisms, testing the food for the pathogen may be appropriate.

Lastly, the type of testing selected should consider if there is a validated test for the pathogen of concern in the specific food matrix and the speed of detection that allows timely decisions regarding corrective actions or product disposition.

Charge 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?

The frequency of testing for a finished product depends on a variety of factors, including ingredients used in the food, whether or not the food has had a validated robust lethality process, whether the food is packaged to prevent recontamination, whether the food is intended for a high-risk population, sanitation controls, and whether environmental monitoring suggests the potential of recontamination (see Appendices A-F of this document for specific examples). Buchanan and Schaffner *(8)* indicate that two key factors related to frequency of testing are the frequency at which a testing criterion will be exceeded and the response time that is needed in declaring a system is out of control, which are typically determined as part of a "process control study." Testing more frequently will be more effective in identifying a loss of process control. Testing frequency should be increased when there is indication of loss of control in order to assist in root cause analysis and to more quickly determine when control has been restored *(8)*.

In the case of products with a terminal, validated lethality process in the package (e.g., cook-in-bag, high-pressure pasteurization of the package, or hot-fill) or those filled in a closed system (e.g., pasteurized milk), routine testing of finished product for pathogens may not be needed. Rather pathogen testing may be limited to situations where process control parameters are not met (e.g., when evaluating deviations for controls such as kill temperatures/time, cooling rate, or storage temperature). Typically, testing can be limited to spoilage microorganisms that are indicators of shelf-life related to quality of ingredients used or additional verification of process control such as such as *Pseudomonas* spp. in pasteurized milk or lactic acid bacteria in cook-in-bag products.

For products that have a microbial reduction processing step but that are subsequently exposed to the environment prior to packaging (e.g., products made with roasted nuts, butter or soft cheeses made with pasteurized cream or milk, baked cakes), lot testing for indicator organisms is frequently used as the primary verification of process control (see appendices for examples). Pathogen testing of finished product may be useful as a periodic check for process control (such as quarterly or as risk assessed). More frequently, finished product pathogen testing is indicated if investigative testing from an Environmental Monitoring Program (EMP) for *Listeria* or *Salmonella*, suggests there is potential cross-contamination to the product from the environment, either inherently due to design and construction of the facility or equipment or due to the recurring presence of these pathogens in zones 2 or 1. In these

cases, the implicated product is held and tested for the pathogen using a statistically based sampling program and validated detection method to determine contamination.

However, in cases of short shelf-life foods (e.g., prepared sandwiches, cut melon, deli salads), testing of finished product for pathogens is impractical because the held product may be at the end of shelf life by the time results are confirmed. For these types of products, supplier control programs and EMP are more effective than finished product testing for pathogens. Microbial testing of product is focused on trending indicator organisms to identify loss of process control as a supplement to supply chain control for ingredients and robust sanitation/environmental controls (refer to appendices for examples).

For most products considered in this document, that have a long shelf stable shelf-life, unless there is a loss of process controls during production, environmental monitoring indicating a problem, or breakdown in supplier control programs, finished product testing might consist primarily of periodic testing for spoilage organisms for shelf-life verification or for microbial indicators of loss of process control (including sanitation processes).

One situation where pathogen testing of RTE foods or ingredients with a long shelf life may be appropriate is for products that have a history of microbial contamination (e.g., milk powders). In these cases, hold and testing may be frequent, such as for lot-disposition. In general, the frequency of lot testing of the final product is determined by an assessment of risk. If the time for processing after lethality is long (such as days), or if product has multiple points of exposure to recontamination after the lethality step, frequency of testing will be greater than if the product is rarely handled and risk of exposure is limited.

Charge Question 6. Generally microbial testing by a company to verify process control is conducted on "finished product." Are there situations in which testing at sites other than at the end of the process can achieve the goal of verifying the adequacy of control of microbial hazards? Describe the situations and the testing that would be appropriate.

There are situations where testing or verification other than microbial testing at the end of the process (i.e., finished product testing) can achieve the goal of verifying the adequacy of microbial hazard control (see Table 2 for comparison of testing for various commodities and Appendices A-F of this document for details). Alternative sites and strategies include, but are not limited to, ingredient testing by suppliers or processors, robust environmental monitoring, and in-process product measurement of food qualities (such as rate of acid development during fermentation) that affect microbial growth. Selection of strategies will be influenced significantly by commodity/food characteristics (for example pH or a_w values in food that are able to support growth vs. being inhibitory), use of a validated microbial kill-step, and the degree of post-lethality handling.

In some cases, an ingredient is used in manufacturing a food where there is no additional control applied for a hazard associated with that ingredient. In such instances, microbiological testing of the ingredient prior to use can be an important measure in ensuring control of a hazard. Such testing is often conducted by the supplier (usually the supplier contracts with an independent accredited laboratory for the testing) and a certificate of analysis (COA) is provided to the customer. COAs provide assurance of the suppliers' control processes at the time of sampling and testing. COAs may not be needed for each shipment of an ingredient. The frequency of such testing depends on many factors, including the likelihood and severity of illness if the hazard were present in the ingredient, knowledge about the food safety system implemented by the supplier (e.g., obtained through an audit), and the safety history of the ingredient received from the supplier. It is recommended that testing ingredients from a supplier be periodically performed by the customer to verify the efficacy of the supplier's control programs. The frequency of periodic testing should provide confidence that suppliers' programs are indeed effective. Written procedures for the sampling plan should include how to collect and prepare the samples, and describe the analytical methods used. Testing of ingredients is not warranted when the manufacturer uses the ingredient in a product for which there is a process control measure that would address that hazard (e.g., a kill step), unless the manufacturer's control measure is dependent on the ingredient containing a low pathogen load (which could be reflected by samples testing negative for a pathogen).

Testing of food characteristics such as pH or a_w can also be performed on in-process product or finished product and can replace microbiological testing of finished product. For example, during a fermentation process, the pH of in-process product could be measured to monitor the acid production that can control microbial hazards. When characteristics such as pH and a_w are relevant to the safety of the product, periodic testing intervals of the food product batches should be established. Using food characteristics as process control parameters requires establishing and maintaining records to include equipment calibration, monitoring and verification of the parameters, review of the process control records, and any corrective actions. As noted above, the rapid reduction of pH may be important in controlling pathogen growth in a food fermentation process; similarly, the reduction of moisture or a_w during a drying process may be important to monitor. If these steps are under control, testing for pathogens such as *S. aureus* or *B. cereus* or their enterotoxins (if these are a concern for the products) would not be needed.

Testing of product during validation studies of process controls can provide the data needed to show that microbiological hazards of concern can be consistently controlled. The microbiological data obtained during validation prior to implementing a process and during the initial stages of implementation to demonstrate consistent control may indicate that finished product testing is not needed as long as the monitoring of the process parameters that were validated indicates the process remains in control.

Charge Question 7. The CGMP & PC rule requires environmental monitoring for an environmental pathogen (e.g., Listeria monocytogenes, Salmonella) or for an appropriate indicator organism as a verification activity if contamination of an RTE food with an environmental pathogen is a hazard requiring a preventive control (such as sanitation controls). What impact does environmental monitoring have on frequency and extent of product testing verification activities by companies?

Environmental monitoring as a verification of sanitation controls is more effective than solely testing finished product, but it may not eliminate the need for finished product testing. The results of environmental monitoring could indicate that product contamination may have occurred (e.g., a product contact surface tests positive for *Listeria* spp. and follow-up tests indicate the potential for product contamination) and this could lead to product testing as part of actions to identify the root cause and correct the problem *(52)*.

Determinations of potential harborage sites for pathogens through periodic testing for the pathogen or an indicator organism (e.g. food contact surfaces, zone two is non-food contact surfaces in close

proximity to food contact surfaces, zone three is non-food contact surfaces not proximal to zone one, and zone four is areas remote from production) is recommended *(12, 13, 20, 26, 27, 41, 52)*. Samples should be taken several hours into processing, or at the end of the day prior to sanitation. The degree of environmental monitoring is impacted by, but not limited to product characteristics, process type (wet v. dry), facility and equipment design, process and product history, supplier monitoring program, and target of environmental program (indicator, pathogen, non-microbial). Manufacturers should refer to commodity-specific guidance for environmental monitoring programs *(2, 11, 21, 22, 26, 27, 52)*. While *Salmonella* is frequently the target pathogen for control in dry environments and *Listeria monocytogenes* in wet environments, both microorganisms may need to be considered in many processing environments.

Environmental monitoring can influence frequency and extent of product testing. An Environmental Monitoring Program (EMP) should be designed to detect pathogens or indicator organisms in zones one and two or other areas that pose a risk of cross-contamination to product. When contamination of an RTE food by *Salmonella* or *Listeria monocytogenes* from the processing environment is a primary concern, a robust EMP should reduce the need for product testing (e.g., frequency, number of samples). This is particularly the case for RTE foods that receive a validated lethality treatment but may subsequently be exposed to the environment (e.g., after the lethality treatment but prior to final packaging) where cross-contamination is possible. Examples of RTE foods where EMP can reduce the need for final product testing include cheeses made from pasteurized milk, butter, cultured dairy products, dried dairy products, ice cream, roasted nuts and nut products (for summary, see Table 3; details are found in Appendices A-F of this document).

For some food products, an EMP is the primary means for verification of effective sanitation control programs, and finished product testing is not typically conducted unless triggered by other data (e.g., zone 1 or zone 2 environmental positives). Examples here include RTE baked items (time-temperature controlled for safety, TCS, and non-TCS), RTE cereals, RTE grained-based baked products, RTE cold pressed bars (Appendix B), RTE meals and sandwiches with short shelf life (Appendix C), and fresh cut fruits and vegetables with short shelf life (Appendix E).

In some cases, an EMP is implemented in conjunction with routine finished product testing, although the results from the EMP may still influence the degree and level of finished product testing. For example, there are regulatory requirements for finished product testing for powdered infant formula (i.e., powdered infant formula must be tested for *Cronobacter* spp. (30 X 10 g) and *Salmonella* spp. (60 X

25 g) in accordance with 21 CFR 106.55). Powdered infant formula may be subject to contamination by *Cronobacter* spp. from the environment and an EMP may indicate the need for additional product testing for *Cronobacter*. Other examples of products where both an EMP and routine finished product testing is appropriate could include raw milk cheeses, certain soft cheeses (e.g., soft ripened; Appendix A), RTE nuts not processed for lethality (Appendix D), and nut butters (Appendix D).

Charge Question 8. (1) What criteria should a company apply in determining that microbial testing results indicate a loss of process control? (2) What actions should a company take if test results indicate a loss of process control? (3) 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?

Answer Q8 -1. What criteria should a company apply in determining that microbial testing results indicate a loss of process control?

For this document, process control refers to the entire operation (e.g., entire food safety system/process). It is not restricted to process preventive controls.

A food safety system and the manufacturing process managed by that system are in control when, within the limits of a stable and predictable process variation, all food safety hazards are controlled to an acceptable level. Building on this definition, the development of measurable attributes that indicate whether a process maintains or surpasses an acceptable degree of hazard control or falls below that level is required *(29)*.

One measure of process control is the adherence to microbiological limits established in the food safety system for verification of activities such as those used for sanitation and processing controls intended to mitigate microbiological hazards. Failure to meet prescribed microbiological testing limits for indicator organisms or pathogens could constitute a loss of control. A food manufacturer should determine limits relevant to its specific products and processes. Guidance, not regulatory limits, is provided in this section and in Appendices A-F.

The measurable attribute and the type of microbial testing used to measure process control will vary with the product, the hazard being controlled, and the location of the control along the process

744 continuum. Once actionable limits for test results are established at points along the entire
745 manufacturing process, a company can then respond to those results based on food safety impact.
746
747 Measurement of process control is based on the following *(35)*.

748 1. Sampling and assessing the output of the process for key microbial targets should occur at a
749 frequency that limits the amount of time that a loss of control goes unrecognized. Frequency of
750 sampling is predicated on the propensity for the system to lose control, the prevalence of the
751 microbial target and practicality, balancing rapid recognition of a system out of control with the
752 cost of sampling and testing. Sampling sites are selected that are representative of the product
753 as it passes through the process or as it exits the process. Larger sample sizes add statistical
754 relevancy. Testing frequency and sample size taken should be risked based. More intensive
755 testing is needed for foods where there is little information, e.g., for new suppliers, a new
756 processing line or product, or for individual foods or ingredients that have been shown to have
757 higher prevalence of microbial risks e.g., for spices obtained in certain regions. As a firm builds a
758 data base of microbial results, testing frequency can be refined based on an understanding of
759 how often product will be outside microbial limits that have been identified to verify that the
760 process is in control.
761 2. Process control performance limits and testing targets (e.g., specifications) are predefined for
762 the type of food product, type and extent of processing, RTE status, chemical and physical
763 characteristics of the food product, and the history of the process. Microbial criteria for food
764 safety or food quality need to be relevant to signaling a hazard in a specific product and be
765 attainable.
766 3. A system for documentation and review of results is in place that allows corrective action with
767 the appropriate level of immediacy.
768 4. A predetermined plan of action (POA; a corrective action plan) is developed based on a scaled
769 response considering public health impact, deviation from relevant limits, and frequency of the
770 deviation. For example, a typical set of POA choices might be take no action, move to increased
771 sampling frequency or sample size, conduct a predetermined internal or external audit of the
772 process that is typical for out-of-control variability, and identify an assignable cause through
773 root-cause analysis and take corrective and preventive actions. The corrective actions specified
774 must be subsequently verified to ensure they reduce or prevent future deviations. The proper
775 action should be decided upon based on the severity and frequency of the deviation.

5. The microbial measurement of insanitary conditions through environmental testing could also indicate the loss of process control or contribute to an overall assessment of loss of control.

An adequate process control indicator is an attribute that can be measured with objectivity and for which limits that indicate a need for corrective action can be established. The primary strength of process control indicators is signaling the need for a more comprehensive analysis of the system and to take corrective action before a noncompliance occurs. An ideal indicator of process control is one that allows corrective actions to be taken before a loss of control represents a threat to public health. USDA FSIS reviewed the use of process indicators in its public health risk-based inspection system *(29)*. The agency proposed two basic types of process indicators: those that may predict a future loss of control (e.g., exceeding a specific rate of out of specification (OOS) results) and those that reveal outcomes of a past loss of control (e.g., finding a pathogen in an RTE food product, recall of a product for safety reasons).

Limits (criteria) that are chosen as indicators of process control should take this distinction into consideration, as the type of process control indicator will determine the criticality of the corrective action. For instance, the presence of an indicator organism could reflect normal variation within acceptable parameters and not necessarily demonstrate that a process is out of control. In this case, the frequency of finding an OOS result becomes important in determining loss of control. However, the finding of a pathogen-contaminated product indicates an overt loss of process control that could have occurred in the past, unrecognized by the facility or inadequately addressed by actions taken in response to a prior failure.

The following factors should be considered when analyzing an OOS result and determining whether a loss of process control has occurred. These include, as appropriate:

- the target organism and levels detected, i.e., a qualitative pathogen (e.g., presence of *Salmonella* in a 375 g sample or environmental sample), quantitative pathogen (e.g., the number of *Staphylococcus aureus*) or an indicator organism (e.g., the number of coliforms).
- the type of sample analyzed, i.e., ingredient, in-process, environmental or finished product.
- the location of the sampling site and proximity to finished product.

- the extent to which the target organism deviated from the limit for a quantitative microbiological result.
- the frequency with which OOS results are obtained.

All or some of these factors can be used to determine a level of criticality that will drive scalable reactions from recleaning a piece of equipment to discarding product. For instance, the finding of a pathogen in product or in close proximity to product would warrant an immediate and aggressive reaction as compared to an OOS indicator level in in-process product.

Identifying and ranking process control indicators can be challenging. The relative importance of different predictors will vary with the products produced, the state of the processing facility, raw ingredient sources and several other variables. Appendices A through F in this document describe six commodity groups and provide a comparison of microbial limits for determining whether processes are out of control depending on the product manufactured. Two examples of microbial limits drawn from Appendices A and D are shown below. Additional information on establishing microbiological safety criteria can be found in *Scientific Criteria to Ensure Safe Food (36)*.

Example 1. Appendix A - Dairy Products.

When there is a loss of systemic process control for soft cheeses as recognized by the finding of a pathogen in product or a frequent occurrence of OOS indicator organism results, a root cause analysis should be performed, including looking at heat-treatment of milk, cheese vat/make procedures, acidification rate, finishing table, brine tanks, block formation, aging, cutting, and packaging to determine the source(s) of loss of control and to implement corrective action. The findings of the root cause analysis will dictate corrective actions and whether verification testing that includes finished product is indicated (Table A-1).

831 Table A-1. Microbial targets, limits, and recommended actions if limits are exceeded, for soft cheeses made with pasteurized milk. Additional testing may be indicated for cheeses made with
832 raw milk *(5, 23)*.

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms or Enterobacteriaceae	≤100/g	Investigate reason for exceeding limit and implement corrective action; consider testing for *E. coli* (≥10/g) if coliforms are detected	Routine testing
S. aureus	≤100/g	If ≥10^4/g, reject lot due to potential for enterotoxin production. Due to heat stability of enterotoxin, diverting to further processing is not recommended	Investigative testing if routine pH monitoring of a vat during fermentation suggests acid development is slow and culture is not active. Investigate, implement corrective action
Listeria monocytogenes	Negative in 125 g analytical units (5 x 25-g samples)	Reject lot. Investigate cause of contamination. Determine if other lots are involved. Determine steps to prevent reoccurrence.	Investigative testing as response to EMP that suggests likely contamination of product or routine testing for products that can support growth of *L. monocytogenes*
Salmonella	Negative in 375 g analytical units (15 x 25 g samples)	Reject lot. Investigate cause of contamination. Determine if other lots are involved. Implement corrective action to prevent reoccurrence.	Investigative testing as response to EMP that suggests likely contamination of product or routine testing for cheeses made with raw milk and aged for 60 days

833

Example 2. Appendix D - Nuts (including tree nuts and peanuts) and Nut/Seed Products.

Microbiological limits for Ready-to-eat (RTE) chopped raw tree nuts.

Producers of RTE chopped raw tree nuts and some types of whole RTE nuts rely on preventive controls that include sanitation controls and a supply-chain program. Control is based on the expectation that processers beyond the grower are compliant with sanitation and supply-chain programs under the Preventive Controls for Human Food Rule (21 CFR Part 117)*(51)* and that growers that supply the raw unprocessed nuts are compliant with the Produce Safety Rule (21 CFR Part 112)*(50)*, where applicable, and Good Agricultural Practices (GAPs) *(53)*. Finished product testing is conducted to verify that sanitation controls are in place and effective within the manufacturing facility. Product testing for *Salmonella* and generic *E. coli* provides highly relevant verification data and is appropriate for the level of risk associated with the raw nuts. One indication of loss of control would be the finding of a positive pathogen result. When a pathogen is detected from a sample taken at the end of the production line, the recommended action is to divert for reprocessing with a kill step or destroy the lot of raw nuts represented by the sample, as appropriate. The repeated finding of an indicator organism such as generic *E. coli* above a threshold level can also indicate a loss of sanitation control and the potential for pathogen ingress into the process. However, in this case, testing provides an opportunity to adjust the process and avoid public health implications. Actions taken would follow a tiered approach based on numbers and frequency of occurrence (Table D-1).

Table D-1. Microbial targets, limits, and recommended actions if limits are exceeded, for ready-to-eat nuts not processed for lethality.

Target Microorganism	**Microbiological Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
E. coli (generic)	≤0.36 MPN/g	Investigate, implement corrective action	If 2 of 10 samples are ≥0.36 MPN/g, follow CPG Sec 570.450 *(48)*
Listeria monocytogenes	Negative in 25 g	Reject. Investigate and implement corrective action	
Salmonella	Negative in two 375 g samples	Reject. Investigate and implement corrective action	Two 375 g analytical units derived from 30 x 25 g samples

Answer Q8-2. What actions should a company take if test results indicate a loss of process control?

Microbiological and chemical limits for foods for use by the United States Department of Defense to assess process control and insanitary conditions were evaluated and published by a previous NACMCF committee *(35)*. The microbiological limits reported for indicator organisms in that document are not lot acceptance criteria, unless there is a regulatory limit associated with that value, such as limits for coliforms in milk or generic *E. coli* in nuts (see NACMCF-DOD Appendices *(35)*. The 2018 NACMCF-DOD document was developed for inspectors or auditors to evaluate whether a food was produced under sanitary conditions without having full knowledge of the processing conditions. However, the target microorganisms and limits included both product and environmental monitoring that would be useful to the manufacturer that their process is in control. Therefore, both the NACMCF-DOD guidance and this document provide guidance to evaluate sanitary conditions and process control for foods, including appropriate target microorganisms and limits in foods, as well as recommended actions to be taken if the limits are exceeded. In many instances, actions include investigating to determine a root cause, implementing corrective and preventive actions, and conducting follow-up sampling and testing to determine if the corrective and preventive actions have been effective. These actions were categorized as "Investigate" or "Implement Corrective Actions." The 2018 NACMCF-DOD document indicated that investigative and corrective action procedures would likely be unique to each situation. Given the scalable approach recommended for determining loss of control, actions taken would also depend on the type of hazard created by a loss of control.

As an example, samples taken of a low water activity product (e.g., a cold pressed bar) at several in-process points during production are found to be out of specification for coliforms; however, levels decrease over the course of the process run. If the process had been wet cleaned prior to start-up, the investigation might focus on water left behind due to inadequate drying and outgrowth on the equipment and/or a review of coliform levels in ingredients. The fact that the coliform levels decreased over time would appear to support elevated levels due to outgrowth at start-up that were removed as the process progressed. The company could take the following actions:

1. Review sanitation activities and implement corrective actions if found inappropriate or inadequate (e.g., modify cleaning and sanitizing procedures, revise sanitation verification activities).

2. Review coliform levels in ingredients and implement corrective actions if found to be elevated beyond the ingredient specification (e.g., address issue with supplier, use alternative supplier).

3. Consider whether pathogen testing of finished product could be appropriate. (As an indicator of post-process contamination, high levels of coliforms might also indicate a pathway for pathogen ingress).

4. Decide on product disposition.

In another example, samples are taken at the end of the production line and tested for a target pathogen. If the pathogen is detected, this represents a serious loss of process control that warrants stopping the process line until a root analysis is completed, the hazard is mitigated, and the hazard is assured to be eradicated. The root cause analysis could include a review of all processing records, questioning production workers about whether there were any unusual occurrences during processing, testing ingredients for the pathogen, environmental sampling, additional testing of product from throughout the production, etc. Specific corrective actions depend on the findings of the root cause analysis. Unless the product can be reprocessed using a validated process, product destruction is indicated. An essential activity is to assess whether contaminated product has left the company's control (public health risk) and take the necessary actions to recall the product.

Answer Q8-3. 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?

The number of in-process, finished product, or environmental samples to take and test on a routine basis is determined by a review of the process and product, and the information derived from the analysis. In general, taking more samples increases the probability of pathogen detection; and larger numbers of samples taken for pathogens can increase the confidence of detecting pathogens present at a low prevalence. Analytical unit weights for testing should be a minimum of 25 grams; for pathogen testing, the analytical unit is usually a composite weight such as 375 grams (15 X 25 gram samples to result in a 375 gram analytical unit) *(3)* When there has been a loss of control, the number of samples, the size of the sample, and the frequency of verification testing can all increase.

If a root cause is not readily apparent, investigational testing should span the entire process, including ingredient, in-process product and a sampling of finished product produced over contiguous runs or produced during a time frame bracketed by breaks in the process for full sanitation ("clean breaks"). The intent is to find ingress points and establish a timeframe for the contamination event.

When a root cause investigation and corrective/preventive activities are completed, the decision to resume normal production is based, in large part, on microbiological testing that verifies control has been restored. Predetermined testing strategies (frequency and numbers of samples) for a process in control (standard "surveillance" level of testing), a process trending away from control (increased "heightened" level of testing) and a process that is out of control (investigative testing) should be part of a microbiological testing program. The increased number of samples and the frequency with which they are taken to initially investigate the root cause can be scaled back in a stepwise manner, first to a heightened level of microbiological testing and, eventually, to fewer samples, smaller sample sizes and fewer sample sites consistent with surveillance testing used with a process in a steady state of control. This step-down approach requires a commitment to testing at each step for a defined amount of time to collect sufficient data that demonstrates the process is moving toward a consistent state of control.

930 **LIST OF TABLES**

931 ***Table 1. Comparison of responses to Charge Question 1 by commodity.*** *What principles and criteria should a company apply in determining the need for and in designing an effective microbial*

932 *testing program to verify that processes are effectively controlling microbial pathogens?*

	Dairy	Grain-based products	Meals & Entrees	Nuts, Seeds & products	Fruits & Vegetables	Spices & Herbs
1.1 Have pathogens been associated with the food or its ingredients and whether the food has been involved in foodborne illnesses?	All raw commodities in these groups have been associated with pathogens and/or foodborne illness. Post-lethality contamination and long-term survival of low infectious dose pathogens, such as *Salmonella* in low moisture foods (spices, dry dairy, grains, nuts/seeds) are problematic; presence/growth of *L. monocytogenes* in perishable refrigerated foods (RTE meals, high moisture cheeses, cut fruits/vegetables) has occurred. Other pathogens such as Shiga-toxin producing *E. coli* have been associated with leafy greens and cheeses made with unpasteurized milk.					
1.2 Is it likely that ingredients are contaminated, given the nature of the ingredient and the robustness of the supplier programs?	The likelihood that ingredients are contaminated depends on whether they have previously received a robust lethality process (kill step). For example, foods with cooked components or have lower probability of being contaminated due to the lethality process but rely on supplier programs to prevent post-lethality contamination. RTE meals/salads with fresh produce depend on supplier control programs to prevent contamination being introduced into the ingredient and hence the final product.					
1.3 Are the processing control procedures robust.	This is product dependent.					

1.3.a. Is there a kill step? Other microbial reduction step? (Not having a kill/microbial reduction step increases risk. Kill step in the package mitigates the risk and may eliminate the need for finished product testing.)	Except for cheese made with raw milk, milk is pasteurized for use in dairy products.	Most bakery products have a kill step (baking); however, process should be controlled to prevent growth of bacteria such as *S. aureus* and *B. cereus* that produce heat-stable enterotoxins. Other grain-based products such as cold-pressed bars have no kill step for the final product	Some foods are fully cooked, including a cook-in-bag. However, some are combination products with raw ingredients (e.g., sandwiches containing raw produce).	Roasted or otherwise treated provide microbial reduction. When this is not needed, suppliers should comply with the Produce Safety Rule (21 CFR Part 112) where applicable, or GAPs.	Antimicrobials in produce washes are typically used to prevent cross contamination in the wash water and not as a microbial reduction step on the product surface. Suppliers of fruits and vegetables for fresh-cut or drying should comply with the Produce Safety Rule (21 CFR part 112) where applicable, or GAPs. Some drying processes may have	Depending on the intended use. Some will be treated with gas, steam, radiation, etc.; others are not processed for lethality

					sufficient heat to inactivate pathogens.	
1.3.b. Does formulation result in a reduction of microorganisms (based on the characteristics of the food, e.g., pH, acid type, aw)?	Cultures used in dairy products produce sufficient lactic acid (e.g., pH <4.6) that bacterial pathogens will be slowly inactivated during storage; hard cheeses rely on combination of acidity and reduced moisture/a_w and extended aging as a gradual pathogen reduction.	Grains and grain-based foods typically do not have formulations that rapidly inactivate pathogens	Most RTE meals are not formulated to inactivate pathogens	Dried nuts and seeds are not formulated to inactivate pathogens; some slow inactivation of pathogens can occur over time in low a_w foods, but survival may be months	Some citrus fruits may have sufficiently low pH to inactivate pathogens, but lethality will be slow; fresh produce is typically not formulated to ensure lethality	Dried and fresh spices and herbs do not have formulations that inactivate pathogens

1.4. Is there a potential for recontamination from the handling or the environment?	Except for foods that are hot-filled, filled within a closed system, or which receive an in-package lethality step, all commodities have the risk of contamination from handling or from the environment.					
1.5. Does the product support survival or growth?	Variable; all products within this category will support survival to a degree over shelf life, but populations of pathogens may decrease over time, such as during aging of hard cheese or exposure to high acid content in cultured dairy products. Growth largely depends on product pH, a_w, presence of antimicrobial ingredients (e.g.,	Foods with low a_w can allow pathogen survival but do not support growth. Other foods with higher a_w (>0.88) and pH >4.6 may support growth and require temperature-time control for safety.	Foods in this category are typically within pH and a_w ranges that support growth	Pathogens can survive for extended periods in dry nuts/seeds/products. Nut-milks may support growth if not properly refrigerated.	Pathogens will survive on fresh cut fruits/vegetables; growth is likely to be slow if refrigerated. Pathogens may survive on dried fruits and vegetables but are unlikely to grow due to pH and low water activity.	Dried spices and herbs are low a_w that allow survival but do not support growth.

	potassium sorbate), and presence of competitive microbiota (e.g., starter cultures), as well as storage conditions					
1.6. Is this product intended specifically for higher risk population?	In most instances the product is being made for the general population but may be consumed by individuals in higher risk populations. Exceptions are milk powders used for infant formula and cereals that are intended for infants.					
1.7. What is the shelf life of the product?	**Butter:** 3-9 months **Dried:** months-years **Cheese Hard:** several years **Cheese fresh:** 60-90 days **Cultured pH<4.8:** 60-90 days **Cultured pH 4.8-5.4:** 60-90 days	**Filled pastry, soft cookies and bread** 1-3 weeks at ambient temp. **Frozen products** (e.g., waffles or filled pastry) can be 18 months. **Dried products** (e.g., cereals and cold pressed bar; hard cookies) 18 months.	**Variable.** **RTE Salad:** 1-2 weeks **Sandwich:** 1-2 days. Several months frozen. Several days thawed. **Heat & Eat Entrée:** Several days	**Nuts no lethal process:** 6 months ambient temp., 1 year refrigerated, 1-2 years frozen. **Nuts processed for lethality:** Months to years **Nut products:** Almond milk 2-3	**Fresh cut fruits:** 1 week **Fresh cut vegetables:** 1 week **Dried:** 1- 2 years	**Spices NOT processed for lethality:** 1-2 years **Spices processed for lethality:** 1-2 years **Dried chopped herbs:** 6-9 months

	Frozen desserts: months - years **Fluid milk:** HTST pasteurized up to 3 weeks		refrigerated. Several months frozen	months HTST, 8 - 10 months UHT. Nut cheese 6 months. **Nut and seed butters:** 1 year		
1.8. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Variable depending on the product. **Butter:** unlikely that storage conditions will alter risks associated with salted butter. *S. aureus* may grow in unsalted or whipped butter if unrefrigerated. **Dried:** Unlikely that storage will affect risk for	Variable depending on the product. Items with high a_w components, e.g., custard filling, can support growth of pathogens such as *L. monocytogenes or S. aureus.* If frozen products are thawed and held extended periods at refrigeration or ambient temperatures	Variable depending on the product. **RTE Salad:** *L. monocytogenes* can grow @ refrigeration if pH >4.4. *B. cereus* can grow in cooked rice if not refrigerated. Consumers can hold @ room	Unlikely that consumer handling or storage will increase risk unless condensate is allowed to form on the product to increase the a_w.	**Fresh cut fruits and vegetables:** Increase risk if improperly handled or temperature abused. **Dried**: Bulk containers at retail add risk for cross-contamination but due to low a_w, dry storage outside chilled storage or beyond	**Dried spices and herbs** are typically shelf-stable due to low a_w. No changes to risk if handling or storage conditions at the retail or consumer level are not as intended.

	dried product. If rehydrated and temperature abused, *Cronobacter* and *Salmonella* can grow. **Cheese Hard:** Combinations of acidity, a_w and residual competitive starter culture will inhibit pathogen growth if temperature abused. **Cheese fresh:** Storage >3C or extended storage will promote growth of *L. monocytogenes*.	pathogens may grow. Temperature abuse or extended refrigerated storage of rehydrated infant cereal may allow growth of pathogens	temper for several hours. **Sandwich:** Holding refrigerated sandwich for several days can increase risk of *L. monocytogenes* growth. **Heat & Eat Entrée:** Low risk. Fully cooked. Potential for pathogen growth if re-contaminated and temp. abused by consumer.		use-by date will not increase food safety risk.	

	Cultured pH<4.8: no changes in risk. **Cultured pH 4.8-5.4**: potential for growth of *L. monocytogenes* if temperature abused, particularly if not formulated with preservatives. **Frozen desserts:** No change in risk as long as product remains frozen **Fluid milk:** not likely. Spoilage microorganisms likely to out compete pathogens.					

933 ***Table 2. Comparison of responses to Charge Question 6 by commodity.*** *Generally, microbial testing by a company to verify process control is conducted on "finished product." Are there*
934 *situations in which testing at sites other than at the end of the process can achieve the goal of verifying the adequacy of control of microbial hazards? Describe the situations and the testing**
935 *that would be appropriate.*

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
Butter, Margarine: Yes. Testing aerobic colony count and Enterobacteriaceae or coliforms can be done during production, as well as for environmental testing. Cheese, hard: and	RTE, baked, refrigerated or time-temperature controlled for safety (TCS): Yes. Testing of a custard filling prior to being filled into the pastry may be more appropriate than enumerating *S. aureus* in the finished product. Enumeration of toxin producers *S. aureus* and/or *B.*	RTE Deli salads: Yes. Monitoring and verification of processing steps such as the cook step for certain components of deli salads to ensure validated process controls are appropriately implemented, combined with testing of the ingredients of concern (e.g., those that have not received	RTE nuts not processed for lethality: No. RTE nuts and seeds processed for lethality, and RTE nut and seed products processed for lethality, and	RTE fresh-cut fruits, and RTE fresh-cut vegetables: Yes. Pre-harvest testing or activities associated with supplier verification, assays and/or electronic monitoring of wash water system or at receiving of the processing facility may be considered as alternative to finished product testing.	RTE spices and spice blends, not processed for lethality: No. RTE spices and spice blends, processed for lethality: Yes. Consider quantitative Enterobacteriaceae testing of the raw, unprocessed spices or herbs.

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
Cheese, fresh, soft, soft-ripened, semi-soft, or veined: Yes. Monitoring the pH of curd can detect slow fermentation and testing for *S. aureus* (<10^4 CFU/g) may be relevant if acidification proceeds slowly. Testing for indicator organisms (e.g., molds, yeasts, Enterobacteriaceae, or *Listeria*-like microorganisms) in brine or in curd for *E. coli* (<100 CFU/g) in cheese made	*cereus* in raw waffle batter may be necessary, since testing of the finished frozen waffle would not be appropriate due to the kill step in baking the waffle. RTE, baked, shelf stable or non-TCS: No.	a lethality treatment) could be an alternative to finished product testing. Sandwiches: Yes. Microbial testing and COAs from suppliers (or periodic testing of ingredients by the receiving facility) may be appropriate in some circumstances, but may not be warranted (or may be limited) if a firm can verify a supplier has	RTE nut/seed butters not processed for lethality beyond initial nut processing: No. For processes that are not enclosed, finished product testing is recommended along with additional points of verification testing including: • Environmental monitoring. • Inbound raw material testing – depends on processed state of	RTE dried/dehydrated fruits: Pathogen testing (pre-harvest or testing at receiving) may be necessary depending on the commodity, if there is an emerging issue, a risk associated with the farming or harvesting system (i.e., absence of water treatment for overhead irrigation) or for a new supplier or change of supplier. Lot acceptance	Dried, chopped herbs: No.

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
from heat-treated milk may be useful to verify process control and hygiene conditions. Cultured, pH < 4.8: and Cultured, pH > 4.8 and <5.4: Yes. pH testing during fermentation to monitor acid production should be done routinely to ensure adequate acid production to control microbial hazards. Testing	RTE Cereals: No. For ingredients added post-lethality, COAs should be received from suppliers and supplier control programs verified. RTE, cold-pressed bars: No	adequate process controls and control of environmental contamination verified with an EMP. "Heat and Eat" Entrées and Meals: Yes. Monitoring of the process controls that have been validated for products that are fully cooked provides more assurance of safety than microbiological testing of finished product. However, if	ingredients and COA data. Lot-by-lot testing if supplier is deficient in pathogen mitigation interventions and hazards are not controlled by a process. • Sanitation/hygiene verification testing.	testing could be considered, as the shelf-life allows for this type of testing to be applied. Additional points of verification may not eliminate the need for finished product testing but are important including pathogen environmental monitoring and sanitation/hygiene verification testing.	

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
for indicator organisms, and environmental monitoring programs are verification of process control and sanitation. <u>Dried products or ingredients</u>: Yes. Sampling plans for APC/SPC, coliforms, *Salmonella*, or Enterobacteriaceae should include representative samples taken after the drying step up to the filling		the food is exposed to the environment after the process, as with egg rolls and baked pot pies, an EMP is critical.			

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
operation. Sampling points are sifter tailings from after dryer/after cooler or from tipping stations of intermediate products and filling machines. <u>Frozen desserts</u>: Yes. Samples for coliforms or APC are typically taken from the mixing and maturation tanks, at the filler or after hardening tunnels. Particular attention needs to be paid to					

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
build-up of residues or condensation spots where growth may occur. <u>Milk and Milk products (fluid)</u>: No.					

936 ***Table 3. Charge Question 7.*** What impact does environmental monitoring have on frequency and extent of product testing verification activities by companies?

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
For products that utilize pasteurized milk and have product composition (pH, a_w, competitive microbiota) such that growth is inhibited, environmental monitoring for *Listeria* species will identify the potential for product contamination and will reduce the need to test product.	For RTE baked items (TCS or non-TCS) and RTE cereals, pathogens would most likely come from environmental recontamination to packaging. Therefore, ongoing environmental monitoring to verify sanitation controls provides the most relevant information on product safety. A robust EMP should reduce the need for finished product testing.	For RTE deli salads, sandwiches and meals with short shelf life, finished product testing for pathogens is impractical. A robust EMP is needed to verify sanitation controls and to identify potential for cross contamination. For heat-and-eat entrees and meals, EMP is a key factor in	RTE nuts processed and not processed for lethality require EMP but this will not diminish the need for finished product testing. EMP for RTE nut products processed for lethality in closed systems (e.g., almond "milk" beverages) will inform sanitation efficacy as final product testing may not be necessary.	For fresh-cut, RTE fruits and vegetables, a robust EMP should reduce the need for finished product testing, since the main pathogens of concern are *L. monocytogenes* or *Salmonella* (depending on commodity), which can come from environmental contamination. Furthermore, the short shelf life of these foods may make pathogen	For spices/herbs not treated for lethality, EMP does not impact product testing because untreated spice may be the source of contamination. a) After treatment, spices and herbs are usually in some form of container, limiting environmental exposure and the need for environmental monitoring.

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
Products that have potential for post-process contamination and rely on storage temperature to inhibit pathogen growth (such as soft cheeses with high pH) may require both a robust EMP and include finished product testing. The results of the EMP can impact the frequency and number of product samples. Frozen dessert may still require finished product testing because of the potential of	For RTE grain-based products without a lethality step (such as cold-pressed bars), environmental monitoring and supplier control for ingredients can reduce frequency of finished product testing.	not conducting finished product testing.	For other nut products where processes are not enclosed, a robust environmental monitoring program should be present or deployed targeting the post-lethality areas. Application of EMP, however does not replace finished product verification testing. For nut/seed butters that are not processed for lethality beyond initial nut/seed,	testing of the finished product impractical. For RTE dried/dehydrate fruits/vegetables, environmental monitoring for pathogens of concern (likely *Salmonella* and *Listeria*) is warranted if drying process is conducted in a closed environment and aided by equipment that can facilitate cross-contamination. However, if the process is an	b) If there is an opportunity for environmental exposure of the spice or herb after the application of the microbiological intervention, then an environmental monitoring program may be appropriate. c) An environmental monitoring program may result in a short term movement to investigational sampling, when an event in the environmental program

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
growth if the product were stored in unfrozen state. Dairy powders: Since the major cause of presence of *Salmonella* or increased levels of Enterobacteriaceae in finished products is recontamination from the processing environment, sampling and testing of environmental samples plays a key role in verifying the effectiveness of the preventive measures. It			environmental testing and supply chain verification activities can reduce the need for finished product testing.	outdoor process such as "sun-drying" then all reasonable precautions need to be followed to prevent contamination. Lot acceptance testing may be appropriate because of the limitations in deploying an environmental monitoring program and sanitation controls.	indicates a potential for contamination.

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
should be noted that testing for Enterobacteriaceae alone is not suitable since even low levels do not necessarily guarantee the absence of the pathogen. Frequency and extent of product testing should be increased if the results from environmental monitoring show the presence of *Salmonella*, or increased levels of EB, or if product is intended for immunocompromised individuals.					

Dairy	Grain-based products	Meals and Entrees	Nuts, Seeds & Nut/Seed products	Fruits and Vegetables	Spices and Herbs
Finished product testing (micro) of fluid milk is not necessary if records are kept verifying that pasteurization was effective. Typically, fluid milk is considered not to be exposed to the environment during filling. However, firms usually identify/implement sanitation controls and perform environmental monitoring					

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938 **REFERENCES**

939 1. Ahamad, N., and E. H. Marth. 1989. Behavior of *Listeria monocytogenes* at 7, 13, 21, and 35-
940 degrees-C in tryptose broth acidified with acetic, citric, or lactic-acid. *J. Food Prot*. 52:688-695.
941 2. American Spice Trade Association. 2017. Clean, Safe Spices: Guidance from the Amercian Spice
942 Trade Association. https://www.astaspice.org/food-safety-technical-guidance/best-practices-and-
943 guidance/clean-safe-spices-guidance-document/, Accessed April 3, 2021.
944 3. Andrews, W. H., and T. Hammack. 2003. BAM Chapter 1, Food Sampling and Preparation of
945 Sample Homogenate. *In*, FDA Bacteriological Analytical Manual, https://www.fda.gov/food/laboratory-
946 methods-food/bam-chapter-1-food-samplingpreparation-sample-homogenate.
947 4. Anthon, G. E., and D. M. Barrett. 2002. Kinetic parameters for the thermal inactivation of
948 quality-related enzymes in carrots and potatoes. *J. Agric. Food. Chem.* 50:4119-4125.
949 5. Artisans Dairy Producers Food Safety Initiative. 2019. Food Safety Systems Guide to Raw Milk
950 Cheese Production. https://www.usdairy.com/getmedia/a87ecbc3-4fcc-4ff2-bb3f-
951 e0b848b167bb/food_safety_systems_guide_to_cheese_production_final_2019.pdf.pdf, Accessed 12
952 December 2020.
953 6. Breidt, F., E. L. Andress, and I. B. 2018. Recommendations for designing and conducting cold-fill
954 hold challenge studies for acidified food products *J. Food Prot.* 38:322-328.
955 7. Breidt Jr, F., K. Kay, J. Cook, J. Osborne, B. Ingham, and F. Arritt. 2013. Determination of 5-log
956 reduction times for *Escherichia coli* O157: H7, *Salmonella enterica*, or *Listeria monocytogenes* in acidified
957 foods with pH 3.5 or 3.8. *J. Food Prot*. 76:1245-1249.
958 8. Buchanan, R., and D. W. Schaffner. 2015. FSMA: Testing as a tool for verifying preventive
959 controls. *Food Prot. Trends*. 35:228-237.

960 9. Buchanan, R. L., M. H. Golden, and R. C. Whiting. 1993. Differentiation of the effects of pH and
961 lactic or acetic-acid concentration on the kinetics of *Listeria monocytogene*s inactivation. *J. Food Prot*.
962 56:474-&.
963 10. Busta, F. F., T. V. Suslow, M. E. Parish, L. R. Beuchat, J. M. Farber, E. H. Garrett, and L. J. Harris.
964 2006. The use of indicators and surrogate microorganisms for the evaluation of pathogens in fresh and
965 fresh-cut produce. *Compr Rev Food Sci F*. 2:179-185.
966 11. California Leafy Green Products Handler Marketing Agreement (LGMA). 2020. Food Safety
967 Practices *In*, https://lgmatech.com/wp-content/uploads/2020/08/CA-LGMA-Metrics-August-
968 2020_Final_Clean_9-18-20.pdf.
969 12. Chen, Y. H., V. N. Scott, T. A. Freier, J. Kuehm, M. Moorman, J. Meyer, T. Morille-Hinds, L. Post, L.
970 Smoot, S. Hood, J. Shebuski, and J. Banks. 2009. Control of *Salmonella* in low-moisture foods II: Hygiene
971 practices to minimize *Salmonella* contamination and growth. *Food Prot. Trends*. 29:435-445.
972 13. Chen, Y. H., V. N. Scott, T. A. Freier, J. Kuehm, M. Moorman, J. Meyer, T. Morille-Hinds, L. Post, L.
973 Smoot, S. Hood, J. Shebuski, and J. Banks. 2009. Control of *Salmonella* in low-moisture foods III: Process
974 validation and environmental monitoring. *Food Prot. Trends*. 29:493-508.
975 14. Craven, H. M., M. J. Eyles, and J. A. Davey. 2003. Enteric indicator organisms in foods. *In* A.D.
976 Hocking (ed.), Foodborne Microorganisms of Public Health Significance, 6th ed. Australian Institute of
977 Food Science and Technology Inc., Tempe, Australia.
978 15. D'Amico, D. J., M. J. Druart, and C. W. Donnelly. 2008. 60-day aging requirement does not
979 ensure safety of surface-mold-ripened soft cheeses manufactured from raw or pasteurized milk when
980 Listeria monocytogenes is introduced as a postprocessing contaminant. *J. Food Prot*. 71:1563-1571.
981 16. D'Amico, D. J., M. J. Druart, and C. W. Donnelly. 2010. Behavior of *Escherichia coli* O157:H7
982 during the manufacture and aging of Gouda and stirred-curd Cheddar cheeses manufactured from raw
983 milk. *J. Food Prot*. 73:2217-2224.

17. Farakos, S. M. S., R. Pouillot, and S. E. Keller. 2017. *Salmonella* survival kinetics on pecans, hazelnuts, and pine nuts at various water activities and temperatures. *J. Food Prot*. 80:879-885.

18. Glass, K. A., and J. R. Bishop. 2007. Factors that contribute to the microbial safety of commercial yogurt. *Food Protection Trends*. 27:380-388.

19. Glass, K. A., L. M. McDonnell, R. C. Rassel, and K. L. Zierke. 2007. Effect of cooling rate on pathogen survival in post-process contaminated yogurt. *Food Protection Trends*. 27:16-21.

20. Grocery Manufacturers Association. 2014. *Listeria monocytogenes* guidance on environmental monitoring and corrective actions in at-risk foods. https://ucfoodsafety.ucdavis.edu/sites/g/files/dgvnsk7366/files/inline-files/208833.pdf, Accessed April 12, 2021.

21. Grocery Manufacturers Association (GMA). 2009. Control of Salmonella in Low-Moisture Foods.http://www.gmaonline.org/downloads/technical-guidance-and-tools/SalmonellaControlGuidance.pdf.

22. Grocery Manufacturers Association (GMA). 2010. Industry Handbook for Safety Processing of Nuts. https://ucfoodsafety.ucdavis.edu/sites/g/files/dgvnsk7366/files/inline-files/227728.pdf, Accessed April 29, 2021.

23. Health Canada, and U. S. Department of Health and Human Services. 2015. Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada. *In*, https://www.fda.gov/media/90488/download.

24. Ibrahim, G. 1981. A simple sensitive method for determining staphylococcal thermonuclease in cheese. *J. Appl Bacteriol*. 51:307-312.

25. Ingham, G. A., M. Pan, F. Ranelli, and B. H. Ingham. 2017. Efficacy of a hold-time at 10 degrees C for achieving a 5-log reduction of *Escherichia coli* O157: H7, *Salmonella enterica*, and *Listeria monocytogenes* in prepared mustard. *Food Prot. Trends*. 37:8-15.

1008 26. Innovation Center for U.S. Dairy. 2015. Control of *Listeria monocytogenes*: Guidance for the U.S.
1009 Dairy Industry. https://www.usdairy.com/getmedia/aee7f5c2-b462-4f4f-a99d-
1010 870f53cb2ddc/control%20of%20listeria%20monocytogenes%20guidance%20for%20the%20us%20dairy
1011 %20industry.pdf.pdf, August 12, 2020.
1012 27. Innovation Center for U.S. Dairy. 2019. Controlling Pathogens In Dairy Processing Environments:
1013 Guidance for the U.S. Dairy Industry https://www.usdairy.com/getmedia/cdafaa12-e765-4432-820c-
1014 de5240e6c7ff/pathogen%20guidance_final.pdf.pdf, December 12, 2020.
1015 28. Institute of Food Technologists. 2000. Special supplement: Kinetics of microbial inactivation for
1016 alternative food processing technologies - IFT's response to task order #1, US Food and Drug
1017 Administration: How to quantify the destruction kinetics of alternative processing technologies. *J. Food*
1018 *Sci*. 65:4-108.
1019 29. Institute of Medicine. 2009. Review of Use of Process Control Indicators in the FSIS Public Health
1020 Risk-Based Inspection System: Letter Report. The National Academies Press, Washington, DC.
1021 30. International Commission for the Microbiological Specifications for Foods (ICMSF). 2011.
1022 Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance. Springer,
1023 New York.
1024 31. International Commission on Microbiological Specifications for Foods (ICMSF). 2018.
1025 Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. 2nd edition. Springer
1026 International Publishing, New York.
1027 32. Kornacki, J. I., J. B. Gurtler, and B. A. Stawick. 2013. Ch. 9 Enterobacteriaceae, Coliforms, and
1028 *Escherichia coli* as Quality and Safety Indicators. *In*, Compendium of Methods for the Microbiological
1029 Examination of Foods, 5th ed. APHA Press, an imprint of American Public Health Association,
1030 https://doi.org/10.2105/MBEF.0222.

33. Lobo, A., C. Zuniga, R. W. Worobo, O. I. Padilla-Zakour, and J. Usaga. 2019. Fate of spoilage and pathogenic microorganisms in acidified cold-filled hot pepper sauces. *J. Food Prot*. 82:1736-1743.

34. Minor, T. E., and E. H. Marth. 1972. Fate of *Staphylococcus aureu*s in cultured buttermilk, sour cream, and yogurt during storage. *J Milk Food Technol*. 35:302-306.

35. National Advisory Committee for 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. *J. Food Prot*. 81:115-141.

36. National Research Council. 2009. Scientific Criteria to Ensure Safe Food. The National Academies Press, Washington, DC.

37. Peng, J., J. Tang, D. M. Barrett, S. S. Sablani, N. Anderson, and J. R. Powers. 2017. Thermal pasteurization of ready-to-eat foods and vegetables: Critical factors for process design and effects on quality. *Crit Rev Food Sci Nutr*. 57:2970-2995.

38. Rankin, S. A., A. Christiansen, W. Lee, D. S. Banavara, and A. Lopez-Hernandez. 2010. Invited review: The application of alkaline phosphatase assays for the validation of milk product pasteurization. *J. Dairy Sci*. 93:5538-5551.

39. Salazar, J. K., V. Natarajan, D. Stewart, Q. Suehr, T. Mhetras, L. J. Gonsalves, and M. L. Tortorello. 2019. Survival kinetics of Listeria monocytogenes on chickpeas, sesame seeds, pine nuts, and black pepper as affected by relative humidity storage conditions. *Plos One*. 14:10.

40. Sanyal, B., J. J. Ahn, J. H. Maeng, H. K. Kyung, H. K. Lim, A. Sharma, and J. H. Kwon. 2014. An improved approach to identify irradiated spices using electronic nose, FTIR, and EPR spectroscopy. *J. Food Sci*. 79:C1656-C1664.

41. Scott, V. N., Y. U. H. Chen, T. A. Freier, J. Kuehm, M. Moorman, J. Meyer, T. Morille-Hinds, L. Post, L. Smoot, S. Hood, J. Shebuski, and J. Banks. 2009. Control of *Salmonella* in low-moisture foods I: Minimizing entry of *Salmonella* into a processing facility. *Food Prot. Trends*. 29:342-353.

42. Smittle, R. 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. *J Food Prot*. 63:1144-53.

43. Thevaraja, M., K. Govindaraju, and M. Bebbington. 2021. Modelling the effect of sampling methods on detection tests for powdered products*. Food Control*. 120:107512.

44. Thiyagarajan, S., and P. Vasanth. 2011. *Staphylococcus aureus* thermonuclease detection is a tool to investigate enterotoxin production in raw and processed food products. *Indian J. Appl. Microbiol.* . 13:1-9.

45. U. S. Department of Health and Human Services. 2019. Grade "A" Pasteurized Milk Ordinance, Including Provisions from the Grade "A" Condensed and Dry Milk Products and Condensed and Dry Whey--Supplement I to the Grade "A" Pasteurized Milk Ordinance. *https://www.fda.gov/media/140394/download*.

46. U. S. Food and Drug Administration. 2015. FSMA Final Rule for Preventive Controls for Human Food-Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food; Final Rule*.*

47. U.S. Department of Health and Human Services. 2015. FSMA Final Rule on Produce Safety-Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Final Rule. http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm, Accessed August 10, 2021.

48. U.S. Food and Drug Administration. 2005. CPG Sec 570.450 Tree Nuts - Adulteration with filth, involving the presence of the organism *Escherichia coli.* https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-570450-tree-nuts-adulteration-filth-involving-presence-organism-escherichia-coli.

49. U.S. Food and Drug Administration. 2016. FY 2014 – 2016 Microbiological sampling assignment summary report: raw milk cheese aged 60 days*.* https://www.fda.gov/media/99340/download, Accessed March 20, 2021.

50. U.S. Food and Drug Administration. 2021. 21 CFR Part 112—Standards for the growing, harvesting, packing, and holding of produce for human consumption. https://ecfr.federalregister.gov/current/title-21/chapter-I/subchapter-B/part-112?toc=1, April 11, 2021.

51. U.S. Food and Drug Administration. 2021. 21 CFR Part 117—Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food. https://www.ecfr.gov/cgi-bin/text-idx?tpl=/ecfrbrowse/Title21/21cfr117_main_02.tpl.

52. U.S. Food and Drug Adminstration. 2017. Control of *Listeria monocytogenes* in Ready-To-Eat Foods: Guidance for Industry. https://www.fda.gov/files/food/published/Draft-Guidance-for-Industry--Control-of-Listeria-monocytogenes-in-Ready-To-Eat-Foods-%28PDF%29.pdf, Accessed April 26, 2021.

53. Western Agricultural Processors Association. 2015. Good Agricultural Practices (GAPs) For Production of Tree Nuts. https://ccgga.org/wp-content/uploads/2016/11/Good-Agricultural-Practices-for-Nut-Growers_WAPA-9-11-15-Final.pdf, Accessed February 5, 2021.

54. World Health Organization, F. 2019. Statistical aspects of microbiological criteria related to foods: A Risk Manager's Guide. https://apps.who.int/iris/rest/bitstreams/1060496/retrieve.

55. Zwietering, M. H., L. Jacxsens, J. M. Membre, M. Nauta, and M. Peterz. 2016. Relevance of microbial finished product testing in food safety management. *Food Control*. 60:31-43.