Microbiological Testing by Industry of Ready-to-Eat Foods Under FDA's Jurisdiction for Pathogens (or Appropriate Indicator Organisms): Verification of Preventive Controls[1]

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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. Advise 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

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 appendix, 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 could be used to 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 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 are given in Table 1. Detailed answers to questions for each commodity are provided in Appendices A-F. Criteria to consider include:

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.

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 (TNase) 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 those 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 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

492 frequently, finished product pathogen testing is indicated if investigative testing from an Environmental
493 Monitoring Program (EMP) for *Listeria* or *Salmonella*, suggests there is potential cross-contamination to
494 the product from the environment, either inherently due to design and construction of the facility or
495 equipment or due to the recurring presence of these pathogens in zones 2 or 1. In these cases, the
496 implicated product is held and tested for the pathogen using a statistically based sampling program and
497 validated detection method to determine contamination.

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

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

509 One situation where pathogen testing of RTE foods or ingredients with a long shelf life may be
510 appropriate is for products that have a history of microbial contamination (e.g., milk powders). In these
511 cases, hold and testing may be frequent, such as for lot-disposition. In general, the frequency of lot testing
512 of the final product is determined by an assessment of risk. If the time for processing after lethality is long
513 (such as days), or if product has multiple points of exposure to recontamination after the lethality step,
514 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 continuum. Once actionable limits for test results are established at points along the entire manufacturing process, a company can then respond to those results based on food safety impact.

Measurement of process control is based on the following *(35)*.

1. Sampling and assessing the output of the process for key microbial targets should occur at a frequency that limits the amount of time that a loss of control goes unrecognized. Frequency of sampling is predicated on the propensity for the system to lose control, the prevalence of the microbial target and practicality, balancing rapid recognition of a system out of control with the cost of sampling and testing. Sampling sites are selected that are representative of the product as it passes through the process or as it exits the process. Larger sample sizes add statistical relevancy. Testing frequency and sample size taken should be risked based. More intensive testing is needed for foods which there is little information, e.g., for new suppliers, a new processing line or product, or for individual foods or ingredients that have been shown to have higher prevalence of microbial risks e.g., for spices obtained in certain regions. As a firm builds data base of microbial results, can refine understanding product will be outside microbial limits that have been identified to verify that process is in control.
2. Process control performance limits and testing targets (e.g., specifications) are predefined for the type of food product, type and extent of processing, RTE status, chemical and physical characteristics of the food product, and the history of the process. Microbial criteria for food safety or food quality need to be relevant to signaling a hazard in a specific product and be attainable.
3. A system for documentation and review of results is in place that allows corrective action with the appropriate level of immediacy.
4. A predetermined plan of action (POA; a corrective action plan) is developed based on a scaled response considering public health impact, deviation from relevant limits, and frequency of the deviation. For example, a typical set of POA choices might be take no action, move to increased sampling frequency or sample size, conduct a predetermined internal or external audit of the

process that is typical for out-of-control variability, and identify an assignable cause through root-cause analysis and take corrective and preventive actions. The corrective actions specified must be subsequently verified to ensure they reduce or prevent future deviations. The proper 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.
- location of the sampling site and proximity to finished product.
- to what extent did the level deviate from the limit for a quantitative microbiological result?
- 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,

705 acidification rate, finishing table, brine tanks, block formation, aging, cutting, and packaging to determine
706 the source(s) of loss of control and to implement corrective action. The findings of the root cause analysis
707 will dictate corrective actions and whether verification testing that includes finished product is indicated
708 (Appendix Table A-1).
709
710 Example Appendix Table A-1. Microbial targets, limits, and recommended actions if limits are exceeded,
711 for soft cheeses made with pasteurized milk. Additional testing may be indicated for cheeses made with
712 raw milk *(5, 23)*.

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms or Enterobacteriaceae	$\leq$100/g	Investigate reason for exceeding limit and implement corrective action; consider testing for *E. coli* ($\geq$10/g) if coliforms are detected	Routine testing
S. aureus	$\leq$100/g	If $\geq 10^4$/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

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
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

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 (Appendix Table D-1).

Example Appendix 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

734

735 **Answer Q8-2.** ***What actions should a company take if test results indicate a loss of process***
736 ***control?*** Microbiological and chemical limits for foods for use by the United States Department of Defense
737 to assess process control and insanitary conditions were evaluated and published by a previous NACMCF
738 committee *(35)*. The microbiological limits reported for indicator organisms in that document are not lot
739 acceptance criteria, unless there is a regulatory limit associated with that value, such as limits for coliforms
740 in milk or generic *E. coli* in nuts (see NACMCF-DOD Appendices *(35)*. The 2018 NACMCF-DOD document
741 was developed for inspectors or auditors to evaluate whether a food was produced under sanitary
742 conditions without having full knowledge of the processing conditions. However, the target
743 microorganisms and limits included both product and environmental monitoring that would be useful to
744 the manufacturer that their process is in control. Therefore, both the NACMCF-DOD guidance and this
745 document provide guidance to evaluate sanitary conditions and process control for foods, including
746 appropriate target microorganisms and limits in foods, as well as recommended actions to be taken if the
747 limits are exceeded. In many instances, actions include investigating to determine a root cause,
748 implementing corrective and preventive actions, and conducting follow-up sampling and testing to
749 determine if the corrective and preventive actions have been effective. These actions were categorized
750 as “Investigate” or “Implement Corrective Actions.” The 2018 NACMCF-DOD document indicated that
751 investigative and corrective action procedures would likely be unique to each situation. Given the scalable
752 approach recommended for determining loss of control, actions taken would also depend on the type of
753 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.

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.

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APPENDIX A - CATEGORY: DAIRY

Apart from some cheeses, the dairy food categories listed below are made with pasteurized milk to eliminate common vegetative bacterial pathogens. Therefore, the presence of any pathogen represents post-process contamination, loss of process control, or insanitary conditions. *Salmonella, E. coli* O157:H7, and *L. monocytogenes* are considered adulterants in RTE dairy products. In the United States, some dairy products such as fluid milk and yogurt are regulated by States under the Grade "A" Pasteurized Milk Ordinance (PMO)*(63)*. Pasteurization of milk and milk products is required under 21 CFR 1240.61. FDA has enforcement policies for microbial pathogens and indicators of inadequate pasteurization or post-pasteurization contamination of dairy products identified in the dairy compliance guidelines *(64)*. Pathogens such as *Salmonella* and *E. coli* O157:H7 must be absent. Actionable limits for *S. aureus* and *B. cereus* are set at 10^4 CFU/g, whereas limits for generic *E. coli* or coliforms are product specific *(64)*. Other resources for microbiological specifications and guidelines include the *Compendium of Methods for the Microbiological Examination of Foods (4)* and the *Standard Methods for the Examination of Dairy Products (43, 72)*. Alkaline phosphatase concentrations in dairy products other than cheese and related cheese products are limited to less than 2.0 micrograms phenol equivalents per gram in one or more subsamples, whereas cheeses may have higher limits *(1, 64)*. In many cases, coliforms or Enterobacteriaceae are acceptable for routine testing *(40)*. The widely used microbiological count method is the standard plate count (SPC; usually referred to as aerobic plate count or APC) agar method that estimates the microbial population in various dairy products to determine contamination during processing. The SPC is the reference method standard by the National Conference on Interstate Milk Shipments (NCIMS) and PMO for raw and pasteurized milk and milk products *(72)*.

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 $\leq$ 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)

Butter, Margarine

Examples include sweet cream butter (salted and unsalted), cultured sour butter, whipped butter, whipped butter with herbs. For the purposes of this document, the Committee did not include examples for margarine. While margarine may mimic butter in appearance and use, the product composition, production methods, and microbial ecology are different than butter. Principal methods for controlling pathogens in butter are microbial quality of ingredients, pasteurization of raw materials (cream and milk), hygiene during production and packaging, minimal size and even distribution of the water droplets in the fat matrix, and the presence of salt *(41)*. Starter cultures, if used, are not typically a source of contamination. Ingredients such as salt, coloring agents, and neutralizers are usually free of microbial contamination. Water used post pasteurization (e.g., for washing) should be of potable quality. Ingredients should be sourced from suppliers meeting specifications.

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

50

Criterion/Factor	Butter
A. Are pathogens associated with the food or ingredients?	Yes. Outbreaks due to *L. monocytogenes* and *S. aureus* have occurred *(24, 28, 44, 45, 49)*. Also, of concern are *Salmonella, Campylobacter,* and *E. coli* O157:H7, but these pathogens are less likely unless adjunct ingredients such as herbs are added after the cream pasteurization step *(19, 62)* .
B. Are the ingredients likely to be contaminated?	No. Cream is pasteurized to inactivate vegetative pathogens. If fresh herbs are added to whipped butter, they could be contaminated with pathogens such as pathogenic *E. coli, Salmonella*, and *Cyclospora*.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes. Critical ingredients such as cream and milk are pasteurized. Verification of supplier control of biological hazards should be used for any ingredient added post-lethality.
D. Is there a potential for recontamination from the handling or the environment?	Yes. Butter is made with pasteurized cream, but churning, salting, and packaging occurs after pasteurization. Product may be exposed to pathogens from employees during handling and from the environment.

Criterion/Factor	Butter
E. Does the product support survival or growth?	Yes, studies have shown the potential for pathogens such as *S. aureus* or *L. monocytogenes* to grow in butter are impacted by the characteristics of the butter (pH, a_w, salt, cultures), as well as the storage temperature *(24, 28, 47, 71)*. Microbial spoilage of butter is caused mainly by yeasts and molds, and sometimes bacteria. These may be introduced through poor hygiene before or during packaging, or during use. Whipped butter made by the addition of milk, water, or incorporation of herbs can alter the emulsion and thereby support the growth of pathogens. Whipped butter made by the addition of gas only, does not significantly alter the ability of pathogens to survive or grow compared to unwhipped butter. Unsalted butter that has a higher water activity than salted butter can have increased microbial stability if pH is reduced as a preventive control during production.
F. Is this product meant for higher risk population?	Butter is not specifically intended for higher risk populations but will be consumed by the elderly and immunocompromised.
G. What is the shelf life of the product?	Refrigerated shelf life of butter varies between 3 and 9 months, depending on the level of salt, pH, or other preservatives present.
H. Will consumer handling and use increase or decrease likelihood of	The scenarios are unlikely to affect risk for salted butter but holding some types of unsalted or whipped butter at non-refrigerated

Criterion/Factor	Butter
pathogen survival, growth, or toxin production and risk of consumer illness?	conditions could allow growth of pathogens such as *S. aureus* if it is present.

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

For certain products, moisture content, salt content and distribution, and the water droplet size/distribution are important for microbiological stability, therefore, testing for moisture, fat and salt serve as both quality and safety parameters; pH is an important parameter for testing in cultured butter and unsalted butter that is acidified as an additional barrier to microbial growth. If the temperature of incoming milk or cream is >45°F/7.2°C, load would typically be rejected rather than testing for enterotoxin.

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

Yes, if pasteurization and other process controls, as well as environmental control, are verified, finished product may not need microbial verification testing other than for quality as required by customers. However, if the product is open to cross-contamination, results from environmental monitoring will direct whether product testing is appropriate.

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

Because butter is made with pasteurized cream and may contain salt, routine product testing is limited to indicators of process control and sanitation (e.g., enumeration of Enterobacteriaceae or coliforms and aerobic plate count). Customer requirements, such as for butter produced in plants operating under USDA AMS inspection and grading services, may include additional quality-based microbial specifications (proteolytic count, not more than 100 CFU/g; yeast and mold count, not more than 20 CFU/g; coliform count, not more than 10 CFU/g, found in 7 CFR 58.345 and for whipped butter, proteolytic count, not more than 50 CFU/g; yeast and mold count, not more than 10 CFU/g; coliform count, not more than 10 CFU/g; found in 7 CFR 58.346).

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?

Environmental exposure during processing, environmental monitoring program results, and ability to meet processing limits (such as pH, salt, or moisture as determined by the hazard assessment), should be used to determine type and frequency of finished product testing. In general, routine finished product testing for Enterobacteriaceae or coliforms (or other microbial targets identified by 7 CFR 58.345 or 7 CFR 58.346) should be used to verify overall process control and sanitation.

Several situations will indicate that additional product testing is necessary. For example, investigative testing may be needed when populations of indicator organisms exceed specified limits (e.g., >10 CFU/g coliforms or Enterobacteriaceae) suggesting insufficient sanitation, or if environmental

monitoring for *Listeria* spp. suggests that contamination by *L. monocytogenes* may have occurred during the production process (test for absence of *L. monocytogene*s; see product testing recommendations in *(70)*). Other investigative testing includes if there is a failure to achieve formulation parameters (such as insufficient acidification for unsalted or cultured butter) or processing time (such as extended interruptions during production) that may have allowed for growth of *S. aureus;* in such cases, test finished product to ensure $\leq 10^4$ CFU/g *S. aureus* as appropriate.

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

Yes, testing aerobic colony count and Enterobacteriaceae or coliforms can be done on product obtained during production, as well as environmental testing. ATP detection is a useful and quick tool to verify that cleaning and sanitation removed organic matter off lines and product contact surfaces.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

Efficiency of cleaning should be verified for equipment and environment before process start-up, such as by visual inspection, ATP detection, or by testing aerobic colony count at an appropriate frequency. If microbial testing for indicator organisms exceeds limits, investigate source of contamination and implement corrective actions for sanitation and test final product to ensure corrections have been made. As an RTE product exposed to the environment, EMP for *Listeria* spp. should be implemented to reduce the need for testing finished product for *L. monocytogenes*. If EMP suggest that contamination of product by *L. monocytogenes* may have occurred, refer to guidance for testing product *(68).*

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

Table A-1 of this appendix, the Compliance Policy Guide for Dairy products *(64)* and the Department of Defense *(50)* outline microbial limits for various microbes and populations in finished product that can be used as verification of process control. If routine testing for indicators such as Enterobacteriaceae or coliforms in the finished product, or *Listeria* spp. in the environment, are out of specification and indicate loss of process control, facility should investigate source of failure to meet limits and implement corrective action; reference industry and government guidance documents for environmental testing for *Listeria* spp*.,* product testing for *L. monocytogenes,* and follow-up testing needed to ensure process is back in control *(30, 31, 63, 68)*. Products at risk of being contaminated with pathogens should be placed on hold and kept in the company's control until follow-up testing is completed and lot is cleared.

In addition, to microbial testing, other indicators of process control include monitoring of chemical and physical parameters of finished product (such as salt-in-moisture and pH) and production time-temperature (such as extended runs or production down time). Delays during production or out of specification temperatures, particularly for unsalted butter with high water activity and high pH, could allow pathogen growth. Under these circumstances, investigative testing in implicated finished product (e.g., testing for *Staphylococcus aureus*) is recommended.

Table A-1. Microbial targets, limits, and recommended actions if limits are exceeded, for butter either refrigerated or formulated with sufficient salt or lactic acid to prevent growth; products containing added seasoning/herbs/spices may have additional requirements.

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms or Enterobacteriaceae	≤10 CFU/g	Investigate reason for exceeding limit and correct	Routine testing
Aerobic plate count (APC, SPC)	≤10^3 CFU/g	Investigate reason for exceeding limit and correct	This assay is not appropriate for cultured butter that will have higher counts due to use of starter culture
Proteolytic	≤100 CFU/g ≤50 CFU/g whipped butter	Investigate reason for exceeding limit and correct	Testing for USDA AMS specifications, in 7 CFR 58.345, 7 CFR 58.346
Yeast and mold	≤20 CFU/g ≤10 CFU/g whipped	Investigate reason for exceeding limit and correct	Testing for USDA AMS specifications, in 7 CFR 58.345, 7 CFR 58.346

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
S. aureus	≤100 CFU/g	Investigate, implement corrective action. If ≥10^4 CFU/g, reject lot due to potential for enterotoxin production	Investigative testing if loss of process control is suspected.
Listeria monocytogenes	Absent in 25 g	Destroy lot or divert to appropriate use with a lethality step. 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

***Recommendations* for butter**:

- Because there is a pasteurization step for cream, no finished product testing for pathogens is needed when an effective EMP program is in place and other non-microbial monitoring (physiochemical and temperature/time controls) verifies that the process is in control.
- Microbial testing of product should be focused on indicator organisms that reflect post-process contamination.
    - Enterobacteriaceae or coliforms populations should be ≤10 CFU/g.

- Plants operating under USDA AMS inspection and grading services may have additional customer requirements for aerobic colony counts, yeast/mold, and/or proteolytic microbes

- In addition to microbial testing for indicator organisms, monitor physiochemical properties of the butter that are important in formation control of microbes (such as pH, salt-in-moisture, fat) and temperature-time for processing.
- Microbial testing for pathogens in product is part of investigative actions (e.g., in response to out of compliance environmental results, inadequate formulation control, or inadequate temperature/time control), rather than routine testing.
  - If EMP suggest that contamination of product by *L. monocytogenes* may have occurred, refer to industry and government guidance documents for testing product.
  - If results for formulation parameters or time-temperatures controls indicate a loss of process control, enumeration *S. aureus* in product that exceeds 10^4 CFU/g will determine disposition of the product

Cheese

Cheese is made by coagulating milk with acid (developed by starter or direct acidification), acid in combination with heat, or rennet. Classifying cheese by texture (hard, grating, semi-soft, soft) is driven by moisture, with water holding capacity influenced by pH, pressing of curd, and aging. Both moisture and salt content impact water activity (a_w), with a_w 0.87 and 0.92 inhibitory to *S. aureus* enterotoxin production and *L. monocytogenes* growth, respectively, and a_w <0.95 inhibitory to *Salmonella* and *E. coli* O157:H7. Cheese matrices may be inhibitory at higher water activities (e.g., >0.95) depending on product pH, acid type, presence of other competitive microbes, and antimicrobial compounds produced by starter or

adjunct cultures *(3, 21, 42)*. However, pathogens can survive for extended months at reduced a_w, depending on other stress conditions.

While moisture is typically stable or decreases during ripening, the pH of cheeses frequently increases during aging due to growth of molds on surface-ripened and blue-veined cheeses or by deamination of proteins by starter and non-starter lactic acid bacteria proteolytic enzymes. For example, the pH of the curd immediately after pressing may be pH 5.2 but may increase to 5.8 or 7.0 in Gruyere and Camembert, respectively, after the ripening process. Because of the dynamic conditions for many cheeses, evaluating hazards for a given cheese should consider contaminating pathogens at the end of initial production (curd at pressing) and at packaging, as well as throughout aging and storage.

Classification	**Examples**	**Typical Moisture ranges[1]**
Extra hard, grating	Parmesan, Medium and Old Asiago	<37%
Hard	Cheddar, Colby	<40%
Semi-soft	Edam, Gouda, Brick, Muenster, Provolone, Blue, Low-Moisture Part Skim Mozzarella, Swiss	40-50%
Soft, ripened,	Camembert, Brie,	50-60%
Soft, unripened	Queso fresco, Queso de Crema, Queso de Puna, fresh mozzarella, cream cheese, feta, ricotta	>50%

A special note is for cheese made with unpasteurized milk because risks may be present in these cheeses that are not present in cheeses made from pasteurized milk. Specifically, cheeses made with

[1] While individual cheeses may have limits for moisture identified in CFR standards of identity, moisture within general classifications based on texture or ripening is not regulated.

unpasteurized milk should consider additional testing of pathogens in finished product depending on thermization or other heat treatment, make procedures, cultures, product moisture, pH, brining, and aging conditions. Current regulations in the United States allow the sale of certain cheeses with standards of identity to be made from unpasteurized milk if the cheese is aged for at least 60 days at a minimum temperature of 35°F (1.7°C). However, aging may not be sufficient to eliminate low infectious dose pathogens, such as *E. coli* O157:H7 *(18)* and, depending on product moisture and pH, certain cheeses could allow growth of pathogens such as *L. monocytogenes (26, 61)*

Cheese, hard (e.g., Cheddars), extra hard, grating (e.g., Parmesan, Romano)

Microbiological safety issues are extremely rare in hard cheeses made with pasteurized milk and active starter cultures and adequate environmental controls. If cheese milk is contaminated (through use of unpasteurized milk or through post-pasteurization contamination in the vat), pathogens populations can increase/concentrate in the curd; *Salmonella,* STEC, and *Listeria monocytogenes* do not grow but can survive for several months during aging. Cheeses with slow starter activity (slow acidification) have been associated with growth of *S. aureus* and staphylococcal enterotoxin that can remain active during the aging process *(76)*.

Very hard cheeses, such as intact Parmesan and Romano, are inhibitory to growth of bacterial pathogens due to low a_w (e.g., <0.92); validation studies have shown that these cheeses are typically non-TCS (non-time/temperature control for safety)*(3, 21, 38, 42)*. Hard cheeses, such as Cheddar and Gruyere, with water activity <0.95, initial pH <5.6, and residual starter activity are similarly inhibitory to pathogens but are typically refrigerated for quality *(3, 42)*. Low-salt hard cheeses that have water activity >0.95 and pH >5.6 may require temperature control to ensure stability unless validation studies suggest otherwise.

The presence of active cultures in hard cheeses makes the use of routine microbiological testing for aerobic plate count (known as APC or SPC) impractical as a tool for evaluation of process controls and sanitary conditions. In contrast, testing for coliforms or Enterobacteriaceae, which are destroyed by pasteurization, can serve as an indication of post-process contamination from insanitary conditions for cheeses made with pasteurized milk. Testing for *S. aureus* or generic *E. coli* is useful under special circumstances such as investigation when production has occurred without adequate process control. For cheeses made with unpasteurized milk, additional pathogen testing is recommended both for the milk as well as for finished product. For all cheeses, regardless of the use of pasteurized milk, regular environmental testing of the food production environment for the presence of *Listeria* spp. and *Salmonella* is recommended as a verification step for sanitation programs.

Example 1 - Extra hard cheese made with pasteurized milk (e.g., Parmesan, Asiago for grating) finished product a_w typically <0.91

Example 2 - Hard cheese made with unpasteurized milk (e.g., raw milk Cheddar); finished product a_w typically <0.95

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

Criterion/Factor	Extra hard cheese made with pasteurized milk (e.g., Parmesan, Asiago for grating) finished product a_w typically <0.91	Hard cheese made with unpasteurized milk (e.g., raw milk Cheddar); finished product a_w typically <0.95
A. Are pathogens associated with the food or ingredients?	No, pasteurization of milk used for cheesemaking will eliminate vegetative bacterial pathogens, rendering it safe for use. Food safety issues for this cheese type are rare. Use of adjunct ingredients, such as fresh herbs or spice rub, could serve as a source of contamination.	Yes, raw milk may contain multiple pathogens, including *Salmonella, E. coli* O157:H7 and other STEC, *L. monocytogenes*, *S. aureus*, and *Brucella*. Outbreaks have been associated with raw milk cheeses due to survival of low infectious dose pathogens beyond 60-day aging *(16, 29)*
B. Are the ingredients likely to be contaminated?	Hard cheeses made with pasteurized milk are rarely contaminated. Ingredients such as spices and herbs, the environment, or food handlers may be a source of *Salmonella, E. coli* O157:H7*, L. monocytogenes* or *S. aureus*.	Surveys suggest 1-2% of raw milk samples used for artisan cheeses contain one or more pathogens *(17)*. Bulk milk samples can have higher rates of contamination *(58)*.
C. Are there robust processing control procedures such as a	Milk pasteurization is a robust kill step for most vegetative pathogens.	Mild heat treatments, such as thermization may reduce pathogens by only 1 or 2 logs,

Criterion/Factor	Extra hard cheese made with pasteurized milk (e.g., Parmesan, Asiago for grating) finished product a_w typically <0.91	Hard cheese made with unpasteurized milk (e.g., raw milk Cheddar); finished product a_w typically <0.95
kill step or other reduction methods/controls?	Suppliers should have a validated lethality treatment for ingredients/inclusions (spices, herbs, fruits, etc.) added post lethality. Sanitation will reduce microbes on food contact surfaces and in the environment.	unless temperature-time combinations been validated for efficacy. The aging/ripening process for hard and very hard cheeses will reduce pathogen load over time, but 60-day aging may be insufficient to qualify as a robust reduction step in raw milk cheeses. Rate of inactivation relies on combined stresses such as drying (low water activity), acidity, residual starter activity, and storage temperatures >3°C to accelerate lethality during aging. Although the aging process of cheeses inactivates pathogens over time, some low infectious dose pathogens, such as *E.* coli O157:H7, have been shown to survive months. Sanitation will

Criterion/Factor	Extra hard cheese made with pasteurized milk (e.g., Parmesan, Asiago for grating) finished product a_w typically <0.91	Hard cheese made with unpasteurized milk (e.g., raw milk Cheddar); finished product a_w typically <0.95
		reduce microbes on food contact surfaces and in the environment.
D. Is there a potential for recontamination from the handling or the environment?	Yes, there can be potential for recontamination of the cheese milk during curd development and handling, brining, during the aging process, portioning, or packaging.	Yes, there can be potential for cross-contamination of the cheese milk during curd development and handling, brining, during the aging process, portioning, or packaging.
E. Does the product support survival or growth?	Most extra hard cheeses (finished product) do not support growth of pathogens during aging and storage due to combinations of reduced water activity, pH/acidity, and residual starter culture activity. If acidification rate is compromised due to slow or failed starter culture activity during the cheesemaking process, pathogens such as *S. aureus* may grow and produce enterotoxin. While populations of	Most hard cheeses (finished product) do not support growth of pathogens during aging and storage due to combinations of reduced water activity, pH/acidity, and residual starter culture activity. These stresses will typically result in inactivation of pathogens during aging, but hard cheeses made with contaminated milk may have pathogen survival for >60 days.

Criterion/Factor	Extra hard cheese made with pasteurized milk (e.g., Parmesan, Asiago for grating) finished product a_w typically <0.91	Hard cheese made with unpasteurized milk (e.g., raw milk Cheddar); finished product a_w typically <0.95
	vegetative cells will decline during aging of the cheese, enterotoxin will be stable.	If acidification rate is compromised due to slow or failed starter culture activity during the cheesemaking process, pathogens such as *S. aureus* may grow and produce enterotoxin. While populations of vegetative cells will decline during aging of the cheese, enterotoxin will be stable.
F. Is this product meant for higher risk population?	This food is not specifically intended for high-risk populations, but people from high-risk populations may choose to consume this type of product	This food is not specifically intended for high-risk populations, but people from high-risk populations may choose to consume this type of product
G. What is the shelf life of the product?	Variable, several years.	Variable, several years.

Criterion/Factor	Extra hard cheese made with pasteurized milk (e.g., Parmesan, Asiago for grating) finished product a_w typically <0.91	Hard cheese made with unpasteurized milk (e.g., raw milk Cheddar); finished product a_w typically <0.95
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	Because many cheeses within these categories are non-TCS, temperature abuse by the retailer or consumer or holding food beyond use-by date will have little impact on pathogen survival, growth, or toxin production and hence no increased risk for consumer illness.	Because many cheeses within these categories are non-TCS, temperature abuse by the retailer or consumer or holding food beyond use-by date will have little impact on pathogen survival, growth, or toxin production and hence no increased risk for consumer illness.

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

Although alkaline phosphatase (ALP) can serve as an indicator of pasteurization, residual heat in very large wheels of raw milk hard cheese can lead to inactivation of ALP *(52)*.. Pathogen growth is inhibited in milk during cheese making by robust starter culture activity and acid development. Therefore, monitoring pH during acidification of the curd will detect slow fermentation that may result in a product that can support the growth of pathogens. Testing in-process or finished product for moisture, salt and/or water activity is important to verify formulation control. Testing for alkaline phosphatase can serve as an indicator of pasteurization *(63)*.

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

When the process is in control, microbial testing of indicator organisms is an appropriate verification activity because there are several points in the manufacturing process where post-pasteurization contamination could occur (curd development, pressing, brining, packaging, conversion to slices/shreds, etc.).

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

In general, intact hard and extra hard cheeses are unlikely to support the growth of pathogens due to a combination of suboptimal water activity, total acidity, and competitive microbiota. A company should use published or commissioned validation studies or use "safe harbors" such as a_w or pH growth limits *(32, 67, 68)* to identify pathogen growth inhibition parameters for specific cheese types, particularly for non-refrigerated conditions. Under these situations, routine enumeration of indicator organisms in pasteurized milk, along with determining that the growth inhibition parameters are met for the cheese, may be sufficient for verification of process control.

Converting cheese into sliced, shredded, or grated forms may disrupt the inherent safety system. In general, finished product testing is not an effective verification tool in hard cheeses due to the distribution and low levels of potential contaminants. However, finished product testing may be appropriate in cases where the converted cheese (such as slices for prepared refrigerated sandwiches,

pieces for deli salads, etc.) could potentially deliver contaminants, such as *L. monocytogenes*, in foods that could support growth of pathogens (e.g., test presence/absence *L. monocytogenes* in 25 g samples).

Regardless of product composition, aging and storage, environmental monitoring for *Listeria* spp. and *Salmonella (68)* and routine enumeration for Enterobacteriaceae or coliforms (<100 CFU/g) in finished product can be used as indicators of post-process contamination. If acid production is slow, it is advisable to test for coagulase-positive staphylococci or *S. aureus* ($<10^4$ CFU/g)

Cheeses made with raw or thermized milk are more likely to have pathogens derived from the milk that have survived during aging; therefore, testing finished product for presence/absence of *E. coli* O157:H7 and/or *Salmonella* can be used as verification that preventive controls have been adequately implemented.

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?

Because of the great diversity of cheese types produced in many regions, as well as production, consumption, and distribution practices, it is difficult to recommend specific universally applicable testing for all cheese types. Testing frequency will be facility and line dependent, and should consider the

- Potential for recontamination (such as sanitation, extended runs, brine management, etc.)
- Intrinsic properties of the cheese (pH, water activity, microbiota, validation studies that identify pathogen growth, survival, or die-off), aging/ripening conditions, product history of recalls or related illness
- Intended use (retail, food service, institutions)
- Facility-specific process; extent of exposure and handling post-lethality prior to packaging

- Facility-specific infrastructure condition (floors, walls, ceilings, separation of raw/RTE, traffic control, etc.)
- Facility-specific sanitary condition of equipment/processing lines
- Internal testing history (product and environmental)

For example, for cheeses that do not support growth of *L. monocytogenes (61)* and in facilities with a robust environmental monitoring program, routine testing (such as on a lot basis) for indicators of post-process contamination and sanitation, such as Enterobacteriaceae, may be more effective to verify that the food safety system is in control than finished product testing. For cheeses made with raw milk, having a COA or testing incoming milk for *Salmonella* and *Listeria* can provide insight for likelihood of contamination of the finished cheese after minimum aging. If cheese is positive for pathogens at any point during aging, reject the lot unless cheese can be reconditioned to eliminate the pathogen.

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

Environmental monitoring and in-process testing (e.g., in curd after pressing) before aging provides more useful information to evaluate the safety of the product. Monitoring the pH of curd can detect slow fermentation and indicate that testing for *S. aureus* ($<10^4$ CFU/g) may be relevant if acidification does not proceed as anticipated. Testing for indicator organisms (e.g., molds, yeasts, Enterobacteriaceae, or *Listeria*-like microorganisms) in brine or curd for *E. coli* (<100 CFU/g) in cheese made from heat-treated milk may be useful to verify process control and hygiene conditions. For raw milk cheese, pull milk samples from the cheese vat after a homogeneous mix or utilize a disposable filter sock on the milk discharge pipe to the vat, after fill. In addition, supplier control of milk can be achieved through herd management,

mastitis control, focus on feeding regimens, and sanitation during milking, storage and transportation to the cheese makers *(2)*. Microorganisms are likely to decline during the aging process of hard and very hard cheeses; in particular for raw milk cheeses that rely on the aging process to control microbial hazards, testing finished product for pathogens will serve as a final verification that process was effective before release of the product.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

Hard cheeses do not support growth of pathogens, but routine environmental monitoring for *Listeria* species (*Listeria*-like microorganisms as an indicator for *L. monocytogenes*) and Enterobacteriaceae (as an indicator of sanitation), and/or *Salmonella* can determine if post-process contamination problem spots exist *(30, 31, 68, 75)*. If environmental monitoring identifies positive samples, follow guidelines for corrective action and resampling *(30, 68)*. Cheese that has been made from raw milk should be tested for *Listeria* spp., *E. coli* (STEC) and *Salmonella* at 60-day age prior to release for distribution *(2)*.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

The Compliance Policy Guide for Dairy products *(64)* and Department of Defense *(50)* outlines microbial limits for various pathogens and recommendations for indicator organisms. A facility should track/trend test results (using a 3-class sampling plan for indicators such as *E. coli*, Enterobacteriaceae,

etc.) and have identified action limits e.g., for pass, warning, and fail. Reacting to results trending up, in the warning level, will prevent a loss of control. In addition, a company should reference federal and industry guidance documents for environmental testing for *Listeria (30, 31, 63, 68)*.

When there is a loss of systemic process control, there should be a root cause analysis performed, including looking at sanitation procedures, environmental controls, heat-treatment of milk, supplier controls (such as for inclusions, rubs, surface cultures, or raw 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 implement corrective action. The findings will dictate the extent to which verification testing is increased.

Table A-2. Microbial targets, limits, and recommended actions if limits are exceeded, for hard cheeses made with pasteurized milk. Additional testing may be indicated for cheeses made with raw milk where low infectious dose pathogens, such as *E. coli* O157:H7 may survive 60-day aging requirements *(2)*.

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms or Enterobacteriaceae	≤100 CFU/g	Investigate reason for exceeding limit and implement corrective action; consider testing for *E. coli* (<10 CFU/g) if coliforms are detected	Routine testing
S. aureus	≤100 CFU/g	Investigate reason for exceeding limit and implement corrective	Investigative testing for *S. aureus* if pH monitoring of

		action. If $\geq 10^4$ CFU/g, reject lot due to potential for enterotoxin production *(64)*. Due to heat stability of enterotoxin, diverting to further processing is not recommended	a vat suggests acid development is slow. Investigate reason for slow pH drop, implement corrective action.
Listeria monocytogenes	Absent in 25 g	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
Salmonella	Negative in 375 g analytical units (15 x 25-g samples)	Reject lot. Testing may be in-process vat sample due to the aging process for natural cheese	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.

Recommendations for hard, extra hard, and grating cheese.

- For hard and very hard cheeses made **with pasteurized milk** and that are known to inhibit growth of pathogens during normal storage, no routine finished product testing for pathogens is needed when an effective EMP program is in place and other non-microbial monitoring (such as monitoring acid development) suggests process is in control.

- Routine enumeration testing of product for indicator organisms of adequate sanitation (e.g., Enterobacteriaceae or coliforms) and environmental monitoring for *Listeria* spp. should be conducted to identify loss of process control and to identify risks of post-process contamination *(30, 68)*.
- Investigative microbial testing of finished products for pathogens (e.g., *L. monocytogenes* or *Salmonella*) should be conducted if other testing suggests inadequate environment control and likely product contamination.
    - If EMP suggest that contamination of product by *L. monocytogenes* or *Salmonella* may have occurred, refer to industry and government guidance documents for testing product.
- If monitoring of acidification rate suggests poor starter activity, which can allow growth of infectious and toxigenic microorganisms enumeration *S. aureus*, investigative testing of pressed curd for *S. aureus* should be conducted.
    - Destroy lots where enumeration of *S.* aureus in product exceeds 10^4 CFU/g
- In addition to above, for hard and very hard cheeses made with **unpasteurized** milk *(2)* testing of milk supply and finished product for pathogens with low infectious dose and long survival times, such as *E. coli* O157:H7 and *Salmonella*, is appropriate to verify that thermization and ripening processes are sufficient to eliminate these pathogens.
- Microbiological testing of finished product for pathogens is also recommended if an ingredient that has a potential to contain pathogens such as herbs, spices, or other inclusions, are added post-pasteurization; ingredients added after pasteurization should be obtained from approved suppliers and subjected to supplier verification activities. Ingredients from a new supplier with little history may require addition verification testing

Cheese (soft, semi-soft, surface ripened)

Examples: fresh (Queso fresco), soft, soft-ripened (Camembert), semi-soft (Edam, Gouda), veined cheeses (Roquefort, Gorgonzola)

This category represents a broad range of cheeses. Contaminated soft and semi-soft (ripened and unripened) cheeses made with pasteurized or raw milk have been associated with listeriosis; illnesses from *Salmonella* and shiga-toxin producing *E. coli* have been attributed to consumption of semi-soft cheeses made with raw milk.

Routine environmental monitoring for *Listeria* spp. in the environment and coliforms in finished product should occur for all products in this category to identify loss of process control. However, for higher pH (>pH 5.4) soft cheeses known to support the growth of *L. monocytogenes* and that have been implicated in illness, use of pasteurized milk, and aggressive environmental controls and monitoring, as well as periodic finished product testing for indicators and pathogens is appropriate *(30, 56, 61, 68)*. Testing for *S. aureus* and generic *E. coli* may be used when processing or insanitary conditions indicate a potential increased microbiological risk. For cheeses made with unpasteurized milk, testing milk will provide insights for likelihood of residual pathogens that may be present after aging.

Example 1 - Cheese, fresh (Queso fresco), typical aw >0.98

Example 2 - Cheese, semi-soft, Gouda made with unpasteurized milk; typical aw 0.96-0.98

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

Principles that apply to finished product testing of this RTE food:

Examples

Criterion/Factor	Cheese, fresh (Queso fresco) Typical a_w >0.98	Cheese, semi-soft, Gouda made with unpasteurized milk; typical a_w 0.96-0.98
A. Are pathogens associated with the food or ingredients?	Pasteurization of milk used for cheesemaking will eliminate vegetative bacterial pathogens, rendering it safe for use. However, recontamination of curd/finished cheese from the environment or by contaminated adjunct ingredients has been associated with outbreaks from *Salmonella,* STEC, and *Listeria monocytogenes.* Fresh (such as queso fresco) and soft, surface-ripened cheese with high pH (>5.2) are known to support growth of *L. monocytogenes*, whereas soft cheeses with pH <5.2 (lactic acid predominant) are typically	Raw milk may contain multiple pathogens, including *Salmonella, E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *Brucella*. If cheese milk is contaminated, microbial populations can increase/concentrate in the curd. Gouda made with unpasteurized milk has been associated with long survival of *E. coli* O157:H7 and with illness. 60 days aging may be insufficient to inactivate low infectious dose pathogens. Cheeses with slow starter activity (slow acidification) have been associated with growth of *S. aureus;* and

Criterion/Factor	Cheese, fresh (Queso fresco) Typical a_w >0.98	Cheese, semi-soft, Gouda made with unpasteurized milk; typical a_w 0.96-0.98
	inhibitory to pathogens when refrigerated.	staphylococcal enterotoxin that can remain active.
B. Are the ingredients likely to be contaminated?	Pasteurized milk is rarely contaminated, but curd can be recontaminated during the make procedure, brining, or packaging. Adjunct ingredients such as spices and herbs or the environment may be a source of *Salmonella* or *L. monocytogenes*.	Surveys suggest 1-2% of raw milk samples used for artisan cheeses contain one or more pathogen*(17)*. Bulk milk samples can have higher rates of contamination *(58)*.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Legal pasteurization is sufficient kill step for vegetative pathogens. If curd is recontaminated, pathogens are likely to survive or grow during storage, depending on product pH.	Unless validated for lethality, mild heat treatments such as thermization may reduce pathogens by as little as 1 or 2 logs (target suggested to be 3-log reduction). The aging/ripening process for semi-soft cheeses can reduce pathogen load, but 60-day aging may be insufficient to qualify as a

Criterion/Factor	Cheese, fresh (Queso fresco) Typical a_w >0.98	Cheese, semi-soft, Gouda made with unpasteurized milk; typical a_w 0.96-0.98
		robust reduction step. Rate of inactivation relies on combined stresses such as drying, acidity, and residual starter activity and storage temperatures >3°C to accelerate lethality during aging. Some low infectious dose pathogens, such as *E.* coli O157:H7, have been shown to survive months.
D. Is there a potential for recontamination from the handling or the environment?	Yes, there can be potential for recontamination during the curd stage or packaging.	Yes, there can be potential for recontamination during production, aging, portioning, or packaging.
E. Does the product support survival or growth?	Yes, *L. monocytogenes* grows in soft cheeses, particularly with pH >5.2 during normal refrigerated storage. Refrigeration will inhibit growth of mesophilic pathogens, but survival times may be months.	Gouda does not support pathogen growth due to low initial pH (<5.3) and levels of undissociated lactic acid *(74)*, but low infectious dose pathogens such as *E. coli* O157:H7 have been shown to survive for months during aging *(18)*.

Criterion/Factor	Cheese, fresh (Queso fresco) Typical a_w >0.98	Cheese, semi-soft, Gouda made with unpasteurized milk; typical a_w 0.96-0.98
F. Is this product meant for higher risk population?	This food is not specifically intended for high-risk populations, but people from high-risk populations may choose to consume this type of product."	This food is not specifically intended for high-risk populations, but people from high-risk populations may choose to consume this type of product."
G. What is the shelf life of the product?	Typically, 60-90 days	Gouda may be aged from 60 days to several years
Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	Storage at temperatures >3°C increases the risk of *L. monocytogenes* growth in fresh and certain other soft cheeses with pH>5.2, depending on predominant acid. Slight temperature abuse or extended storage times can increase the risk of growth and consumer illness. Other mesophilic pathogens will survive, but not grow, in these cheeses held at <7°C.	Storage temperature and time that exceed marked conditions are unlikely to affect safety if composition is inhibitory to pathogens; however, quality will be diminished. Validation studies for specific formulation may be necessary to determine risks if held at temperatures greater than 4°C

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

Testing for alkaline phosphatase can serve as an indicator of pasteurization of milk used for making cheese *(63)*. Testing in process or finished product for moisture, salt and/or water activity is important to verify formulation control. Pathogen growth is inhibited in milk during cheese making by rapid acidification. Therefore, monitoring formulations (such as volume of acid per volume of milk for direct acid set production) and monitoring pH during acidification of the curd will detect inadequate acidification/fermentation that may result in an unstable product.

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

No, because there are several points in the manufacturing process where post-pasteurization contamination could occur. Microbial testing is an appropriate verification activity.

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

A company should consider the potential of individual cheeses to support growth of pathogens, under refrigerated and non-refrigerated conditions, if it were contaminated. Validation of growth inhibition for specific cheese types, particularly for non-refrigerated conditions, is useful in determining potential risks and therefore, pathogens of concern.

Regardless of product composition, aging and storage, environmental monitoring for *Salmonella* and *Listeria species (68)* and routine testing of finished product for Enterobacteriaceae or coliforms (<100 CFU/g) can be used as indicators of post-process contamination. If acid production is slow, it is advisable to test for coagulase positive staphylococci or *S. aureus* ($<10^4$ CFU/g). Cheeses made with raw milk should consider additional pathogen testing of the final product depending on results for raw milk, environmental positive tests, and levels of indicator organisms. Soft cheeses made with pasteurized milk should also be considered for finished product pathogen testing because of the risk of cross-contamination post kill step, the ability of pathogens to grow in the product, and the history of safety issues with this product type.

Converting cheese into slice, shred, or grated forms may disrupt the inherent safety system. In addition, the end use of the cheese (such as slices used as an ingredient for prepared refrigerated sandwiches, pieces for deli salads, etc.) needs to consider the potential for delivering contaminants, such as *L. monocytogenes*, to foods that may support growth of pathogens or spoilage microbes. In this case, finished product testing for *L. monocytogenes* may be indicated.

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?

Because of the great diversity of cheese types produced in many regions; as well as production, consumption, and distribution practices, it is difficult to recommend specific universal applicable testing for all cheese types. For example, for cheeses that support growth of *L. monocytogenes*, finished product should routinely be tested for indicator organisms and for *L. monocytogenes*. For cheeses that are made with pasteurized milk and do not support pathogen growth, greater reliance on indicator organism testing and environmental monitoring should be sufficient to assure process control.

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

Environmental monitoring provides useful information to determine the potential for post-pasteurization contamination. Monitoring the pH of curd can detect slow fermentation and testing for *S. aureus* (<10^4 CFU/g) may be relevant if acidification does not proceed as anticipated. Testing for indicator organisms (e.g., molds, yeasts, Enterobacteriaceae, or *Listeria*-like microorganisms) in brine or curd for *E. coli* (<100 CFU/g) in cheese made from heat-treated milk is useful to verify process control and hygiene conditions. For raw milk cheese, pull milk samples from the cheese vat after a homogeneous mix or utilize a disposable filter sock on the milk discharge pipe to the vat, after fill. In addition, supplier control of milk can be achieved through herd management, mastitis control, focus on feeding regimens, and sanitation during milking, storage and transportation to the cheese makers *(2)*.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

Testing for *Listeria* spp. in processing environments is important to verify the effectiveness of implemented hygiene control measures; verification of rigorous environmental controls can be justification for reduced finished product testing. Frequency and extent of product testing verification should be based on history and established criteria. Refer to guidance documents for frequency and locations for environmental monitoring *(30, 31, 68)*.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

Refer to sources such as the PMO, federal and industry guidance documents, and Department of Defense microbiological limits for indicators of loss of process control and actions to be taken if results indicate a loss of process control *(30, 31, 50, 63, 68)*.

When there is a loss of systemic process control, there should be a root cause analysis performed, including heat-treatment of milk, supplier controls (such as for inclusions or raw milk), cheese vat/make procedures, acidification rate, finishing table, brine tanks, block formation, aging, cutting, and packaging to determine the source(s) of loss and implement corrective action. The findings will dictate increases in verification testing.

Table A-3. 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 raw milk *(2, 26)*.

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms or Enterobacteriaceae	$\leq$100 CFU/g	Investigate reason for exceeding limit and implement corrective action; consider testing for *E. coli* (10 CFU/g) if coliforms are detected	Routine testing
S. aureus	$\leq$100 CFU/g	Investigate reason for exceeding limit and implement corrective action. If $\geq 10^4$ CFU/g, reject lot	Investigative testing for *S. aureus* if pH monitoring of a vat suggests acid

		due to potential for enterotoxin production. Due to heat stability of enterotoxin, diverting to further processing is not recommended	development is slow. Investigate reason for slow pH drop, 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

476

477 **Recommendations for soft cheese.**

478 • Routine testing of product for indicator organisms (e.g., Enterobacteriaceae) and environmental
479 monitoring for *Listeria* spp. *(30, 68)* are recommended to identify loss of process control.

- Investigative finished product testing for pathogens (e.g., *L. monocytogenes* or *Salmonella*) should be conducted if testing suggests inadequate environment control and likely product contamination.
    - If EMP suggest that contamination of product by *L. monocytogenes* or *Salmonella* may have occurred, refer to industry and government guidance documents for testing product.
- Additionally, routine testing (e.g., daily, weekly, or quarterly depending on facility) of finished product for *L. monocytogenes* may be appropriate for products that can support growth of this pathogen during shelf-life.
- If monitoring of acidification rate suggests poor starter activity which can allow growth of infectious and toxigenic microorganisms enumeration *S. aureus*, investigative testing of pressed curd for *S. aureus* should be conducted.
    - Destroy lots where enumeration of *S.* aureus in product exceeds 10^4 CFU/g
- Microbiological testing of finished product for pathogens is also recommended if an ingredient that has a potential to contain pathogens such as herbs, spices, or other inclusions, are added post-pasteurization.
    - Ingredients added after pasteurization should be obtained from approved suppliers and subjected to supplier verification activities.
    - Ingredients from a new supplier with little history may require addition verification testing
- Finished cheeses made with raw milk should also consider routine testing of pathogens such as *L. monocytogenes*, *Salmonella,* and *E.* coli O157:H7 in raw milk supply and finished product *(2)*

Cultured, pH ≤ 4.8

Examples include buttermilk, sour cream, kefir, koumiss, and yogurts. These products are inherently safe due to pasteurization of the milk to eliminate vegetative pathogens, robust fermentation (lactic acid production) to prevent growth and enterotoxin production and use of qualified ingredients used as inclusions. These finished products do not support growth of pathogens, and with sufficient lactic acid present they can inactivate vegetative pathogens during storage.

Briefly, the products described in this section are produced by fermenting milk at mild temperatures (e.g., 110-115°F) to convert lactose to lactic acid by the metabolism of the specific beneficial bacterial cultures that are added for preservation and flavor. The production of the lactic acid reduces the pH of the milk to the isoelectric point of casein (pH 4.6), but curd will be formed at slightly higher pH of 4.8. The time to achieve a pH <4.8 can range between 4 and 12 hours (>12h for kefir), depending on fermentation temperature and cultures used. Robust fermentation has been shown to outcompete various pathogens, including *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, *Staphylococcus aureus*, and *Bacillus cereus,* particularly when pH is reduced to <5.0 with lactic acid *(12, 22, 48, 57)*. Products are filled into cups and chilled and stored refrigerated for the duration of shelf-life.

Monitoring fermentation rate is the primary indicator that the fermentation is inhibitory to pathogens. Furthermore, products with pH <4.8 (where lactic acid is predominant) have been shown to not support pathogen growth and will slowly inactivate pathogens during storage. If the rate of pH decrease is compromised, testing for pathogens should be considered. The product may be exposed to the environment during preparation and filling of containers and contaminants could be introduced by additions after pasteurization (such as fruit puree, caramel or chocolate sauce, nuts, etc.).

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

524

Criterion/Factor	Cultured Dairy, pH <4.8
A. Are pathogens associated with the food or ingredients?	Although raw milk and inclusions used in cultured dairy products can contain pathogens, cultured dairy products made with pasteurized milk and rapidly fermented to pH <4.8 (where lactic acid is predominant) are rarely associated with illness. Sporeformers such as *B. cereus* or *C. perfringens* will survive pasteurization; however, germination and outgrowth are controlled through fermentative acidification that produces a rapid pH drop below levels that permit growth. Acid tolerant pathogens such as *E.* coli O157:H7 will have the longest survival time but will not grow. Fermentation and resulting acid production are control measures, but producer should be alert to delay of acid development if starter cultures are compromised, such as due to presence of inhibitory substances such as antibiotics or phages.
B. Are the ingredients likely to be contaminated?	Pasteurized milk is unlikely to be contaminated. Recontamination can occur through the addition of ingredients such as fruit concentrates or pulps, pastes, or syrups, chocolate, nuts, or other inclusions. Ingredients that have been heat treated and acidified have a low probability of pathogens, but may include yeasts and molds,

Criterion/Factor	Cultured Dairy, pH <4.8
	which may change the product pH over time. Supplier verification for these ingredients is advised. Additionally, starter cultures should meet specifications, including lack of phage contamination.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes. Milk pasteurization is a validated reduction method. The acid environment (<4.8, lactic acid) will continue to reduce pathogens over time, but reduction may be slow (e.g., days or weeks at 4°C).
D. Is there a potential for recontamination from the handling or the environment?	Yes. Given the nature of the processing environment, which are frequently wet cleaned, potential for recontamination would most likely be from spoilage microorganisms.
E. Does the product support survival or growth?	No. The acidity of the product (<4.8, lactic acid) does not support growth of bacterial pathogens and will limit their survival. The acid environment will continue to reduce pathogens over time, but reduction will be slow. However, acid tolerant spoilage bacteria, yeasts or molds may grow.
F. Is this product meant for higher risk population?	High-risk populations may consume these foods, but food is not specifically intended for this demographic.

Criterion/Factor	Cultured Dairy, pH <4.8
G. What is the shelf life of the product?	4-6 weeks, depending on the product
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	Extended refrigeration will not affect safety due to the low pH of the product. Holding cultured dairy products at temperatures greater than 4°C will accelerate acid development, which will affect quality but will not compromise safety.

525

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

528 Yes. pH testing during fermentation is essential and should be done continuously or at regular
529 intervals to verify fermentation is sufficiently robust as to prevent growth of pathogens in the milk.

530

531 **Question 3. Are there situations where [microbial] verification testing would not be necessary if there is**
532 **evidence that the appropriate treatment was, in fact, applied?**

533 If records show that the acidification rate to <4.8 was rapid (e.g., within 5 hours or per validation
534 study for given product) and EMP verifies sanitation, no pathogen testing for the white mass is needed.
535 Microbial testing for indicator organisms for sanitation and spoilage organisms is recommended
536 particularly if inclusions/adjunct ingredients are used.

537

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

Because product is made with pasteurized milk and robust fermentation, pathogen testing of product is not needed unless acid development is slow or environmental monitoring program indicate loss of process control or sanitation failure. Enumeration testing for indicators of sanitation for post-pasteurization environment (e.g., Enterobacteriaceae or coliforms) or for spoilage microorganisms such as molds, yeasts or gas-producing lactic acid bacteria, e.g., *Leuconostoc spp*., is useful as a check for supplier control of adjunct ingredients/inclusions.

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?

Ability to meet specifications for acid development, environmental exposure during processing, environmental monitoring program results, and supplier control for adjunct ingredients/inclusions that are added after fermentation, should be used to determine type and frequency of finished product testing. In general, routine finished product testing for Enterobacteriaceae, or coliforms or spoilage/mold/yeasts should be used to verify overall process control and sanitation. End product testing for pathogens is not routinely conducted because monitoring of the fermentation in-process provides the most actionable information.

Ingredients and inclusions (such as fruit puree, caramel or chocolate sauce, nuts, etc.) added after pasteurization should be obtained from approved suppliers and subjected to supplier verification activities. Ingredients from a new supplier with little history may require addition verification testing.

Several situations will indicate that additional product testing is necessary. For example, investigative testing for pathogens should be conducted if slow acid development suggests the potential for pathogen growth in the product if it were contaminated by *L. monocytogenes* or *S.* aureus from the environment or employees during the production process. Investigative testing is needed when populations of indicator organisms exceed specified limits suggesting insufficient sanitation or inadequate supplier control for incoming ingredients used for inclusions. If environmental monitoring for *Listeria* spp. suggests that contamination by L. monocytogenes may have occurred during the production process (test for absence of *L. monocytogenes*; see product testing recommendations in U.S. Food and Drug Administration, 2017 *(69)*).

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

Because acid development is the primary preventive control for cultured products made with pasteurized milk, non-microbial testing should include monitoring the pH of the white mass during fermentation routinely to detect slow fermentation. Environmental monitoring provides useful information to determine the potential for post-pasteurization contamination. Because niches and sanitation requirements may be different for gram negative microbes (e.g., Enterobacteriaceae) compared to gram positive bacteria and fungi, samples for testing of gas-forming lactic acid bacteria, yeasts, and molds are best taken from the adjunct ingredients or from critical pieces of equipment such as intermediate storage tanks, balance tanks, fillers, etc. to verify no buildup of these microorganisms before starting each day's production.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

A robust environmental testing program for indicator and spoilage microorganisms will reduce the need for finished product testing. When determining disposition of cultured product where acid production was delayed, results that demonstrate *Listeria* is under control will reduce the need to test for the pathogen in finished product; follow recommendations for testing outlined for cultured dairy products with pH >4.8 but ≤5.2 and industry and government guidance *(30, 31, 68)*. The EMP should also periodically test for *Salmonella,* which is associated with low moisture ingredients such as dairy powders, nuts, or other adjunct ingredients and could become established in the environment. Verifying that this pathogen is controlled in the environment reduces the risk that it will be a post-process contaminant in the finished product.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

When acid production fails, revealing loss of systemic process control, the company should initially investigate the milk product for inhibitory substances to fermentation. If investigative pathogen testing reveals the potential of heat-stable enterotoxin production produced by *S. aureus* (such as $>10^4$ CFU/g of *S. aureus*), lot should be discarded. If populations of *S. aureus* are below the threshold for enterotoxin production, further review of EM results for *Listeria spp.* and potentially finished product testing for *L. monocytogenes* should be considered (see recommendations outlined for cultured dairy products with pH

606 >4.8 but ≤5.2 for testing). When cause of loss of process control has been corrected, routine verification
607 testing can be resumed.
608
609 **Table A-4**. Microbial targets, limits, and recommended actions if limits are exceeded, for cultured dairy
610 products made with pasteurized milk (e.g., Sour cream, yogurt, buttermilk) and active pH control.

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms or Enterobacteriaceae	≤10 CFU/g	Investigate reason for exceeding limit and implement corrective action; consider testing for *E. coli* if coliforms are detected at >10 CFU/g.	Routine testing, particularly for products with ingredient additions after fermentation is complete
Mold/Yeast	≤10 CFU/g	Investigate, implement corrective action	Testing routinely is dependent on the product if no mold inhibitor is added. The presence of mold and yeast may be influenced by added ingredients such as fruit purees and other inclusions. Fungal growth on the surface of a product can increase the

			pH and disrupt the formulation safety based on pH.
S. aureus	≤100 CFU/g	Investigate reason for exceeding limit and implement corrective action. If ≥10^4 CFU/g, reject lot due to potential for enterotoxin production. Due to heat stability of enterotoxin, diverting to further processing is not recommended	Investigative testing for *S. aureus* if fermentation does not reach pH <4.8 in <5 h (or other rate determined by challenge study data). Investigate reason for slow pH drop, implement corrective action

Recommendations for testing cultured dairy products with pH ≤ 4.8:

- Routine product testing is for rate of acid development.
- Environmental monitoring and finished product for indicator organisms is a verification of process control, supplier control, and sanitation.
- Pathogen testing of product is not recommended unless acid development is slow, which indicates loss of process control
- Ingredients and inclusions added after pasteurization should be obtained from approved suppliers and subjected to supplier verification activities.
    - Ingredients from a new supplier with little history may require addition verification testing

Cultured, pH > 4.8 and <5.4

Examples include acidophilus milk, hot-filled or cold-filled cream cheese or cottage cheese. Cultured dairy products are inherently safe due to pasteurization of the milk to eliminate vegetative pathogens; robust fermentation with lactic acid producing bacteria prevents pathogen growth during the culturing process through acid development and competition with the culture. However, additions of cream or other adjunct ingredients may raise the pH of the finished product that may affect growth potential of pathogens and spoilage microbes.

Briefly, products are manufactured using fluid milk or cream heated to 90^{o}C for several minutes to remove competing bacteria. Pasteurized milk or cream is cultured with a lactic acid bacteria starter culture, frequently to pH 4.8. The addition of the adjunct ingredients (such as cream dressing, vegetable material, salmon, or other inclusions) can raise the equilibrated pH to values between 4.8 and 5.4. While hot filling for certain cream cheese and cottage cheese products eliminates concerns for vegetative pathogens and spoilage microbes, sporeforming bacteria can survive. Although pH 4.6 is considered the limit to prevent growth of sporeformers at room temperature, cultured dairy products with pH <5.4 using lactic acid will slow growth (extend lag and generation times) and therefore product can tolerate limited extended cooling (challenge study data is required to validate that cooling profile is sufficient to inhibit spore outgrowth for product of concern). However, other pathogens, such as *L. monocytogenes* can potentially grow in these products if present, even during refrigerated storage, unless formulation adjustments have been made.

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

Criterion/Factor	Cultured, equilibrated pH > 4.8 and <5.4
A. Are pathogens associated with the food or ingredients?	Although raw milk can contain pathogens, products are made with pasteurized milk or cream are rarely associated with illness. Sporeformers such as *B. cereus* or *C. perfringens* may survive pasteurization; however, germination and outgrowth are controlled through fermentation or direct acidification that produces a rapid pH drop below levels that permit growth. On rare occasion, the presence of inhibitory substances such as antibiotics or phages can delay acid production, allowing growth of pathogens if present.
B. Are the ingredients likely to be contaminated?	Pasteurized milk and cream are unlikely to be contaminated with vegetative pathogens, but spores can survive heat. Recontamination from the environment can occur, particularly during addition of adjunct ingredients to the curd. Ingredients that may be cold blended into the curd such canned smoked salmon, pasteurized fruit concentrates or pulps, heat treated pastes or syrups, nuts, chocolate, brined peppers, dehydrated carrots, dried chives, or natural and artificial flavors are infrequently contaminated, but supplier verification is advised. Additionally, starter cultures should meet specifications, including lack of phage contamination.
C. Are there robust processing control procedures such as a kill	Yes. Milk and cream are heated to pasteurization or ultra-pasteurization temperatures to eliminate vegetative pathogens and other microbes that may interfere with the culturing process. Surviving spore outgrowth is slowed by competition with starter

Criterion/Factor	Cultured, equilibrated pH > 4.8 and <5.4
step or other reduction methods/controls?	culture and by rapid lactic acid development to reduce pH to <5.2. Hot-filling products, such as for some types of cream cheese or cottage cheese products, will eliminate vegetative pathogens but will allow survival of spores.
D. Is there a potential for recontamination from the handling or the environment?	Yes. Given the nature of the processing environments, which are frequently wet cleaned, potential for recontamination would be from spoilage microorganisms or *L. monocytogenes*. Addition of ingredients post fermentation process or cold filling into packaging can introduce contaminants.
E. Does the product support survival or growth?	Growth of pathogens is inhibited by a combination of pH ≤5.0 with lactic acid, temperature-control, and/or addition of synthetic or clean label antimicrobials. Products with pH >5.0 and no antimicrobials have potential to support growth of *L. monocytogenes* even with refrigerated temperature control.
F. Is this product meant for higher risk population?	High-risk populations may consume these foods, but food is not specifically intended for this demographic.
G. What is the shelf life of the product?	60-90 days
H. Will consumer handling and use increase or decrease likelihood of pathogen survival,	For intact packages of hot-filled products, such as brick cream cheese, extended refrigerated holding beyond use-by date is unlikely to increase risk. However, cold-filled product without preservatives, such as certain cottage cheese products with pH >5.0, can support

Criterion/Factor	Cultured, equilibrated pH > 4.8 and <5.4
growth, or toxin production and risk of consumer illness?	growth of *L. monocytogenes;* extended refrigerated storage beyond use-by date or holding at temperatures greater than 4°C can increase the risk that food may support growth of *L. monocytogenes* and increase the risk of illness.

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

Yes. pH testing during culturing of the milk at regular intervals is essential to ensure robust fermentation and adequate pathogen control.

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

Yes. Microbial verification testing is not necessary for hot-filled products (with a validated thermal process) that have had a robust fermentation and have met cooling requirements.

However, for products that are cold filled and therefore are exposed to the environment or via addition of ingredients, testing for indicator organisms should be conducted in addition to the environmental monitoring and supplier control programs.

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

For products that are not hot filled, enumeration of Enterobacteriaceae or coliforms (e.g., less than 10 CFU CFU/g) can be used as an indicator of post-pasteurization contamination. For products that also have inclusions/adjunct ingredients, consider the recommendations for appropriate target microbes and microbiological limits in the appendices of this document for those ingredients. For example, while the limit for coliforms is <10 CFU CFU/g in dried chives or spice blends, acceptable populations of Enterobacteriaceae is 10^2 CFU CFU/g; therefore, when added to cold-blended cream cheese, the final populations of Enterobacteriaceae may exceed 10 CFU/g and use of coliforms may be a better indicator organism.

The presence of molds and yeasts may be influenced by added ingredients such as fruit purees and dehydrated vegetables or herbs. For products that do not contain antimycotic agents, testing for molds (<10 CFU/g) may be necessary because mold growth on the product surface can increase the pH of the product, disrupting the safety system established by the reduced pH.

Environmental testing of *Listeria* species, *Salmonella* is recommended for products that are not hot filled; additional testing for mold/yeasts (air) are useful for facilities with products that do not contain mold growth inhibitors or packaging that excludes mold growth.

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?

Ability to meet specifications for acid development, environmental exposure during processing, temperature at which product is filled, environmental monitoring program results, and supplier control for adjunct ingredients/inclusions that are added after fermentation, should be used to determine type and frequency of finished product testing.

Products that have a validated lethality step and hot fill do not require microbial testing for finished product. For products that are filled at temperatures are lower than that needed for lethality, but in facilities with a robust environmental control and supplier control programs, pathogen testing is not routinely conducted but routine finished product testing for indicators of sanitation (e.g., Enterobacteriaceae or coliforms) or spoilage/mold/yeasts should be used to verify overall process control and sanitation.

Several situations will indicate that additional product testing is necessary. For example, investigative testing for pathogens (e.g., *S. aureus* and/or *L. monocytogenes*) should be conducted if slow acid development suggests the potential for pathogen growth in the product. Investigative testing is needed when environmental monitoring for *Listeria spp.* or populations of indicator organisms in finished product exceed specified limits suggesting insufficient sanitation or inadequate supplier control for incoming ingredients used for inclusions.

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

In addition to microbial testing at the end of process, pH testing of the milk/cream during fermentation to monitor acid production should be done routinely to ensure adequate acid production to control microbial hazards. Samples for testing of spoilage microbes as indicators of sanitation (e.g., heterofermentative gas-forming lactic acid bacteria, yeasts, and molds) can be taken from critical pieces of equipment such as intermediate storage tanks, balance tanks, fillers, etc., particularly during extended runs.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

A robust environmental monitoring program that demonstrates that *Listeria* (and molds, as relevant) are in control will reduce the need for finished product testing because they are microorganisms of concern in cultured dairy products with pH >4.8. The EMP should also periodically test for *Salmonella,* which is associated with dairy powders, dehydrated vegetables, herbs, and spices, or other low moisture ingredients, and which could contaminate and become established in the environment. Verifying that this pathogen is controlled in the environment reduces the risk that it will be a post-process contaminant in the finished product

If results from environmental monitoring program suggests potential for contamination of the finished product, it could result in the increased need for microbiological testing of product or ingredients for *L. monocytogenes* as part of investigative testing or root cause analysis *(30, 31, 68)*.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

Because cold-filled cultured dairy products are expected to contain high populations of starter culture, testing the environment and monitoring other process controls (e.g., acid production and cooling rates), are more actionable tests of loss of process control. When acid production is slow or stalls or cooling deviations occur revealing loss of systemic process control, the company should initially investigate causes. Product should be placed on hold and evaluated to ensure there was no potential for growth of toxigenic

729 pathogens to levels that could cause illness. Follow FDA Draft Guidance on environmental monitoring to
730 verify control of *L. monocytogenes (68)*
731
732 **Table A-5**. Microbial targets, limits, and recommended actions if limits are exceeded, for cultured dairy
733 products with pasteurized milk, pH >4.8 and < 5.4, moisture >50%; active pH control required (Ex. Cottage
734 cheese, cream cheese)

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms or Enterobacteriaceae (EB)	≤10 CFU/g	Investigate reason for exceeding limit and implement corrective action. If >10 CFU/g and regulated under PMO, reject lot due to regulatory limit	Routine testing. However, products that have added dried herbs and vegetables may have populations of Enterobacteriaceae that are higher than 10 CFU/g. See Appendix F Spices/Herbs for guidance for testing.
S. aureus	≤100 CFU/g	Investigate reason for exceeding limit and implement corrective action. Investigate, implement corrective action.	Investigative testing for *S. aureus* if fermentation is slow where the pH of the curd does not reach pH <4.8 in <8 h (or other rate depending on challenge

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
		if $\geq 10^4$ CFU/g, reject lot due to potential for enterotoxin production	study data). Investigate reason for slow pH drop, implement corrective action.
Molds	$\leq$10 CFU/g	Investigate, implement corrective action	Testing is dependent on the product. Mold can raise the pH of the product, disrupting the safety system of the product. Considerations are the type of inclusion/ingredient, whether product is hot filled, has effective antimycotic agents added, or packaging excludes oxygen to inhibit molds during storage.

735

Recommendations for testing cultured dairy products with pH > 4.8 and <5.2:

- Routine product testing for acid development
- Routine testing for indicator organisms (e.g., coliforms or Enterobacteriaceae, and molds as appropriate)
- Environmental monitoring programs (e.g., for *Listeria* spp., Salmonella and molds as appropriate) as verification of sanitation
- Pathogen testing of product is not recommended unless environmental monitoring program suggests risk of post-pasteurization contamination or acid development is slow, which suggests loss of process control
- Ingredients and inclusions added after pasteurization should be obtained from approved suppliers and subjected to supplier verification activities.
    - Ingredients from a new supplier with little history may require addition verification testing

Dried Dairy Products

Examples include dry milk powder (e.g., non-fat dried milk), lactose, whey products, buttermilk powder, dried cheese powder, and infant formula (21 CFR part 106). Pasteurization of the fluid milk will kill vegetative pathogens, but spores will survive, including throughout the drying process. Whey derived from cheese making may be stored in silos prior to processing (fractionation, concentration, concentration, drying), with temperature/time limits to prevent growth of bacteria such as *S. aureus* and *B. cereus* that can produce heat stable enterotoxin; these temperature/time limits should be included in the food safety plan. Dried dairy products are common ingredients in products that will not receive a kill step prior to consumption such as chocolate, cold pressed energy bars and energy drink powders that are rehydrated with cold water. *Salmonella* and *L. monocytogenes* are potential hazards for these products, with additional concerns for ingredients used in infant formula including *Cronobacter spp.*

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

Criterion/Factor	Dried Dairy Products
A. Are pathogens associated with the food or ingredients?	Epidemiological data suggest that *Salmonella* is a significant hazard that needs to be controlled during the manufacture of dried dairy products intended for consumption by all populations. Outbreaks of *Salmonella* have been reported in dried milk and infant formula *(11, 14, 54, 73)*. *S. aureus* and *B. cereus* can be present in dairy powders and can present an issue if the product is reconstituted and abused. *(36)*. *Cronobacter* and *Clostridium botulinum* are hazards in products

Criterion/Factor	Dried Dairy Products
	for infants, although testing specifically for *C. botulinum* is not useful as a process control *(5, 34, 65)*. *Listeria monocytogenes* is a hazard for dried milk products that have RTE uses.
B. Are the ingredients likely to be contaminated?	Ingredients such as caseinates, whey powder, and other milk derivatives, vitamins, trace elements and minerals or lecithin may be added during processing. Certain ingredients, such as milk derivatives, have a known history of presence of *Salmonella*. While ingredients added before the heat treatment, (pasteurization) do not represent an issue, those added after the kill step represent a risk and therefore need to fulfill the same microbiological requirements as the finished product. Products are dried after milk pasteurization and thus may be contaminated from the environment.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes. Pasteurization of the fluid milk would precede spray drying. However, the drying process itself (evaporation and spray/roller drying) is not considered a pathogen kill step, as *Salmonella, L. monocytogenes,* and C*ronobacter* can survive drying. Spores will survive both pasteurization and spray drying *(59)*
D. Is there a potential for recontamination from the handling or the environment?	Yes, *Salmonella* contamination from the environment is a concern, as is *L. monocytogenes* for RTE dried milk products. *Cronobacter* contamination from the environment is a concern in products intended for infants. Increased levels of Enterobacteriaceae in finished products can be used as an indicator of recontamination

Criterion/Factor	Dried Dairy Products
	from the processing environment even though when *Cronobacter* populations are low, Enterobacteriaceae may be undetectable *(15, 20)*. Other hazards such *as S. aureus or B. cereus* (or the presence of preformed staphylococcal enterotoxins) are normally only present at very low levels and do not pose a risk as long as the products are not mishandled prior to pasteurization or after reconstitution and before consumption. Mishandling (holding time and temperature) would allow growth and toxin formation.
E. Does the product support survival or growth?	Dry product (a_w 0.3-0.4) does not support microbial growth, but *Salmonella* and *Cronobacter* survive for extremely long periods (months).
F. Is this product meant for higher risk population?	Yes, if used in infant formula.
G. What is the shelf life of the product?	Extended shelf life (months to years) at room temperature.
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin	None of these is likely; however, the product can be rehydrated when used by the consumer. Pathogens such as *Cronobacter* and *Salmonella* can grow in the rehydrated product if they are present and temperature abuse occurs.

Criterion/Factor	Dried Dairy Products
production and risk of consumer illness?	

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

No. Testing for indicator organisms and/or pathogens is the appropriate verification activity.

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

Yes, for example, when the dried dairy product is used exclusively as an ingredient in foods that receive a validated lethality treatment.

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

Enterobacteriaceae (enumeration) and *Salmonella* (absence) are the specific indicator and pathogen, respectively, used for testing in dried dairy products *(35)*. In addition, a consideration for testing finished product is whether it is intended for high-risk populations, such as infants, the elderly, or immunocompromised individuals. In that instance, *Cronobacter* spp. is added to finished product testing (*Cronobacter* is mainly a concern in infants less than 12 months old)*(13, 35)*. For ingredients used in infant formula, enumeration of mesophilic spores or sulfite-reducing *Clostridia* are used frequently in the industry

as an indicator of process hygiene because spores can survive pasteurization and can be concentrated in dry ingredients *(34)*.

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?

Given the history of contamination associated with dry dairy products and the difficulty in eliminating pathogens from the processing environment using dry cleaning methods, testing frequency for this commodity typically is greater than other dairy products described in this appendix. For products that have received a lethality step (e.g., initial milk pasteurization), increased levels of Enterobacteriaceae in finished products indicates recontamination from the processing environment. Because *Salmonella* falls within the family of Enterobacteriaceae, testing for this group of organisms can be performed as a hygiene indicator in the environment *(23)*. However, while there is a correlation between finding Enterobacteriaceae and other gram-negative pathogens, Enterobacteriaceae is not always detected when *Salmonella* or *Cronobacter* are present in low numbers *(6, 20, 33)*.

Listeria monocytogenes is a hazard for dried milk products which are included in RTE products; therefore, environmental monitoring should be conducted to identify food surface, packaging, or other potential environmental contamination especially when a listericidal control is not consistently applied for final product testing *(68)*.

For manufacturers that test both in-process and environmental samples, a low frequency of end - product testing for *Salmonella* is performed as a verification for a low number of samples for end-product testing. Depending on the product use and customer requirements, *Salmonella* testing in finished product may be conducted on each lot pre-shipment. The frequency of finished product testing will be impacted by the results of the environmental monitoring program, as well as the hygienic design of the line, the

ability to exclude water in sensitive processing areas, length of time for dryer or evaporator run, and the addition of ingredients after pasteurization. Note that customers who use dried milk products as ingredients in RTE foods may require testing of each lot for pathogens such as *Salmonella*.

There are microbial specifications for Grade A dry milk powder, whey and buttermilk produced under the PMO, as well as dairy powders eligible for USDA grading or inspection services. Manufacturers of infant formula are required to perform testing for *Salmonella* and *Cronobacter* spp. on each production aggregate per 21 CFR 106.55. Although correlation between infant botulism and dried infant formula is not well established, testing for sulfite-reducing *Clostridium* spores is frequently used as an indicator of process hygiene for these ingredients *(5, 34, 39)*.

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

In-process sampling and testing can confirm effective control measures. Sampling plans should include representative samples taken after the drying step through the filling operation; automatic samples are often used in dry milk filling operations. Product contact surfaces where residues accumulate could indicate areas of moisture condensation, and thus potential for microbial growth. Sampling points include sifter tailings from the after dryer/after cooler or from tipping stations of intermediate products and filling machines.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

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. One recommendation is testing Enterobacteriaceae in dry processing areas, such that target levels is <100 CFU/swab and the action level is >1000 CFU/swab, depending on the proximity to product and product risk level *(31)*. It 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.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

The limits for non-pathogenic indicator organisms listed in this document are intended to provide guidance for acceptable limits where little information is available for a process or product. However, it is expected that each facility will collect and analyze quantitative data to establish statistical process control. A loss of systemic process control is indicated when indicator data repeatedly exceed the limits established for a stable process operating within predictable process variation, or exceptionally high indicator levels are observed pathogens. Loss of control also includes detecting *Salmonella, L. monocytogenes* and *Cronobacter* (for infant formula) in finished or in-process product.

When a loss of process control is indicated, the implementation and efficacy of preventive controls should be investigated before and after the drying process. These include sanitation, temperature and/or

hygiene segregation controls prior to spray drying fluid pasteurized product and sanitation, water and hygiene segregation controls after the spray drying process.

When a pathogen is detected, the recommended action is to reject the lot of dried product represented by the sample tested and any contiguous runs not separated by a clean sanitation break. Due to lengthy process runs, special consideration needs to be given to lot definition and the establishment of a clean break if the product is contaminated with a pathogen such as *Salmonella*. The repeated finding of indicator organisms such as coliforms or Enterobacteriaceae above a threshold level can also indicate a loss of sanitation control although actions taken would follow a tiered approach based on numbers and frequency of occurrence.

Finished product testing for *Salmonella* using FDA category II sampling is typically used on a per lot basis to screen for contamination *(66)*. FDA Category II includes foods that would not normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption. The parameters of Category II are: 30 analytical units/ 25 g samples. The samples may be aggregated into 375 g analytical units. If the ingredient is intended for a high-risk consumer (e.g., infants), testing of 60 X 25 g samples should be considered (see Category I food classification on Sample Schedule Chart 1 of Investigations Operations Manual *(66)*. Product for *L. monocytogenes* testing is sampled in 25 g aliquots. Multiple 25 g samples are taken over a production lot and may be composited into a 125 g sample if this sample size is validated for the matrix. Dried milk products are sampled for Cronobacter in 10g increments over the course of a production run and may be composited into a 300 g sample. If any product tests positive for *Salmonella* or other pathogens, it should not be released for consumption, regardless of results from follow-up testing.

873 Many manufacturers use autosamplers to incrementally take small samples of dry material
874 throughout a production run prior to packaging. These aggregated samples are tested in composites
875 sample sizes (i.e., 375, 125 or 300 grams) appropriate for the pathogen analyzed.

876 Control of toxigenic microorganisms such as *S. aureus* or *B. cereus* is limited to investigative testing
877 if hold temperature and time exceed limits for liquid in-process milk products prior to drying identified in
878 a food safety plan.

879

880 **Table A-6**. Microbial targets, limits, and recommended actions if limits are exceeded, for Dairy-Dried
881 Products (Ex. NFDM, whey powder, including dried dairy ingredients used in infant formula).

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Aerobic plate count (APC, SPC)	$\leq 10^4$ CFU/g	Investigate, implement corrective action	Routine testing. Acceptable aerobic plate count populations can be set by critical evaluation of trends for process control for individual line and facility
Coliforms or Enterobacteriaceae	$\leq$10 CFU/g	Investigate, implement corrective action	Routine testing.
Salmonella	Negative in 375 g	Divert for reprocessing, if	Routine testing. As an alternative sampling option

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
		appropriate, or destroy. Investigate and implement corrective action	to collecting and compositing 15-25 g samples (total 375 g), an auto sampler can be used to collect small amounts of samples throughout a production run for a total of 375g; Recommend 1500 g per lot when high volumes of product are produced per lot (or production day).
L. monocytogenes	Negative in 25 g	Divert for reprocessing, if appropriate, or destroy. Investigate and implement corrective action	Investigative testing as response to EMP that suggests likely contamination of product.
S. aureus	≤100 CFU/g	Investigate, implement corrective action.	Investigative testing, such as if hold temperature/time of components before drying

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
		if $\geq 10^2$ CFU/g, reject lot due to potential for enterotoxin production	exceed limits identified in food safety plan
B. cereus	$\leq$100 CFU/g	Investigate, implement corrective action. if $\geq 10^4$ CFU/g, reject lot due to potential for enterotoxin production	Investigative testing, such as if hold temperature/time of components before drying exceed limits identified in food safety plan
Testing is more stringent for ingredients used in infant formula (21 CFR 106.55) *(34)*; Testing in addition to those described above			
Cronobacter	Negative in 300 g; Composite sample 30 samples x 10 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Routine testing.

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
Mesophilic spores or Sulfite-reducing *Clostridium spores*	<100 spores/g	Divert for reprocessing or alternate use if appropriate or reject. Investigate and implement corrective action	Routine testing.

Recommendations for testing dried dairy products: These products are derived from milk that had been previously pasteurized. However, due to the many processing steps and transfer within and between facilities, post-process contamination can occur. Growth can occur during holding of milk or whey prior to condensation (concentration) or drying unless sufficiently temperature-time controlled.

- Routine testing for standard plate counts and coliforms or Enterobacteriaceae is recommended as indicators of process control.
- Due to the history of contamination, high frequency routine testing of *Salmonella* in RTE dried dairy products is recommended.
- Testing of toxigenic microorganisms such as *S. aureus* or *B. cereus* is limited to investigative testing if hold temperature/time for fluid product before drying exceed limits identified in food safety plan.

- For ingredients that are used in infant formula or for other high-risk individuals, testing is more stringent and additionally includes *Cronobacter* and mesophilic spores or sulfite reducing *Clostridium* spores that may be indicators of loss of process control

Frozen Dairy

Examples include ice cream, frozen yogurt, gelato, frozen custard.

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

Criterion/Factor	Frozen Dairy
A. Are pathogens associated with the food or ingredients?	Yes. There have been outbreaks of *Salmonella* and *L. monocytogenes* associated with ice cream due to environmental contamination or untreated ingredients *(9, 27)*.
B. Are the ingredients likely to be contaminated?	Yes. If using ingredients like raw eggs or untreated flour in raw cookie dough, or untreated fruits or nuts
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes. Pasteurization of the ice cream mix, and treatment for some of the ingredients added after pasteurization, such as nuts.

Criterion/Factor	Frozen Dairy
D. Is there a potential for recontamination from the handling or the environment?	Yes. Pathogen presence in finished products is typically due to post-pasteurization contamination from the processing environment or from the addition of contaminated ingredients.
E. Does the product support survival or growth?	The frozen temperature storage does not allow for growth but does allow for survival of pathogens such as *Salmonella* and *L. monocytogenes;* pathogens can grow if the product is thawed and held at growth temperatures for extended periods.
F. Is this product meant for higher risk population?	No, but is consumed by the elderly and immunocompromised
G. What is the shelf life of the product?	Months to years
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	In general, frozen products are intended to be consumed in the frozen state. However, ice cream can be used in milk shakes, which if left at temperatures that support growth can allow for the growth of *L. monocytogenes*.

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

No for most products; for frozen yogurt, monitoring acid development during the culturing process is important to inhibit growth of pathogens during fermentation.

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

No. This type of product has exposure to the environment, and ingredients are often added after pasteurization.

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

Consideration should be given to the types of inclusions/additions that are added after pasteurization, and the microbial hazards associated with these. Enumeration of coliforms as an indicator organism would be appropriate ($\leq$10 or $\leq$20 CFU CFU/g for plain or frozen desserts with inclusions, respectively). Enumeration of total microbial loads (Aerobic plate count/SPC) may be variable depending on the inclusions and could range up to 4-log CFU/g and still be of acceptable quality SPC testing for frozen yogurt is impractical because the product is made with the additional of starter cultures. Due to the risk of environmental contamination, testing for *Listeria* spp. in the environment can be used as an indicator for *L. monocytogenes.* Products produced under the official USDA Quality Approved Inspection Shield must meet the requirements in 7 CFR 58.646 (no more than 50,000 CFU/g SPC, no more than 10 coliform CFU/g coliform for plain and no more than 20 coliform CFU/gram in chocolate, fruit, nut or other flavors).

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?

Testing frequency for frozen dairy products is dependent upon the level of control during manufacturing. Factors to consider include whether ingredients are added post-pasteurization, the design of the equipment, the condition of the facility, how much manual handling occurs, and the results of the environmental monitoring program. Enterobacteriaceae testing is an effective and simple tool to determine hygiene status of parts of the facility that are primarily dry, whereas *Listeria* spp. may be a better indicator in areas that are wet cleaned, but both microbes can be useful wherever product accumulates.

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

Samples taken at critical steps along the processing line play an important role in determining the effectiveness of preventive measures to control recontamination after the heat treatment. Samples are typically taken from the mixing and maturation tanks (tanks used to cool the pasteurized ice cream mix to 4°C with mixing), at the filler or after hardening tunnels. Particular attention needs to be paid to build-up of residues or condensation spots where microbial growth may occur.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

A robust environmental monitoring program that demonstrates that *Listeria* is in control will reduce the need for finished product testing because they are microorganisms of concern in frozen dessert. The EMP should also periodically test for *Salmonella;* this pathogen is associated with dairy powders or other dry ingredients that are used in the ice cream mix prior to pasteurization and could be introduced

Salmonella into the plant environment. Verifying that this pathogen is controlled in the environment reduces the risk that it will be a post-process contaminant in the finished product. If EMP suggest that contamination of product by *L. monocytogenes* may have occurred, increased finished product testing is recommended; refer to government guidance documents for testing product *(68).*

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

Because cold-filled cultured dairy products are expected to contain high populations of starter culture, testing the environment and monitoring other process controls (e.g., acid production and cooling rates), are more actionable tests of loss of process control. When acid production is slow or stalls or cooling deviations occur revealing loss of systemic process control, the company should initially investigate causes. Product should be placed on hold and evaluated to ensure there was no potential for growth of toxigenic pathogens to levels that could cause illness. Follow FDA Draft Guidance on environmental monitoring to verify control of *L. monocytogenes (68)*

In the event of failure of environmental monitoring, corrective actions should be undertaken, and the verification testing should be increased and expanded to all areas of manufacturing to determine the source or sources of contamination. Once identified and resolved, verification testing can be reduced.

973 **Table A-7**. Microbial targets, limits, and recommended actions if limits are exceeded, for Dairy-Frozen
974 Desserts

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms	≤10 CFU/g for plain ≤20 CFU/g for chocolate, fruit, nut or other flavors § 58.648	Investigate, implement corrective action.	Routine testing. Populations may be influenced by ingredients therefore other coliform levels may be appropriate.
Aerobic plate count (APC, SPC)	≤50,000 for plain ice cream § 58.648	Investigate, implement corrective action.	Routine. Populations are influenced by ingredients; product specific aerobic plate count limits need to be established based on baseline testing. It is impractical to use SPC for Frozen yogurt made with starter culture

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
Listeria monocytogenes	Negative in 25 g	Reject lot; Investigate, implement corrective action.	Periodic testing or investigative testing in response to elevated counts of indicator organisms or in response to environmental monitoring findings suggesting post-process contamination

Recommendations for testing frozen dairy desserts: This product category is made with pasteurized milk or cream, but contamination can be introduced during the churning/freezing process and through ingredient additions (such as fruit, nuts, chocolate, cookie dough, etc.).

- Routine microbial testing for coliforms and/or aerobic plate count (SPC), but microbial limits will depend on the types of inclusions. SPC testing for frozen yogurt is not practical because it is made with starter cultures.
- Pathogen testing for *L. monocytogenes* and *Salmonella* may be limited to investigative testing in response to increasing trends of indictor organisms and environmental testing results that suggest potential post-process contamination of the product
- Ingredients and inclusions added after pasteurization should be obtained from approved suppliers and subjected to supplier verification activities.
    - Ingredients from a new supplier with little history may require addition verification testing

Milk and Milk Products (fluid)

This category represents the various types of fluid milk and milk products such as whole milk, reduced or low-fat milk, skim milk, and flavored milk. Milk and milk products to include all Grade "A" raw milk and/or milk products are required to be properly pasteurized, aseptically processed and packaged, or retort processed after packaging following accordance with mandatory chemical, physical, bacteriological, and temperature standards *(63)*. During the collecting, transporting, and manufacturing processes, measures can be taken to ensure the removal, inactivation, or absence of microbial levels that would contribute to foodborne illness. Bacteria and toxin production are known causative agents of foodborne illness when consuming milk and milk products. In January 2019, the Centers for Disease Control and Prevention (CDC) documented the investigation of *Brucella* strain RB51 exposures due to consuming contaminated raw milk that spanned over 19 states with the origin determined from a farm in, Pennsylvania *(10)*. This investigation is an example of how not exercising pasteurization measures can threaten the public's health by unnecessary exposure and subsequent illness. Additionally, other microorganisms such as *Listeria* and *Salmonella* have caused outbreaks of pasteurized dairy products in pasteurized milk. An outbreak of *Listeria monocytogenes* was identified with consumption of pasteurized milk from a Massachusetts dairy *(8)*. Further, a *Salmonella* outbreak was caused from multi-drug resistant *Salmonella* Typhimurium in pasteurized milk from a dairy *(51)*. Bacteria, such as Enterobacteriaceae and *Pseudomonas*, in processed milk can result from post-pasteurization contamination (PPC)*(46)*. PPC can result from insufficient cleaning or sanitizing, lack of personnel good hygiene and handling practices, and environmental issues such as standing water, milk residue, drains, and condensate *(46)*. Because vegetative pathogens are rarely found in properly pasteurized milk, testing for indicators organisms at appropriate production points should be used as verification of sanitation.

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

Principles that apply to finished product testing of this RTE food:

Criterion/Factor	Whole milk, reduced or low-fat milk, skim milk, and flavored milk
A. Are pathogens associated with the food or ingredients?	Yes, raw milk may contain multiple pathogens, including *Salmonella, E. coli* O157:H7 and other STEC, *L. monocytogenes*, *Campylobacter, S. aureus*, *Yersinia,* and *Brucella (7, 25, 55, 60)*
B. Are the ingredients likely to be contaminated?	Yes, 1-30% of raw bulk tank samples are positive for one or more pathogens including *Campylobacter jejuni, shiga-toxin producing Escherichia coli, Listeria monocytogenes, Salmonella spp.,* and *Yersinia enterocolitica (37)*.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes, High-Temperature-Short-Time (HTST) pasteurization, which uses a combination of time-temperature of 72^{o}C for at least 15 seconds, which in the US is regulated by the PMO *(63)* and 21 CFR 1240.61. Ultra-High Temperature (UHT; Ultra-pasteurization), applies a high temperature (>135^{o}C) for 1-2 seconds and then rapidly chilled.
D. Is there a potential for recontamination from	Low (since product exposure is minimal following pasteurization), if proper precautions are conducted to

Criterion/Factor	Whole milk, reduced or low-fat milk, skim milk, and flavored milk
the handling or the environment?	prevent transient contamination (e.g., worker contact without proper hygiene, exposure to biological aerosols).
E. Does the product support survival or growth?	Yes. Milk and milk products have optimal water activity and pH levels and provide nutrients to support microbial growth.
F. Is this product meant for higher risk population?	These products are primarily for the general public; however, high-risk populations such as children and the elderly can be more severely impacted if these products are contaminated.
G. What is the shelf life of the product?	HTST pasteurization process can extend the shelf-life of milk for up to 3 weeks, depending on the initial microbiological quality of the raw milk. Ultra-pasteurized milk and milk products have a shelf life of 30-90 days under proper refrigeration.
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	Temperature abuse by the consumer or extended storage beyond use-by date could allow growth of *L. monocytogenes* if product is recontaminated after pasteurization. However, spoilage microbes are likely to outcompete psychrotrophic pathogens.

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

Yes. The presence of non-microbiological alkaline phosphatase (ALP; an enzyme that is denatured by milk pasteurization) in milk is an indication that pasteurization of the milk was not achieved *(53, 64)*, allowing microbiological pathogens to persist, if present. However, reactivation of ALP and presence of non-bovine milk ALP has been shown to occur, particularly in UHT milk, which leads to difficulties in using the assay for regulatory purposes *(53)*. The maximum level of alkaline phosphatase is limited to less than 2.0 micrograms phenol equivalents per gram *(1)*; stated limits in the PMO is less than 350 milliunits per L for fluid products and other milk products by approved electronic phosphatase procedures *(63)*.

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

Even though products have a validated lethality step and filled in a closed environment, microbiological verification testing is a well-established process for pasteurized milk and milk products, including requirements for not to exceed 20,000 SPC per ml or gram and not to excel 10 coliform per ml *(63)*. As prescribed under the Pasteurized Milk Ordinance (PMO), microbial risk factors are considered and evaluated at each process step, including primary production, milk collection, storage, pasteurization, packaging, and transportation to determine what steps are required *(63)*.

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

Enumeration of standard plate count (SPC, aerobic plate count) and coliform counts in milk and milk products verifies minimal post pasteurization bacterial contamination *(63)*. Pathogen testing is not typically conducted unless results for EMP indicate risk of contamination by *L. monocytogenes*.

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 finished product testing SPC and coliforms in Grade "A" milk and milk products is prescribed in the PMO *(63)*. Per PMO *(63)*, during any six consecutive months, a minimum of four samples of each product "shall be collected by the Regulatory Agency in at least four separate months, except when three months show a month containing two sample dates separated by 20 days." If the production of milk and/or milk product is not on a continuous monthly basis, and therefore the firm cannot comply with the sampling requirements above, then a sample must be collected during each month of production.

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

None, other than an Environmental Monitoring Program (EMP). Environmental monitoring is critical to ensure microbial contamination is not in finished milk and milk products and is a means of verifying the effectiveness of the overall sanitary conditions in relation to design, method, frequency, and personnel practices (Innovation Center, 2019). The PMO requires a written EMP plan for milk and milk products exposed to environmental conditions prior to packaging; follow industry and government guidance for *Listeria* control *(30, 68)*.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

If *Listeria* spp. is detected in the environment, conduct investigational testing that may include food contact surfaces and product *(30, 68)*. Verification activities will be dependent on an evaluation of the results, including the zone and frequency of *Listeria* spp. positives.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

If microbial limits for indicators identified by the PMO are exceeded, this could indicate loss of process control. The PMO indicates a loss of control to be addressed in a Corrective Action Plan (CAP).

Implement the CAP, which should include increased frequency of testing the product and the environment, as appropriate *(63)*; investigative testing may be product testing for *E. coli* and pathogens, microbial load in water used for cleaning milk contact surfaces, etc.

Table A-8. Microbial targets, limits, and recommended actions if limits are exceeded, for Milk and Milk Products (Fluid finished product)

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Aerobic plate count (APC, SPC)	$<2.0 \times 10^4$/ml or g	Investigate, implement corrective action	Routine testing per PMO *(63)*

Coliforms	<10/ml	Investigate, implement corrective action. if 10 or more per ml	Routine testing per PMO *(63)*
Listeria monocytogenes	Absent in 25 g	Destroy lot or divert to appropriate use with a lethality step. 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

Recommendations for testing fluid milk products: Legal pasteurization in the US is intended to eliminate vegetative pathogens and product exposure is minimal following pasteurization, which is likely to prevent recontamination; risk of exposure is low. However,

- To comply with regulations, routine testing for standard plate count and coliforms is expected.
- Develop a robust environmental monitoring program for *Listeria* spp. as verification of sanitation
- Pathogen testing of product is not recommended unless environmental monitoring program indicates risk of post-pasteurization contamination

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APPENDIX B - CATEGORY: 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

1. RTE, baked items, refrigerated or temperature-time controlled for safety (TCS)

Examples include baked or fried items that are filled after baking with cream or custard fillings such as eclairs or donuts; foods that are made from or coated with batter or have a grain-based wrapper with fillings that have high water activities (>0.94) and a neutral pH and fried or baked batter-dipped vegetables. A third type products that could be included in this category is refrigerated or frozen baked goods such as cakes, pies, muffins, brownies, waffles, pancakes, and pizza. Some of these foods are not homogeneous, and the interface between the distinct food components may allow growth of pathogens that survive cooking (e.g., spore-formers) or are contaminants from the processing environment (e.g., *Listeria monocytogenes*).

Example 1 **– Refrigerated custard-filled chocolate-iced pastry (e.g., donut or éclair)**

Example 2 **– Frozen waffles or other baked or fried foods made with batter with extended run times**

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

24 **Table B1. Criteria/principles for RTE baked items, refrigerated or TCS**

Criterion/Factor	**Refrigerated Custard-Filled Chocolate-iced Pastry**	**Frozen waffles made with batter**
A. Are pathogens associated with the food or ingredients?	Filled bakery products have been implicated in foodborne illnesses involving *S. aureus*, *Salmonella*, *L. monocytogenes* and *Bacillus cereus.* *Salmonella* and STEC have been associated with raw flour *(1, 8, 9, 11, 22)*. *Salmonella* is found in raw eggs, milk powders, yeast, and cocoa powder *(12)*. *S. aureus* is a contaminant that may be associated with food handlers *(19, 23)*. Ingredients used in the custard filling may also contain pathogenic spore-formers such as *Clostridium botulinum, C. perfringens* and *B. cereus* that must be controlled by refrigeration, pH, water activity and/or growth inhibitors (e.g., potassium sorbate or buffered vinegars).	*Salmonella*, *L. monocytogenes*, *B. cereus* and STEC have been associated with raw flour. *Salmonella* is found in raw eggs. *S. aureus* is a contaminant that may be associated with food handlers.

Criterion/Factor	**Refrigerated Custard-Filled Chocolate-iced Pastry**	**Frozen waffles made with batter**
B. Are the ingredients likely to be contaminated?	Yes. Supplier verification programs are necessary for some ingredients such as *Salmonella* in the cocoa powder. Each ingredient needs to be assessed.	Yes. In-shell eggs are a raw agricultural commodity and most flour has not been treated to inactivate pathogens such as *Salmonella*. Milk is pasteurized so there will not be vegetative pathogens associated with it.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Baking of the pastry shell provides pathogen lethality. The custard filling containing eggs will also have a lethality step. All of the ingredients in the chocolate icing would have received a lethal treatment, but icing ingredients are mixed with no kill step applied prior to adding to the baked pastry. Supplier controls are needed for the ingredients using to make the icing.	Yes. The baking process will destroy vegetative pathogens present in the batter. However, there is the potential for *S. aureus* or *B. cereus* enterotoxin formation in the batter during extended production runs *(7, 10, 13, 26)*. The enterotoxin will not be destroyed by the baking process.
D. Is there a potential for recontamination from	Yes. The product is exposed to the environment after baking and during	Yes. There is the potential for *L. monocytogenes* recontamination of

Criterion/Factor	**Refrigerated Custard-Filled Chocolate-iced Pastry**	**Frozen waffles made with batter**
the handling or the environment?	custard and icing production. It is exposed to the environment during the chilling or freezing step prior to packaging. However, sanitation controls and a robust environmental monitoring program (EMP) can reduce the potential for the pastry to be contaminated with microbial pathogens. Of particular importance is preventing contamination of the filling after cooking/cooling with *S. aureus* from workers. Achieving a temperature below which *S. aureus* can grow quickly (e.g., <10°C/50°F) and limiting the time that the filling is above that temperature is important in preventing enterotoxin production *(23)*.	the cooked waffles post-baking during freezing and packaging.
E. Does the product support survival or growth?	Environmental pathogens that may contaminate the product would survive refrigeration/frozen storage.	Vegetative pathogens such as *Salmonella* and *L. monocytogenes* will

Criterion/Factor	**Refrigerated Custard-Filled Chocolate-iced Pastry**	**Frozen waffles made with batter**
	L. monocytogenes may grow slowly in the high-water activity/neutral pH custard if the product is refrigerated for extended periods, but the low water activity of the cake and icing will prevent growth. Since this is a refrigerated or TCS product, it is assumed that the combination of water activity, pH, and/or presence of chemical preservatives of the custard filling would not be adequate prevent the growth of *B. cereus* and *S. aureus* if product were temperature abused.	survive on frozen waffles but will not grow during frozen storage.
F. Is this product meant 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.	In most instances the product is being made for the general population but may be consumed by individuals in higher risk populations.
G. What is the shelf life of the product?	1 week refrigerated; several months frozen	18 months frozen storage

Criterion/Factor	Refrigerated Custard-Filled Chocolate-iced Pastry	Frozen waffles made with batter
H. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Frozen product would be thawed prior to consumption and possibly brought to room temperature. Extended storage at room temperature may allow growth of *S. aureus* or *B. cereus* in the custard filling to levels where enterotoxin would be produced.	If thawed and held refrigerated by the consumer for an extended time, there is the potential for the growth of *L. monocytogenes* that may have recontaminated the waffle during production. Heating the waffles would reduce *L. monocytogenes* that may have recontaminated the waffle during production but may not eliminate it. There is the potential for consumers to allow teething infants to eat frozen waffles without heating (e.g., toasting).

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

There are no practical alternatives for verification testing of these products other than pathogens or indicator organisms. Control of the batter temperature or cooled cooked custard to less than 50°F would prevent enterotoxin production during extended runs *(26)*, but temperature/time limits need to be validated under conditions mimicking production conditions. For extended runs, enumeration of *S. aureus* or *B. cereus* is preferred to enterotoxin testing, partially due to the lack of validated assays for *B.*

cereus enterotoxins, or the specialized equipment needed for some assays *(24)*, such as cereulide (emetic toxin by *Bacillus cereus*).

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

No. Although temperature/times of baking and cooking that are needed for quality exceed those required for lethality of pathogens, these products (baked pastry with custard filling and icing and cooked waffle) are exposed to the environment after lethality treatment, including during filling with cooled, cooked custard and adding icing after baking.

Even though the outside surfaces of these products typically have water activity values that are lower than values that support rapid growth of pathogens, the custard filling is not likely formulated to prevent pathogen outgrowth (*L. monocytogenes* during refrigeration or *S. aureus* if temperature abused). Therefore, testing for indicator organisms of in-process (such as batter or cooled custard) should be conducted in addition to the environmental monitoring and supplier control programs.

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

The temperature and time limits for extended runs should be based on validation studies to ensure <3-log growth of toxigenic microorganisms such as *S. aureus* or *B. cereus (16, 26)*. Verification testing should include enumeration of *S. aureus* of the custard filling either in the finished product or from work in process (WIP) as appropriate, although the short shelf life of this refrigerated product may preclude having test results before shipping the product. Enumeration of *S. aureus* and/or *B. cereus* in the

raw waffle batter during extended production runs will provide an indication of the risk of enterotoxin production during that run and should be $<10^3$ CFU/g *(15)*. There is the risk of product buildup in the kettles/tanks that may be contaminated with *S. aureus* or *B. cereus* that is not removed by the routine flow of fresh product in the line during extended runs. If visible, these areas could be sampled for *S. aureus* and/or *B. cereus.*

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?

Ability to meet temperature/time limits to prevent growth of toxigenic microbes during extended runs (e.g., *S. aureus*, *B. cereus*) and lethality of infectious microorganisms during baking (e.g., *Salmonella*), along with environmental monitoring program results, and supplier control for ingredients that are added after lethality, should be used to determine type and frequency of finished product testing.

Enumeration testing of the raw batter for *S. aureus* or *B. cereus* may be appropriate, depending on the rigorousness of the validation testing and temperature controls of the batter during production. Products that have a validated lethality step do not need routine microbial testing for finished product. Investigative testing is needed when environmental monitoring for *Listeria* spp. or *Salmonella* suggests insufficient sanitation or inadequate supplier control for incoming ingredients used as post-lethality additions (such as cold blended icing or other toppings).

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

Testing of the custard filling for aerobic plate counts at or prior to the filling point into the pastry may be more appropriate than enumerating *S. aureus* and/or *B. cereus* in the finished product as a verification that no post-cook contamination or microbial growth occurred (spores may survive cooking).

Enumeration of *S. aureus* and/or *B. cereus* in the raw waffle batter is a verification that microbes that can produce heat-stable enterotoxin did not grow in the batter as part of an investigation of loss of process control. Aerobic plate counts are not appropriate for waffle batter due to the initial high background flora in the batter.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

RTE baked products have a microbial reduction step (baking, cooking) prior to packaging but recontamination of the final product is possible from the environment. Therefore, a robust environmental monitoring program that demonstrates that *Listeria* and *Salmonella* are in control will reduce the need for finished product testing.

If results from an environmental monitoring program suggest potential for contamination of the finished product, it could result in the increased need for microbiological testing of product as part of investigative testing or root cause analysis *(25, 27)*.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

If *S. aureus* and/or *B. cereus* numbers exceed a set limit in the pastry filling or in the waffle batter, investigation into the cause is warranted. Corrective actions would need to be taken for *S. aureus* and/or *B. cereus* contamination levels exceeding a set limit (see Tables B2 and B3). Corrective actions should be implemented for detection of *Listeria* in the environment; corrective actions followed by repeat positives may indicate the need for product testing for *L. monocytogenes* (see FDA draft guidance on Control of *Listeria monocytogenes* in RTE Foods)*(27)*.

Table B2. Example of product testing for the custard in a filled pastry

Target Microorganism	**Microbial Limit**	**Recommended Action if limit is exceeded**	**Comments**
Aerobic plate count	≤100 CFU/g	Investigate reason for exceeding limit and implement corrective action.	Routine testing. Populations may include sporeforming bacteria that can survive cooking.
S. aureus	≤10^4 CFU/g	Destroy lot. Investigate cause of contamination. Determine if other lots involved. Determine steps to prevent recurrence.	Non-routine testing. Test as part of investigative action if loss of process control (time-temperature control during production) is suspected.
B. cereus	≤10^4 CFU/g		

Table B3. Example of product testing for batter

Target Microorganism	Microbial Limit	Recommended Action if limit is exceeded	Comments
S. aureus	$\leq 10^4$ CFU/g	Destroy lot. Investigate cause of contamination. Determine if other lots involved. Determine steps to prevent recurrence.	Non-routine testing. Test as part of investigative action if loss of process control is suspected, such as exceeding temperature/time limits identified by challenge study. For extended runs, if temperature exceeds 10°C/50°F, routine testing for *S. aureus* and *B cereus* is recommended.
B. cereus	$\leq 10^4$ CFU/g		

Recommendations for RTE, baked items, refrigerated or temperature-time controlled for safety (TCS)

1. Because there is a kill step for the both the refrigerated filled pastries and the frozen waffle, no finished product testing is needed when an effective EMP program is in place. Finished product testing may be implemented when EMP results show potential for contamination of the finished product.
2. Waffle batter temperature should be kept below 10°C/50°F to prevent outgrowth of *S. aureus* and/or *B. cereus* such that toxin production is prevented. For extended runs where batter

temperature is greater than 10°C/50°F, routine enumeration testing of the batter for *S. aureus* and/or *B. cereus* is recommended based on results from validation studies for extended runs.

3. For RTE baked goods where components do not have a microbial reduction step (such as a cold-blended icing or toppings), ingredients should be obtained from approved suppliers and subjected to supplier verification activities. Ingredients from a new supplier with little history may require additional verification testing.

2. RTE, baked items, shelf stable or non-TCS

Examples include fully baked manufactured from dough or batter such as bread (flat and leavened), cookies, crackers, pretzels, wafer or waffle cones, and certain pastries, cakes, and pies with validation studies showing they are exempt from TCS requirements. Some may have inclusions that are baked with the item such as fruits, vegetables, or cheeses or are iced or filled with shelf stable (low water activity) components such as jellies after baking. There may be leavening of the dough or batter, either through yeast fermentation or chemical leavening agents. Stability of these types of products is achieved by one of or combinations of lowered pH, reduced water activity or chemical agents to prevent mold growth.

***Example 1* – Chocolate, creme-filled sandwich cookie**

***Example 2* – Whole wheat, sliced bread**

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

141 **Table B4. Criteria/principles for RTE, baked items, shelf stable or non-TCS**

Criterion/Factor	**Chocolate, creme-filled sandwich cookie**	**Whole wheat, sliced bread**
A. Are pathogens associated with the food or ingredients?	*Salmonella*, *L. monocytogenes*, STEC, and pathogenic spore-formers have been associated with raw flour *(1, 22)*; *Salmonella* has been found in cocoa powder *(6)*.	*Salmonella*, *L. monocytogenes*, STEC, and pathogenic spore-formers have been associated with raw flour and seeds *(28)*
B. Are the ingredients likely to be contaminated?	Yes. The flour is likely to be contaminated with spore-formers, but the incidence is generally low for vegetative pathogens. Cocoa powder could be contaminated if supplier has not applied proper process controls and environmental control programs to prevent recontamination. Flour and cocoa powder will receive a kill step (baking cookie). Processed ingredients used in the crème-filling (sugar, oils) have low likelihood of contamination.	Yes. Most flour is not treated and is likely to be contaminated with vegetative pathogens and pathogenic spore-formers. *Salmonella* and *L. monocytogenes* have been associated with seeds that may be used as bread toppings (applied prebake).

C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Baking of the cookie provides pathogen lethality, but no kill step is applied to the finished product after adding filling and assembly.	Yes. Baking of bread will provide greater than 5-log destruction of vegetative pathogens *(3, 21)*.
D. Is there a potential for recontamination from the handling or the environment?	Yes. The product is exposed to the environment after baking and during icing/filling and prior to packaging. However, sanitation controls and a robust EMP can reduce the potential for the sandwich cookie to be contaminated with pathogens.	Yes. The product is exposed to the environment after baking and prior to packaging. However, sanitation controls and a robust EMP can reduce the potential for the bread to be contaminated with microbial pathogens.
E. Does the product support survival or growth?	Environmental pathogens that may contaminate the product would survive storage. Growth of pathogens would not be possible due to the low water activity of the cookie and filling. Spore-formers can survive, but not grow.	Environmental pathogens that may contaminate the product exterior would survive storage. Growth of pathogens would not be possible due to the low water activity of the exterior crust. Spore-formers can survive, but not grow.

F. Is this product meant 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.	In most instances the product is being made for the general population but may be consumed by individuals in higher risk populations.
G. What is the shelf life of the product?	9-12 months	1-4 weeks depending on use of preservatives
H. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Consumer use is not likely to affect the risk of pathogens on this product.	Consumer use is not likely to affect the risk of pathogens on this product.

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

No other testing is appropriate beyond verification that temperature/time limits for lethality have been met.

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

Although these products are exposed to the environment after a validated lethality process (baking), the products have low water activity that will not support the growth of pathogens from recontamination after the baking kill step and filling of the sandwich cookie throughout their shelf-life. Neither product has been associated with foodborne outbreaks. Microbial testing of finished product is not needed when an effective EMP program is in place and ingredients added after baking are obtained from approved suppliers and subjected to supplier verification activities.

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

Routine finished product testing is not needed for shelf-stable cookies. Mold counts for breads can be helpful as a quality indicator, but frequently retaining loaves for incubation and visual inspections for mold growth within shelf-life is used as an alternative to enumeration.

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?

Ability to meet temperature/time limits for lethality of infectious microorganisms during baking (e.g., *Salmonella*), along with environmental monitoring program results, and supplier control for ingredients that are added after lethality, should be used to determine whether to conduct finished product testing. Results from the EMP for Enterobacteriaceae or *Salmonella* that demonstrate sanitary

control of the processing environment preclude testing of finished product. Air sampling/monitoring of yeast and mold levels within the plant environment will help to gauge potential impact to spoilage.

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

No, if testing occurs, target should be after the microbial reduction step (baking).

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

RTE baked products have a microbial reduction step prior to packaging but recontamination of the final product is possible from the environment. *Salmonella* can be introduced into the plant environment from flour and become established in the facility. Therefore, a robust environmental monitoring program that demonstrates that *Salmonella* (or Enterobacteriaceae as an indicator of hygiene) are in control will reduce the need for finished product testing.

If results from an environmental monitoring program suggests potential for contamination of the finished product, it could result in the increased need for microbiological testing of product as part of investigative testing or root cause analysis *(4, 5, 11, 20, 25)*.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

Microbiological testing of this finished product is not recommended. Monitoring of baking times and temperatures is adequate to find loss of process control. Measurement of finished product moisture can be evidence that an adequate baking process was applied due to the expected moisture loss during baking. EMP results (e.g., repeat positive Enterobacteriaceae or *Salmonella* spp.) could indicate a loss of sanitation control and could lead to investigative product testing.

Recommendations for RTE, baked items, shelf stable or non-TCS*:* Because there is a kill step for both the cookie and bread, and because the crème filling in the cookie is not likely to contain pathogens, finished product testing for pathogens is not needed when a robust EMP is in place. Finished product testing may be implemented when EMP results show repeat positive Enterobacteriaceae or *Salmonella* species.

1. Because there is a kill step for RTE baked goods, no finished product testing is needed when the baking step is under control and an effective EMP program is in place. Finished product testing may be implemented when EMP results show potential for contamination of the finished product.
2. For RTE baked goods where components do not have a microbial reduction step (such as a cold-blended icing), ingredients should be obtained from approved suppliers and subjected to supplier verification activities. Ingredients from a supplier with little history may require additional verification testing.

3. RTE Cereals

Examples include breakfast cereals with or without inclusions such as nuts, and/or dried fruits, infant cereal, oatmeal, and rice cakes. This product has a cook step for some of the ingredients (e.g., grains, nuts, other inclusions) that eliminates pathogens of concern in the ingredients, but also may have added ingredients that have not received a kill step, e.g., dried fruit. The product is also exposed during preparation and filling of containers and could be contaminated with *Salmonella (2, 12, 18)* or *L. monocytogenes*.

Example 1 **– rice-based cereal with processed nut inclusion and dried fruit inclusion with no kill step**

Example 2 **- infant cereal**

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

Table B5. Criteria/principles for cereals

Criterion/Factor	**Rice-based cereal with processed nut inclusion and dried fruit inclusion with no kill step**	**Dry infant cereal**
A. Are pathogens associated with the food or ingredients?	*Salmonella*, *L. monocytogenes*, *B. cereus*, and STEC have been associated with grains. *Salmonella*, STEC, and viral pathogens may be associated with nuts or dried fruit.	*Salmonella* has been associated with dry infant cereal *(17)*. Other organisms such as *S. aureus*, *B. cereus*, or *Cronobacter* spp. may be present at low levels *(29)*.

Criterion/Factor	Rice-based cereal with processed nut inclusion and dried fruit inclusion with no kill step	Dry infant cereal
B. Are the ingredients likely to be contaminated?	Yes	Yes
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes. The grains have a lethality step during processing. The nuts are treated by the supplier. However, no lethality step for dried fruit added post-lethality step.	Yes. The grains have a lethality step during processing.
D. Is there a potential for recontamination from the handling or the environment?	Yes. The product may be exposed to the environment after the lethality processing step prior to packaging.	Yes. The product may be exposed to the environment after the lethality processing step prior to packaging.
E. Does the product support survival or growth?	Yes. Pathogens such as *Salmonella* will survive but will not grow.	Yes. Pathogens such as *Salmonella* will survive but will not grow.
F. Is this product meant for higher risk population?	This product is made for the general population. However, high-risk populations may purchase cereal or be served	Yes. This product is meant for infants aged 4 months and higher.

Criterion/Factor	Rice-based cereal with processed nut inclusion and dried fruit inclusion with no kill step	Dry infant cereal
	cereal in hospital or nursing home facilities.	
G. What is the shelf life of the product?	18 months	18 months
H. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Consumer handling is unlikely to alter pathogen survival or growth.	Consumer handling may alter pathogen survival or growth if the cereal is held for extended time and not temperature-controlled after reconstitution.

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

No

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

No, finished product testing is required for the cereals (except the infant cereal) if supplier verification supports the adequacy of supplier controls for the inclusions and an appropriate environmental monitoring program shows that the process is under control. Dried cereals are low water

activity products that do not support the growth of pathogens. However, dried cereals could allow persistence of pathogens such as *Salmonella* (if present).

For the infant cereal, when environmental testing results are negative for *Salmonella*, testing of end product for *Salmonella* is still appropriate, Table B7 *(12)*.

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

When finished product testing is done (depending on EMP results or if product is intended for high-risk individuals), presence/absence of *Salmonella* would be the appropriate organism. Product should be held until testing is complete.

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?

Routine testing is not needed for shelf-stable cereals not intended for high-risk individuals. However, if environmental testing indicates the presence of *Salmonella*, then finished product should be tested. In addition, testing should occur in zones 1 and 2, including vitamin or sugar spray nozzles, if used. For infant cereal, end product testing is appropriate because infants are a higher risk population and because of the risk for consumer mishandling, see Table B7.

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

Environmental monitoring is needed to demonstrate control of the environment. For post-lethality step added ingredients, COAs should be received from suppliers and supplier control programs verified.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

Environmental monitoring takes place primarily in zones 2 and 3 (and in zone 1 if zone 2 is contaminated). If there is a positive for an indicator (Enterobacteriaceae) and/or *Salmonella* in zone 2 or 3, then additional swabbing using 3D-vectoring should be conducted to look for niches. The production environment should be cleaned and sanitized, followed by subsequent testing to evaluate cleaning procedures and whether equipment needs to be altered to prevent niches (e.g., hollow rollers). If subsequent zone 2 sampling is positive, finished product testing may be warranted. If finished product is tested, the entire line would be cleaned before and after testing and the lot should be held until results confirm as negative.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

If *Salmonella* is found in zone 2, then additional testing (e.g., every 3 or 4 days, or more frequently depending on findings and risks) and cleaning of zone 2 in the area where the positive was found, as well as nearby areas in zone 3, is warranted until consecutive zone 2 samples are negative. Consider testing

zone 1 and finished product. If finished product testing of infant cereal indicates that *Salmonella* is present, the lots must be destroyed, and an investigation into the cause of the contamination must occur.

Recommendations for RTE Cereals:

1. Microbiological testing of certain ingredients (i.e., those that could potentially be contaminated with pathogens), the environment, and, to a limited extent, finished product, should play a role in the verification of control measures for cereal.
2. Ingredients added after lethality step should be obtained from approved suppliers and subjected to supplier verification activities, which may include pathogen testing. Ingredients from a new supplier with little history may require additional verification testing.
3. A robust *Salmonella* environmental monitoring program for all cereal products is recommended.
4. Routine finished product testing for *Salmonella* should be conducted for dry infant cereal. Although there is a kill step for the dry infant cereal, finished product testing is warranted because the ultimate consumer, infants, is a high-risk consumer category.

Table B6. Example of end product testing for cereal if there is suspected loss of process control

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Salmonella	Negative in 10 samples (case 11 sampling plan) *(12)*	Destroy lot, investigate cause of contamination, determine if other lots involved, determine steps to prevent reoccurrence.	Sample size is 25 g for *Salmonella.*

299 **Table B7. Example of end product testing for infant cereal**

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
Salmonella	Negative in 60 samples *(12)*	Destroy lot, investigate cause of contamination, determine if other lots involved, determine steps to prevent reoccurrence.	Sample size is 25 g for *Salmonella*. Typically, four 375 g composites are tested for *Salmonella*.

300

301 **4. Grain-based products: RTE, cold-pressed bars.**

302 Examples include granola bars. Cold-pressed bars are made from cooked grains, carbohydrate-
303 based binders, and inclusions such as fruit, nuts, and/or chocolate. The ingredients should be verified for
304 microbiological safety, since in most cases the bars will not receive a validated lethality step during
305 manufacturing. These added ingredients should come with a COA that includes pathogen testing. The
306 preventive control supplier program also requires an annual onsite audit when the supplier controls a
307 hazard that could cause serious adverse health consequences or death. Recommendations for finished
308 product and environmental testing by suppliers depend on the specific ingredient being supplied.

309

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

313

314

315 **Table B8. Criteria/principles for cereal bars**

Criterion/Factor	Example: cold-pressed bar with chocolate and coconut
A. Are pathogens associated with the food or ingredients?	*Salmonella* has been associated with cereal, coconut, nuts, and chocolate *(14, 20)*.
B. Are the ingredients likely to be contaminated?	Yes, if the supplier does not process ingredients to eliminate pathogens from the product or prevent recontamination.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	No, for the finished product. Many of the ingredients may have received a lethality treatment, e.g., chocolate, treated nuts, cooked grains.
D. Is there a potential for recontamination from the handling or the environment?	Yes
E. Does the product support survival or growth?	Pathogens will survive, but not grow, on dry product.
F. Is this product meant for higher risk population?	This product is made for the general population. However, high-risk populations may purchase or be served this product.
G. What is the shelf life of the product?	18 months

H. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Consumer handling is unlikely to alter pathogen survival, growth, or toxin production.

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

There are no alternatives to microbial testing *per se*. Robust environment monitoring should be implemented. Ingredients are tested for pathogens by supplier, CoA is provided, and supply chain controls should be verified.

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

Cereal bars are low water activity products that do not have a lethality treatment, although many of the ingredients will have previously been treated. But, since cereal is a low water activity food, pathogens such as *Salmonella* (if present) will persist. Exclusion of pathogens from the ingredients and the environment should be verified through supplier controls and environmental controls (i.e., sanitation controls verified with an EMP).

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or

specific indicator organism) and type of test (e.g., presence/absence or enumeration)? What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

Enterobacteriaceae enumeration (e.g., <100/g) can be used as an indicator of potential contamination. When non-routine finished product testing for pathogens is done (e.g., quarterly), *Salmonella* (presence/absence in 375-g analytical unit composed of 15 x 25-g samples) would be the appropriate organism. Product would be held until testing is done.

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 finished product testing would depend, in part, on the history of environmental testing. Consistently low counts of Enterobacteriaceae and infrequent findings of *Salmonella* in the environment support less frequent finished product testing. If environmental testing (e.g., zones 2 and 3) indicates the presence of *Salmonella*, then investigation and testing of zone 1 and finished product should be considered.

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

Since contamination of cereal bars is from the ingredients or the environment, testing should focus on the ingredients and the environment rather than finished product.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

Environmental monitoring is needed. Low level environmental positives for Enterobacteriaceae or other indicator organisms do not result in the need for finished product testing, but may indicate a

need for increased environmental monitoring *(11)*. If an appropriate environmental monitoring plan is implemented, no routine testing of finished product is needed. With cold-pressed cereal bars, safety is primarily addressed through supplier controls for ingredients and controls related to the environment, such as sanitation controls and sanitary operations, verified with environmental monitoring. If environmental monitoring indicates that the process environment is not adequate, further testing is needed to identify the point(s) in the process that need correction. The results may indicate a need for product testing for pathogens such as *Salmonella.*

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

If *Salmonella* is found in zone 2 or 3, then additional testing (e.g., every 3 or 4 days or more frequently) and cleaning of zone 2 or 3 is warranted until consecutive samples are negative. Consider testing zone 1 and finished product.

Recommendations:

Based on the above, we recommend that:

1. Microbiological testing of ingredients, the environment, and, to a limited extent, non-routine testing of finished product, should play a role in the verification of control measures for cold-pressed bars.
2. Cold-pressed bar manufacturers should conduct activities to verify that suppliers have implemented control measures to minimize the potential for pathogens to be present in those

ingredients for which pathogens have been associated. Supplier verification activities could include microbiological testing of ingredients; the frequency of such testing should be based on an assessment of the likelihood of the ingredients supplied being contaminated, considering the likelihood of contamination of the raw material for the ingredients supplied and the control programs implemented by the supplier.

3. Robust environmental monitoring programs should be implemented to ensure that the cold-pressed bars are not contaminated from the processing environment. Having a robust environmental monitoring program minimizes the need for finished product testing for pathogens.
4. Routine finished product testing for pathogens is not recommended. Microbiological testing of finished product for *Salmonella* should be conducted if there is suspected loss of environmental or process control to investigate process and sanitation control.

Table B9. Example of product testing for cold-pressed bars if there is suspected loss of process control

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Salmonella	Negative in 10 samples (see case 11 sampling plan) *(12)*	Destroy lot, investigate cause of contamination, determine if other lots involved, determine steps to prevent reoccurrence.	Sample size is 25 g for *Salmonella*.

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APPENDIX C - CATEGORY: READY-TO-EAT MEALS

This category includes a wide range of multi-component refrigerated or frozen food products, with shelf lives ranging from less than one week to several months. Some of these may be "heat and eat" foods but are still considered RTE; the food has been processed to control pathogens but is intended to be heated for palatability. The microbial diversity and populations depend on the ingredients used and handling prior to packaging, which can introduce contamination. In most cases, microbial testing uses hygiene indicator organisms (e.g., coliforms, Enterobacteriaceae, generic *E. coli*), with defined limits outlined in this document and in other references *(1, 2, 8, 9, 12)*.

RTE foods having no terminal lethality step, and with short shelf life, will rely more heavily on supplier controls and environmental controls, primarily verified using indicator organisms, because of the impracticality of holding samples until pathogen testing can be complete. In contrast, meals with longer shelf life that have been previously associated with *Salmonella* or *L. monocytogenes* may incorporate testing for pathogens as a verification of process control, as well as testing for indicator organisms. Environmental testing for *Listeria* spp. as an indicator for *L. monocytogenes* is common for these foods. If *Listeria* spp. is found in Zone 1 environmental samples, investigational testing for *L. monocytogenes* may be indicated *(20)*.

A. RTE Deli salads

Examples include macaroni salad, potato salad, egg salad, coleslaw, 3-bean salad, and grains-based salads (e.g., quinoa, barley). Most have a low-acid component that has been cooked (using a heat treatment that provides lethality for microbial pathogens), a vinegar- or mayonnaise-based dressing that reduces the pH but may not be sufficient to prevent growth of *Listeria monocytogenes* (if pH >4.4) or *Salmonella* (if pH>3.7). Most also contain added ingredients that may not have received a kill step for pathogens such as *L. monocytogenes* and *Salmonella*, e.g., cut vegetables such as onions, celery, and

peppers (see Appendix E Fruits/Vegetables). Seasonings may include herbs such as cilantro or spices such as black pepper that are known to be contaminated with pathogens such as *Salmonella*, although many of these have been treated to control such microbial pathogens. (See Appendix F Spices/Herbs)

Deli salads with a shelf life greater than two weeks could result in increased risk to consumers due to potential for pathogen growth (especially *L. monocytogenes*). Where feasible, some deli salads contain antimicrobials such as sorbate or "clean label" antimicrobials to inhibit pathogen growth for duration of the desired shelf life, which could thus reduce risk.

Example 1 - potato salad

Example 2 – rice, bean, and corn salad

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

Criteria a facility can apply to determine whether and how often to test ready-to-eat deli salads:

Criterion/Factor	Potato salad (potatoes, onions, celery, mayonnaise, salt, pepper, vinegar)	Rice, bean, and corn salad (cooked brown rice, canned black beans, frozen corn, red peppers, jalapeño peppers, onions, cilantro, sugar, salt, vinegar, oil)
A. Are pathogens associated with the food or ingredients?	Yes - Pathogens such as *Salmonella, L. monocytogenes,* and pathogenic *E. coli* have been associated with raw agricultural commodities such as potatoes,	Yes - Pathogens such as *Salmonella, L. monocytogenes,* and pathogenic *E. coli* have been associated with peppers and onions. *L. monocytogenes* has been

Criterion/Factor	Potato salad (potatoes, onions, celery, mayonnaise, salt, pepper, vinegar)	Rice, bean, and corn salad (cooked brown rice, canned black beans, frozen corn, red peppers, jalapeño peppers, onions, cilantro, sugar, salt, vinegar, oil)
	onions, and celery, and *Salmonella* has been associated with spices such as black pepper *(9)*(Ch. 11). The ingredients (e.g., black pepper, potatoes) may also contain pathogenic sporeformers such as *Clostridium botulinum, C. perfringens,* and *Bacillus cereus*. Pathogens have not been associated with mayonnaise (commercial), salt, and vinegar.	associated with frozen corn. *B. cereus* has been associated with rice. *Cyclospora* has been associated with cilantro. Pathogens have not been associated with sugar, salt, vinegar, and oil. Pathogens are not associated with properly canned black beans.
B. Are the ingredients likely to be contaminated?	Yes, ingredients such as produce that have not received a microbial reduction treatment could be contaminated with pathogens, even though they have been grown in accordance with Good Agricultural Practice/produce	Yes, produce ingredients that have not received a microbial reduction treatment (e.g., peppers, onions, cilantro) could be contaminated with pathogens even though they have been grown in accordance with Good Agricultural

Criterion/Factor	Potato salad (potatoes, onions, celery, mayonnaise, salt, pepper, vinegar)	Rice, bean, and corn salad (cooked brown rice, canned black beans, frozen corn, red peppers, jalapeño peppers, onions, cilantro, sugar, salt, vinegar, oil)
	safety standards to minimize contamination.	Practice/produce safety standards to minimize contamination. Rice is expected to contain *B. cereus* spores.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	There is no kill step applied to the finished product. Potatoes are cooked; black pepper will have been treated (e.g., steam, ethylene oxide, irradiation), which will kill vegetative pathogens such as *Salmonella*, but pathogenic sporeformers will be present. However, some of the ingredients do not have a kill step, e.g., chopped fresh produce, and could still contain pathogens such as *L. monocytogenes* and *Salmonella*.	There is no kill step applied to the finished product. Rice is cooked, but pathogenic sporeformers will be present. Canning will eliminate all pathogens present in black beans. Some of the ingredients do not have a kill step (e.g., chopped fresh produce, cilantro) and could still contain pathogens such as *L. monocytogenes* or *Cyclospora*. Frozen corn has been blanched but may have been recontaminated with *L. monocytogenes* after blanching.

Criterion/Factor	Potato salad (potatoes, onions, celery, mayonnaise, salt, pepper, vinegar)	Rice, bean, and corn salad (cooked brown rice, canned black beans, frozen corn, red peppers, jalapeño peppers, onions, cilantro, sugar, salt, vinegar, oil)
D. Is there a potential for recontamination from the handling or the environment?	Yes, the product is exposed to the environment during ingredient preparation (e.g., chopping) and mixing; however, sanitation controls verified with a robust environmental monitoring program (EMP) can reduce the potential for deli salads such as potato salad to be contaminated with microbial pathogens such as *L. monocytogenes* and *Salmonella* that can become established in the environment.	Yes, the product is exposed to the environment during ingredient preparation (e.g., chopping) and mixing. Sanitation controls verified with environmental monitoring can reduce the potential for contamination with environmental pathogens such as *L. monocytogenes* and *Salmonella*.
E. Does the product support survival or growth?	Pathogens will survive but growth, if it occurs, is likely to be slow since product is refrigerated and most deli salads, including potato salad, are acidified to a pH of 4.5-4.9.	Rice-based salads may not be acidified to a pH that controls growth of all pathogens that may be present and could support growth of pathogens to hazardous

Criterion/Factor	Potato salad (potatoes, onions, celery, mayonnaise, salt, pepper, vinegar)	Rice, bean, and corn salad (cooked brown rice, canned black beans, frozen corn, red peppers, jalapeño peppers, onions, cilantro, sugar, salt, vinegar, oil)
	Vinegar and mayonnaise will reduce the pH, which could prevent growth of pathogens such as pathogenic sporeformers, and, if present, *Salmonella* and *L. monocytogenes*. Challenge studies can determine whether there is growth or survival of pathogens in the formulation *(13)*	levels. Challenge studies can determine whether there is growth or survival of pathogens in the formulation *(13)*.
F. Is this product meant for higher risk population?	In most instances the product is being made for the general population. However, some facilities may be producing potato salad for hospitals or nursing homes, where the consumers are at higher risk for illness from pathogens such as *L.*	In most instances the product is being made for the general population. However, some facilities may be producing the salad for hospitals or nursing homes, where the consumers are at higher risk for illness from pathogens such as *L.*

Criterion/Factor	Potato salad (potatoes, onions, celery, mayonnaise, salt, pepper, vinegar)	Rice, bean, and corn salad (cooked brown rice, canned black beans, frozen corn, red peppers, jalapeño peppers, onions, cilantro, sugar, salt, vinegar, oil)
	monocytogenes. This pathogen can cause serious illness or death in susceptible consumers (in particular the elderly, the immunocompromised, and pregnant women).	*monocytogenes.* This pathogen can cause serious illness or death in susceptible consumers (in particular the elderly, the immunocompromised, and pregnant women).
G. What is the shelf life of the product?	1-2 weeks, refrigerated	1-2 weeks, refrigerated
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	*L. monocytogenes* can grow (although slowly) during refrigeration if pH is ≥4.4, therefore extended storage time (beyond a use-by date) can lead to higher numbers of the organism and increased illness risk. Consumers could hold potato salad for several hours without	*L. monocytogenes* can grow (although slowly) during refrigeration if pH is ≥4.4, therefore extended storage time (beyond a use-by date) can lead to higher numbers of the organism and increased illness risk. *B. cereus* can grow in cooked rice salads if held without refrigeration, but this would require several

Criterion/Factor	Potato salad (potatoes, onions, celery, mayonnaise, salt, pepper, vinegar)	Rice, bean, and corn salad (cooked brown rice, canned black beans, frozen corn, red peppers, jalapeño peppers, onions, cilantro, sugar, salt, vinegar, oil)
	refrigeration (e.g., at social gatherings). Note: Most deli salads will not be heated. A salad such as German Potato Salad may be served warm, and heating would reduce, but not eliminate, the risk from *L. monocytogenes*.	hours; reduced pH in the salad could extend the time needed for growth to hazardous levels. Consumers could hold the salad for several hours without refrigeration (e.g., at social gatherings). A rice, bean and corn salad may be heated by the consumer for palatability, which would reduce but not eliminate, the risk from pathogens such as *L. monocytogenes*.

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

The diversity of ingredients makes an enzyme-based test impractical. Testing pH can be an important verification activity for process control for many of these products. The pH is important in reducing growth of pathogens, especially under refrigeration, but preventing pathogen growth does not

control the risk of illness from pathogens such as *L. monocytogenes, Salmonella,* and pathogenic *E. coli*, which are relatively acid tolerant and can survive in the product. Testing of pH does not address control of contamination from the environment. The diversity of ingredients and large particles can result in pH variation throughout the product, which makes testing for pH less relevant as a verification activity for some deli salads.

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

Deli salads do not receive a treatment in the final package that is lethal for the pathogens of concern, and the product contains ingredients that have not been subjected to a lethality process. Thus, there is not a treatment that can be monitored that would provide assurance that all pathogens of concern have been controlled. Periodic microbial testing is an appropriate verification activity in ensuring the safety of RTE deli salads, although routine end-product testing for pathogens is not recommended (see ICMSF Chapter 26)*(7)*. Microbial testing of finished product for hygiene indicator organisms (e.g., coliforms, Enterobacteriaceae, generic *E. coli*) can be used for ongoing process control; when indicators suggest a potential problem, pathogen testing relevant to the product and/or ingredients may be considered *(12)*. Companies should consider the risk to the intended consumer when deciding on whether to conduct testing for pathogens; for example, testing for pathogens should be considered when foods are specifically intended for highly susceptible populations (e.g., hospitals). See Question 7 on environmental monitoring for microbial verification testing. See the Appendix E Fruits and Vegetables [RTE fresh-cut vegetables] with respect to testing of produce such as onions, celery, and cilantro to verify supplier controls.

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

As noted in Question 3, microbial testing of finished product for hygiene indicator organisms can be used for ongoing process control. These could include coliforms, Enterobacteriaceae, or generic *E. coli (12)*. Coliforms or Enterobacteriaceae may be better indicators of process control and sanitation than *E. coli*, since they would be present in greater numbers *(10)*. However, they are not appropriate indicators for environmental contamination with *L. monocytogenes* or *Salmonella*. Environmental testing for *Listeria* should be used to assess process control and sanitary conditions *(12)*. Periodic testing of the environment for *Salmonella* may also be warranted. If deli salads are being prepared specifically for at-risk populations (e.g., hospitals, nursing homes), testing of the finished product should include *Listeria monocytogenes.*

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?

A company should consider how robust the control measures are with respect to efficacy and implementation. This includes the company's process control and sanitation control measures, as well as those of suppliers, when applicable. The company should also consider data from verification testing of product for indicator organisms, applicable supplier verification testing, and from environmental monitoring programs. A company could conduct more frequent testing of product initially to obtain baseline information; this testing could include some pathogen testing, as well as indicator organisms. The frequency of finished product microbiological testing can be reduced the longer the production process is found to be under control (See ICMSF Chapter 18; *(7)*. Testing for pathogens should be

increased when verification activities indicate a problem that has the potential to result in pathogen contamination of the food.

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

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 a lethality treatment), could be an alternative to finished product testing. However, in many cases the ingredients of concern may have a short shelf life, and unless the test results can be obtained within approximately 24 hours, such testing may not be practical.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

A robust EMP should reduce the need for finished product testing, since one of the main pathogens of concern for deli salads is *Listeria monocytogenes*, which primarily comes from environmental contamination. The EMP should also periodically test for *Salmonella,* which is associated with the raw vegetables and grains and can become established in the environment. The results of environmental monitoring could result in the need for microbiological testing of product or ingredients (e.g., if a food contact surface tests positive for *Listeria* spp. or *L. monocytogenes,* product or ingredient testing may be part of investigative testing or root cause analysis [FDA, 2017]).

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

A company should consider the finding of a pathogen in an RTE deli salad to indicate a likely loss of process control. In addition, the finding of indicator organisms exceeding the established limits could also indicate a loss of process control. In all cases, investigation is warranted. The investigation could indicate the need for additional testing to determine the root cause of the problem or to determine, in the case of indicator organisms, whether pathogen testing is warranted.

If *E. coli*, coliforms, or Enterobacteriaceae exceed a defined limit investigation into the cause is warranted. In some cases, product testing for pathogens such as *Salmonella* or pathogenic *E. coli* may be warranted when limits for indicator organisms are exceeded. This may depend on the findings of a root cause analysis of the issue, or companies may establish a protocol for when such testing would be done based on the overall food safety system, the likelihood that a pathogen could be present, and the risk to the consumer. Corrective actions should be taken for any finding of *Listeria* in the environment; corrective actions followed by repeat positives may indicate the need for product testing for *Listeria monocytogenes* (see FDA draft guidance on Control of *Listeria monocytogenes* in RTE Foods FDA *(20)*. Similarly, corrective actions for finding *Salmonella* in the environment may indicate the need for product testing for this pathogen.

Recommendations: Based on the above, we recommend that for deli salads:

- Microbiological testing of finished product and the environment should play a role in the verification of control measures.
- Periodic testing of finished product (e.g., quarterly) for pathogens (e.g., *Salmonella*, *L. monocytogenes*) should be conducted to verify process control. In addition, "for cause" pathogen testing is recommended (e.g., when a problem is detected that indicates the potential for the food to be contaminated with a pathogen). Routine testing for *E. coli*, Enterobacteriaceae or coliforms should be conducted more frequently (e.g., daily or weekly) than tests for pathogens. (See Table C-1 for microorganisms and common limits.)
- Deli salad makers should conduct activities to verify that suppliers have implemented control measures to minimize the potential for pathogens to be present in those deli salad ingredients that have been associated with pathogens (e.g., chopped onions and celery).
- Supplier verification activities should include microbiological testing of certain ingredients (e.g., chopped onions and peppers, cilantro and other ingredients that have not received a step lethal to the pathogens of concern) by the supplier or the deli salad manufacturer (e.g., for *Salmonella* and *L. monocytogenes*); the frequency of such testing should be based on an assessment of the likelihood of the ingredients supplied being contaminated, considering the likelihood of contamination of the raw material for the ingredients supplied and the control programs implemented by the supplier.
- Deli salad manufacturers should implement robust environmental monitoring programs for *Listeria* spp. (and periodically for *Salmonella*) to ensure that the salads are not contaminated from the processing environment; having a robust environmental monitoring program minimizes the need for finished product testing.

155 **Table C-1**. Example of product testing for deli salads

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
Coliforms or Enterobacteriaceae	<100 cfu/g	Investigate reason for exceeding limit and correct. Determine if pathogen testing is warranted.	Coliforms, Enterobacteriaceae, or *E. coli* are acceptable for routine testing *(10)*. Only one of these indicators is needed. As noted above, coliforms or Enterobacteriaceae may be better indicator of process control and sanitation than *E. coli*, since they would be present in greater numbers.
E. coli [a]	<10 cfu/g	Investigate reason for exceeding limit and correct. Determine if pathogen testing is warranted.	
Salmonella	negative in 375 g	Destroy lot. Investigate cause of contamination. Determine if other lots	Can composite 15 25g samples into one 375 g analytical

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
		involved. Determine steps to prevent reoccurrence.	unit; Sample size should increase for investigation sampling (e.g., 60 25g samples tested individually or composited into 4 375 g analytical units
Listeria monocytogenes	Negative in 25g	Destroy lot. Investigate cause of contamination. Determine if other lots involved. Determine steps to prevent reoccurrence.	

B. Sandwiches

Sandwiches have many combinations of ingredients, including breads, meats, cheeses, produce (e.g., lettuce, tomato), salads (e.g., chicken, egg), and condiments. Some sandwiches may be prepared for reheating for palatability prior to serving (e.g., an egg and biscuit sandwich, which can also contain meat such as sausage). In many instances the sandwiches are assembled manually, which can result in contamination.

Sandwich example 1: Ham, turkey, or roast beef with bread, cheddar cheese, lettuce, tomato, mayonnaise, mustard (refrigerated)

Sandwich Example 2: Sausage and egg biscuit sandwich (frozen)

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

Criteria a facility can apply to determine whether and how often to test ready-to-eat sandwiches:

Criterion/Factor	Ham, turkey, or roast beef with bread, cheddar cheese, lettuce, tomato, mayonnaise, mustard (refrigerated)	Sausage and egg biscuit sandwich (frozen)
A. Are pathogens associated with the food or ingredients?	Meat has pathogens such as *Salmonella* and *L. monocytogenes* that are addressed through cooking of meat by the supplier and prevention of recontamination after cooking. Pathogenic sporeformers in meat (e.g., *C. perfringens*) are controlled by refrigeration. *Salmonella* in flour is addressed in baking bread. Pathogens associated with cheese	Sausage has pathogens such as *Salmonella* and *L. monocytogenes* that are addressed through cooking of sausage by the supplier and prevention of recontamination after cooking. Pathogenic sporeformers in meat (e.g., *C. perfringens*) are controlled by refrigeration. *Salmonella* and pathogenic *E. coli* potentially present in flour are addressed by

Criterion/Factor	Ham, turkey, or roast beef with bread, cheddar cheese, lettuce, tomato, mayonnaise, mustard (refrigerated)	Sausage and egg biscuit sandwich (frozen)
	are addressed in its manufacture through pasteurization of the milk, production of acids, and reduction of pH through microbial growth of starter cultures, and through aging, as well as through controls to minimize contamination with *L. monocytogenes* from the environment. Lettuce and tomato have the potential to contain pathogens such as *Salmonella* from the growing environment, addressed in part by controls applied during growing and harvesting. Condiments are not likely to contain pathogens.	the supplier in baking the biscuits. *Salmonella* is associated with eggs *(21)*, but the organism will be killed when the egg is cooked. The egg ingredient in the sandwich is likely to be made using pasteurized liquid whole egg, and thus will be subjected to two lethal processes - pasteurization of the liquid whole egg at a USDA establishment and cooking of the liquid egg for the sandwich.
B. Are the ingredients likely to be contaminated?	Suppliers will control the hazards in many of the ingredients used in making sandwiches, such as meats	If the sandwiches are assembled from pre-cooked sausage, eggs, and biscuits from a supplier, the

Criterion/Factor	Ham, turkey, or roast beef with bread, cheddar cheese, lettuce, tomato, mayonnaise, mustard (refrigerated)	Sausage and egg biscuit sandwich (frozen)
	and cheeses. Although the potential is relatively low, pathogens are considered reasonably likely for produce such as lettuce and tomatoes because there is no kill step. There is also the potential for the meats used in these sandwiches to be contaminated with *L monocytogenes* from the supplier's environment (see Criterion/Factor D and Question 3).	ingredients have a low potential to be contaminated, provided that the suppliers properly implement process controls and prevent recontamination from the environment. If the sandwich manufacturer prepares any of the components from raw ingredients such as raw meat, shell eggs, and flour, these raw ingredients should be considered likely to be contaminated. If the manufacturer uses pasteurized liquid whole egg, the potential for *Salmonella* to be present is low.
C. Are there robust processing control procedures such as a kill step or other	Yes, for meats, bread, and cheese, but not for the lettuce and tomato or for the assembled sandwich.	Yes, for sausage, biscuit, and egg, but not for the assembled sandwich.

Criterion/Factor	Ham, turkey, or roast beef with bread, cheddar cheese, lettuce, tomato, mayonnaise, mustard (refrigerated)	Sausage and egg biscuit sandwich (frozen)
reduction methods/ controls?		
D. Is there a potential for recontamination from the handling or the environment?	Yes, for both handling (sandwich assembly) and from the sandwich manufacturing environment; employee GMPs and sanitation controls for the environment, verified with an EMP, are needed to minimize the likelihood of contamination. In addition, there is potential for recontamination of the meat and cheese ingredients in the suppliers' manufacturing environments. Some deli meats may receive a process in the package (e.g., high-pressure processing or a heat treatment) to inactivate low levels of *L. monocytogenes* present due to	Yes, for both handling (during sandwich assembly) and from the environment; employee GMPs and sanitation controls for the environment, verified with an EMP, are needed to minimize the likelihood of contamination. In addition, there is potential for recontamination of the sausage and biscuits ingredients in the suppliers' manufacturing environments.

Criterion/Factor	Ham, turkey, or roast beef with bread, cheddar cheese, lettuce, tomato, mayonnaise, mustard (refrigerated)	Sausage and egg biscuit sandwich (frozen)
	recontamination from environment.	
E. Does the product support survival or growth?	Yes. Refrigeration will slow growth (e.g., for *L. monocytogenes*) or prevent growth (e.g., pathogenic sporeformers) of pathogens. Cheddar cheese is a hard cheese that will not support growth of *L. monocytogenes*, but the organism will survive if the cheese is contaminated from the environment. Meat ingredients may contain inhibitors to growth of *L. monocytogenes* (e.g., lactate and diacetate) *(14)*	Yes, pathogens (e.g., *L monocytogenes*, if present, and pathogenic sporeformers) will survive, although freezing will prevent growth. If the sandwich is thawed, growth of pathogens could occur, depending on the temperature.
F. Is this product meant for higher risk population?	In most instances the product is being made for the general population. However, some facilities may be producing	In most instances the product is being made for the general population. However, some facilities may be producing

Criterion/Factor	Ham, turkey, or roast beef with bread, cheddar cheese, lettuce, tomato, mayonnaise, mustard (refrigerated)	Sausage and egg biscuit sandwich (frozen)
	sandwiches for hospitals or nursing homes, where the consumers are at higher risk for illness from pathogens such as *L. monocytogenes.*	sandwiches for hospitals or nursing homes, where the consumers are at higher risk for illness from pathogens such as *L. monocytogenes.*
G. What is the shelf life of the product?	Short: 1-2 days maximum for ham/turkey/roast beef sandwiches with lettuce, tomato, and cheese. Thus, the time available for pathogen growth will be short.	Several months when frozen, a few days if thawed and refrigerated. No growth will occur during frozen storage; if the product is thawed and held refrigerated, the time available for growth will be short.
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	If the sandwiches are contaminated with *L. monocytogenes*, and the consumer holds the sandwiches under refrigeration for a day or more, the risk of illness from that organism could increase. The risk of growth from pathogenic sporeformers is very low, since	If the consumer keeps the sandwich frozen until heated and consumed, there is no increased risk. Heating the product could potentially decrease the risk of illness from *L. monocytogenes* (if present) by reducing the number of organisms.

Criterion/Factor	Ham, turkey, or roast beef with bread, cheddar cheese, lettuce, tomato, mayonnaise, mustard (refrigerated)	Sausage and egg biscuit sandwich (frozen)
	significant temperature abuse would have to occur, and the sandwich is likely to spoil and not be consumed.	

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

No, in particular the diversity of ingredients makes an enzyme-based test impractical. The diversity of ingredients also makes testing for pH and a_w not relevant as a verification activity for most sandwiches.

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

There is no single treatment applied to sandwiches that can be monitored and verified as a means of demonstrating that pathogens have been controlled. Because of the short shelf life of most sandwiches, companies need to rely on supplier controls, process controls, control of employee practices, and environmental (sanitation) controls rather than microbial testing of sandwiches for verification of pathogen control. Environmental monitoring to verify sanitation controls will be needed. In these examples, there are robust process controls (lethality steps) in the manufacture of the meats (ham,

turkey, roast beef, sausage), cheese, bread, biscuit, and egg that would not need to be verified by routine microbial testing. For example, USDA FSIS has lethality requirements for a 6.5 log reduction of *Salmonella* in roast beef (9 CFR 318.17(a)(1)), cooked poultry products must be processed to achieve at least a 7-log reduction of *Salmonella* (9 CFR 381.150(a)(1)), and uncured meat patties must be processed to meet or exceed the times and temperatures listed in 9 CFR 318.23, which will achieve a 5-log lethality *(15)*. Typical commercial processes for baking (e.g., whole wheat bread, hamburger buns) have been shown to achieve a significant reduction (e.g., >5 logs) of *Salmonella (5, 6)*, although suppliers should provide validation information for the specific baked goods. USDA requires pasteurized egg products to be produced to be edible without further preparation to achieve food safety and to be sampled for *Salmonella* spp. (9 CFR 590.570 and 590.580)[1]. In general, FSIS considers a 5-log reduction of *Salmonella* to provide safety in products that are edible without additional preparation to achieve food safety, including egg products *(17)*.

However, many of these ingredients are likely exposed to the environment after the lethality step and, thus, there is the potential for recontamination from the environment. For example, USDA FSIS reported that the *Salmonella* percent positive in pasteurized egg products from 2008 to 2017 was 0.14% (although there have not been any positives in pasteurized liquid whole egg since 2012) *(16)*. USDA FSIS also reported only one (of more than 14,000) samples of RTE meat and poultry tested positive for *Salmonella* in 2017, but 30 samples (0.20 %) tested positive for *L. monocytogenes (18)*. It would be appropriate for the suppliers of the luncheon meat, sausage, pasteurized egg, and the cheese to periodically conduct product testing to verify their process control and their sanitation control measures

[1] USDA has amended the egg products inspection regulations by requiring official plants that process egg products to develop and implement HACCP systems and to process egg products to be edible without additional preparation to achieve food safety, i.e., ensure that the products are free of detectable pathogens (85 *Federal Register* 68640, October 29, 2020); minimum times and temperatures for pasteurization in 9 CFR 590.570 were moved to the FSIS Food Safety Guidelines for Egg Products, September 9, 2020 (U.S. Department of Agriculture Food Safety and Inspection Service, 2020).

based in part on the results of a robust EMP. It would also be appropriate for the sandwich manufacturer to periodically conduct testing of these ingredients as part of a supplier verification program. The frequency of the testing would depend on factors such as results of a supplier audit, history of supplier compliance, association of the ingredient with pathogen contamination and illnesses, etc. Testing of bread/biscuits is not warranted (provided the supplier has been verified to have appropriate process control and sanitation control measures verified by an EMP); recontamination of bread from the environment has not been the cause of foodborne outbreaks.

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

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

Routine finished product testing for pathogens is not practical for short shelf-life sandwiches. There could be benefit from enumerating indicator organisms such as coliforms, Enterobacteriaceae or generic *E. coli* to identify changes in microbiological counts that warrant investigation of process, sanitation, and supplier controls, as well as facility CGMP practices. Since sanitation controls are essential to prevent contamination from food handlers and the environment during assembly of sandwiches, verification of sanitation controls provides greater benefit than finished product testing. ATP swabs after cleaning surfaces (including utensils) provide a useful tool to verify cleaning procedures, and environmental testing for *Listeria* spp. is needed to assess whether there are insanitary conditions that could lead to contamination with these organisms from the environment. Likewise, since suppliers control the hazards in many of the components used to make sandwiches, verifying the control measures that suppliers have in place, including process controls and sanitation controls, also provides greater benefit than finished product testing of sandwiches. Verification of supplier controls on ingredients should include

audits and may include microbial testing reported in COAs. Out-of-specification ingredients (for those tested when warranted) could be diverted from use in sandwiches. Microbial testing for pathogens (e.g., for certain ingredients used in sandwiches) should be considered, in particular when the sandwiches are specifically intended for highly susceptible populations (e.g., hospitals).

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?

Routine finished product testing for pathogens is not warranted, as discussed in Questions 3 and 4. As noted in Question 4, enumerating indicator organisms such as coliforms, Enterobacteriaceae or generic *E. coli* could be used to indicate inadequate controls that could result in an increased risk of pathogens being present. The frequency of process control testing will depend on a variety of factors (see Main Document, Question 5). Regardless, testing more frequently will be more effective in identifying a loss of process control, assist in root cause analysis and in determining when control has been restored *(3)*.

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

Supplier controls are critical; 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 adequate process controls and control of environmental contamination verified with an EMP. For example, with respect to the meat and cheese ingredients of the example luncheon meat sandwich, periodic testing for *L. monocytogenes* before use of the ingredients would be appropriate, with the frequency dependent on the strength of the supplier's control measures

ad supplier performance. Similarly, periodic testing for *L. monocytogenes* and *Salmonella* in the sausage and for *Salmonella* in the egg would be appropriate for the example sausage and egg biscuit sandwich.

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

EMP is a key factor in not conducting or in limiting finished product testing. Because the greatest likelihood of pathogens being present comes from environmental contamination (assuming suppliers' control programs are appropriate and properly implemented), environmental monitoring on an ongoing basis to verify sanitation controls provides the most relevant information on product safety. The results of environmental monitoring could result in the need for microbiological testing of product or ingredients (e.g., if a food contact surface tests positive for *Listeria* spp. or *L. monocytogenes*, product or ingredient testing may be part of investigative testing or root cause analysis)*(20)*.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

A company should consider the finding of a pathogen in an RTE sandwich or RTE ingredient for the sandwich to indicate a likely loss of process control. In addition, the finding of indicator organisms exceeding the established limits could also indicate a loss of process control. In all cases, investigation is warranted. The investigation could indicate the need for additional testing to determine the root cause of the problem or to determine, in the case of indicator organisms, whether pathogen testing is warranted.

If problems occur at a supplier (e.g., a meat provider has a problem with a pathogen being detected in RTE meat) and the supplier has taken appropriate corrective action, a company may consider testing that ingredient (or requiring a COA) for a period of time as a verification that the corrective actions have been effective. In addition, corrective actions should be taken by the sandwich manufacturer of by a supplier of an RTE ingredient for any finding of *Listeria* spp. in the environment; corrective actions followed by repeat positives may indicate the need for product testing for *L. monocytogenes* (see FDA draft guidance on Control of *Listeria monocytogenes* in RTE Foods *(20)*.

If testing for *E. coli*, Enterobacteriaceae or coliforms indicates a loss of control, testing frequency should be increased to assist in root cause analysis and to more quickly determine when control has been restored *(3)*

Recommendations: Based on the above, we recommend that:

- Microbiological testing of ingredients, the environment, and, to a limited extent, finished product should play a role in the verification of control measures for sandwiches. (See Table C-2 for microorganisms and common limits that could be applied when testing products.)
- Sandwich makers should conduct activities to verify that suppliers have implemented control measures to minimize the potential for pathogens to be present in those sandwich ingredients that have been associated with pathogens. Supplier verification activities could include microbiological testing of ingredients; the frequency of such testing should be based on an assessment of the likelihood of the ingredients supplied being contaminated, considering the likelihood of contamination of the raw material for the ingredients supplied and the control programs implemented by the supplier.
- Sandwich manufacturers should implement robust environmental monitoring programs for *Listeria* spp.to ensure that the sandwiches are not contaminated from the processing

environment; having a robust environmental monitoring program minimizes the need for finished product testing for environmental pathogens.

- Routine finished product testing for pathogens is not recommended. Microbiological testing of finished product for indicator organisms such as *E. coli*, Enterobacteriaceae or coliforms rather than pathogens should be conducted to verify process and sanitation control and identify changes in microbiological counts warranting investigation.

Table C-2. Example of product testing for sandwiches

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
Coliforms or Enterobacteriaceae	≤100 cfu/g	Investigate reason for exceeding limit and correct. Determine if pathogen testing is warranted.	Routine testing. Coliforms, Enterobacteriaceae, or *E. coli* are acceptable for routine testing *(10)*. Only one of these indicators is needed. As noted above, coliforms or Enterobacteriaceae may be better indicator of process control and sanitation than *E. coli*, since they would be
E. coli	≤10 cfu/g	Investigate reason for exceeding limit and correct. Determine if pathogen testing is warranted.	

Target Microorganism	Microbial Limit	Recommended Action if Limit is Exceeded	Comments
			present in greater numbers.
Salmonella	negative in 375 g	Destroy lot. Investigate cause of contamination. Determine if other lots involved. Determine steps to prevent reoccurrence.	Investigative testing as response to EMP that suggests likely contamination of product. Can composite 15 25g samples into one 375 g analytical unit; Sample size should increase for investigation sampling (e.g., 60 25g samples tested individually or composited into 4 375 g analytical units
Listeria monocytogenes	Negative in 25g	Destroy lot. Investigate cause of contamination. Determine if other lots involved. Determine steps to prevent reoccurrence.	Investigative testing as response to EMP that suggests likely contamination of product

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310 **C. "Heat and Eat" Entrées and Meals**

Examples include macaroni and cheese, vegetable raviolis, soy/vegetable meat analogues (e.g., vegetable patties), samosas, pierogis, egg rolls, tofu vegetable pot pies; these could be refrigerated or frozen. These have been cooked and only require heating for palatability. (If fillings contain non-fully cooked ingredients, the products would be not-ready-to-eat ("Cook and Eat"); these products are outside the scope of the charge.)

Entrée Example 1 - Fried Vegetable Egg Roll (Refrigerated)

Entrée Example 2 - Baked Tofu and Vegetable Pot Pie (Frozen)

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

Criteria a facility can apply to determine whether and how often to test ready-to-eat "Heat and Eat" Entrées and Meals:

Criterion/Factor	Fried Vegetable Egg Roll (Refrigerated) flour (in the wrappers), vegetables (cabbage, carrots, bean sprouts), ginger	Baked Tofu Vegetable Pot Pie (Frozen) flour (wheat, rice), vegetables (potatoes, onions, carrots, peas), butter/cream base (cream, salt), tofu (meat analogue), spices (black pepper).
A. Are pathogens associated with the food or ingredients?	Yes - Pathogens such as *Salmonella, L. monocytogenes,* and pathogenic *E. coli* have been	Yes, pathogens such as *Salmonella, L. monocytogenes,* and pathogenic *E. coli* have been associated with

Criterion/Factor	Fried Vegetable Egg Roll (Refrigerated) flour (in the wrappers), vegetables (cabbage, carrots, bean sprouts), ginger	Baked Tofu Vegetable Pot Pie (Frozen) flour (wheat, rice), vegetables (potatoes, onions, carrots, peas), butter/cream base (cream, salt), tofu (meat analogue), spices (black pepper).
	associated with raw agricultural commodities such as cabbage, carrots, bean sprouts. Cabbage and carrots may also contain pathogenic sporeformers such as *Clostridium botulinum, C. perfringens,* and *Bacillus cereus.* *Salmonella* has been associated with spices such as ginger and with flour.	raw agricultural commodities such as potatoes, onions, carrots, peas. *Salmonella* has been associated with black pepper, and *Salmonella* and pathogenic *E. coli* with flour. The ingredients (e.g., black pepper, potatoes) may also contain pathogenic sporeformers such as *Clostridium botulinum, C. perfringens,* and *Bacillus cereus.* Butter and cream have been associated with *Salmonella* and *L. monocytogenes*.
B. Are the ingredients likely to be contaminated?	Yes, ingredients such as produce that have not received a microbial reduction treatment could be contaminated with pathogens,	Yes, but limited. Black pepper has been treated to eliminate *Salmonella*. Vegetable items are pre-cooked or blanched *(4)* so they

Criterion/Factor	Fried Vegetable Egg Roll	Baked Tofu Vegetable Pot Pie
	(Refrigerated) flour (in the wrappers), vegetables (cabbage, carrots, bean sprouts), ginger	(Frozen) flour (wheat, rice), vegetables (potatoes, onions, carrots, peas), butter/cream base (cream, salt), tofu (meat analogue), spices (black pepper).
	even though they have been grown in accordance with Good Agricultural Practice/produce safety standards to minimize contamination.	are not likely to be contaminated, provided that the suppliers properly implement process controls and prevent recontamination from the environment. Flour may be contaminated, but the incidence is generally low. If the pot pie manufacturer prepares any of the components from raw ingredients such as raw vegetables, these raw ingredients could be contaminated. Tofu could be contaminated with *Listeria monocytogenes*, but it is frequently pasteurized after packaging to extend shelf life. Butter/cream base purchased is

Criterion/Factor	Fried Vegetable Egg Roll (Refrigerated) flour (in the wrappers), vegetables (cabbage, carrots, bean sprouts), ginger	Baked Tofu Vegetable Pot Pie (Frozen) flour (wheat, rice), vegetables (potatoes, onions, carrots, peas), butter/cream base (cream, salt), tofu (meat analogue), spices (black pepper).
		pasteurized so highly unlikely to be contaminated.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes, fully cooked egg rolls receive frying that would be a kill step (but the frying needs to be validated to ensure all components receive sufficient heat).*(11)*	Yes, blanching vegetables (frozen) occurs before preparation of the pot pie (and vegetables will be cooked in the pie during baking). Tofu receives a kill step during manufacture *(11)* . Fully cooked Pot Pies are baked within the crust by the manufacturer (the process should be validated).
D. Is there a potential for recontamination from the handling or the environment?	Yes, from the environment; egg roll is fried after assembly and sent into a cooling chamber where it could be exposed to post-process contamination. Sanitation controls for the environment, verified with	Yes, from the environment; the product is baked and will be exposed to the environment during cooling prior to packaging. Sanitation controls for the environment, verified with EMP for

Criterion/Factor	Fried Vegetable Egg Roll	Baked Tofu Vegetable Pot Pie
	(Refrigerated) flour (in the wrappers), vegetables (cabbage, carrots, bean sprouts), ginger	(Frozen) flour (wheat, rice), vegetables (potatoes, onions, carrots, peas), butter/cream base (cream, salt), tofu (meat analogue), spices (black pepper).
	EMP, are needed to minimize the likelihood of contamination.	both *L. monocytogenes* and *Salmonella*, are needed to minimize the likelihood of contamination.
E. Does the product support survival or growth?	Pathogens will survive (e.g., *L monocytogenes*, if present due to post-process contamination, and pathogenic sporeformers). Refrigeration will slow growth (e.g., for *L. monocytogenes*) or prevent growth (e.g., pathogenic sporeformers) of pathogens.	Pathogens (e.g., *L monocytogenes* and *Salmonella*, if present due to post-process contamination, and pathogenic sporeformers) will survive, but freezing will prevent growth.
F. Is this product meant for higher risk population?	In most instances the product is being made for the general population. However, some facilities may be producing egg rolls for hospitals or nursing homes	In most instances the product is being made for the general population. However, some facilities may be producing pot pies for hospitals or nursing homes

Criterion/Factor	Fried Vegetable Egg Roll (Refrigerated) flour (in the wrappers), vegetables (cabbage, carrots, bean sprouts), ginger	Baked Tofu Vegetable Pot Pie (Frozen) flour (wheat, rice), vegetables (potatoes, onions, carrots, peas), butter/cream base (cream, salt), tofu (meat analogue), spices (black pepper).
	where the consumers are at higher risk for illness from pathogens such as *L. monocytogenes.*	where the consumers are at higher risk for illness from pathogens such as *L. monocytogenes.*
G. What is the shelf life of the product?	Short: a few days to two weeks. Even with a longer shelf life, potential for *L. monocytogenes* growth is minimal due to contamination being on the outside of the egg roll, which has a low water activity.	Several months when frozen, a few days if thawed and refrigerated.
H. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Risk is low since these products are fully cooked. They will be reheated for eating and likely consumed within a short time. Potential for temperature abuse if left at non-refrigerated temperatures, which	Risk is low since these products are fully cooked. They will be reheated for eating and likely consumed within a short time. If product is held at room temperature, any surviving pathogens could grow

Criterion/Factor	Fried Vegetable Egg Roll	Baked Tofu Vegetable Pot Pie
	(Refrigerated) flour (in the wrappers), vegetables (cabbage, carrots, bean sprouts), ginger	(Frozen) flour (wheat, rice), vegetables (potatoes, onions, carrots, peas), butter/cream base (cream, salt), tofu (meat analogue), spices (black pepper).
	could result in growth of pathogens if present.	since the pH is likely >4.6 and the water activity is above 0.95.

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

No, in particular the diversity of ingredients makes an enzyme-based test impractical.

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

Yes – Fully-cooked products that are fried or baked using a validated process do not contain any uncooked ingredients. Control of the cooking process (with monitoring) and preventing recontamination through sanitation controls verified by an EMP indicate routine microbiological testing of product is not warranted (ICMSF, Ch. 26, *(7)*. Heating of product for palatability further reduces risk of illness from consumption of these products.

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

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

For egg rolls and pot pies that are fully cooked routine finished product testing for pathogens is not necessary. Environmental monitoring for *Listeria* spp. and for *Salmonella* is recommended *(7, 9)*.

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

For fully cooked, heat-and-eat meals, finished product testing for pathogens is not needed except for investigative testing if EMP results suggest there is a loss of control. However, routine testing for heat-sensitive indicator organisms is useful for verification that the cooking process was effective; if enumeration limits are exceeded, investigation and corrective actions are needed.

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

For a product with a validated lethality (cooking) process, neither in-process or finished product microbial testing for pathogens is useful as a routine verification activity for product. However, other monitoring activities of the lethality process (e.g., oven or product internal temperature, time) provides more assurance of safety than microbiological testing of the food. If the food is exposed to the environment after the process, as with egg rolls and baked pot pies, an EMP is critical.

Question 7: What impact should [does] environmental monitoring have on frequency and extent of product testing verification activities by companies?

A robust EMP is key factor in not conducting finished product testing of a fully cooked product that is exposed to the environment. If EMP results suggest there is a loss of control, investigative testing of finished product may be part of a root cause analysis.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

Cook step monitoring and an EMP will provide evidence of control for a fully cooked product. If either process or EMP monitoring suggest limits were not met (loss of control), investigative microbial testing for heat-sensitive indicator organisms (e.g., ≥10 CFU/g *E. coli*) in the finished product, and comparison with adjacent/similar lots could identify a root cause of the problem and direct corrective actions. If microbial test results confirm loss of process control, investigative testing for pathogens may be appropriate. The presence of indicator organisms alone, without evidence of insufficient cooking or pathogen survival, is not used to determine release of product. Investigation and corrective actions should be taken for any finding of *Listeria* or *Salmonella* in the environment; repeat positives may indicate the need for product testing for *Listeria monocytogenes* (see FDA draft guidance on Control of *Listeria monocytogenes* in RTE Foods *(20)* or for *Salmonella (19)*. Verification testing can be scaled back when root cause has been identified and corrected and microbial testing confirms correction.

Recommendations: Based on the above, we recommend that:

1. Validation of the cook step and monitoring of parameters that demonstrate control are needed to ensure appropriate log reduction is achieved for vegetative pathogens that may be present in the ingredients.
2. Because there is a validated kill step for the both the refrigerated vegetable egg roll and frozen tofu vegetable pot pie, no finished product testing is needed when process monitoring indicates the process is under control and an EMP program is in place that indicates sanitation controls are effective in preventing contamination from the environment.
3. The results of environmental monitoring could result in the need for microbiological testing of product or ingredients (e.g., if a food contact surface tests positive for *Listeria* spp. or *L. monocytogenes*, product or ingredient testing may be part of investigative testing or root cause analysis *(20)*. Similarly, environmental monitoring indicating the presence of *Salmonella* could suggest the need to test product for *Salmonella (19).*

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APPENDIX D - CATEGORY: NUTS (INCLUDING TREE NUTS AND PEANUTS) AND NUT/SEED PRODUCTS

Nuts are defined as "low-moisture, one-seeded fruit, usually enclosed by a rigid outer casing or shell" and are divided into tree nuts or ground nuts. Nuts are grown around the world and global sourcing is common *(21)*. Tree nuts include almonds, hazelnuts (filberts), pistachios, Brazil nuts, pecans, coconuts, macadamias, chestnuts, pine nuts and walnuts, while ground nuts generally refer to peanuts *(21)*. Processed products made from nuts and seeds include nut butters such as peanut butter and sunflower butter, nut "milks", and nut and seed "cheeses" and spreads.

The hazards associated with peanuts and tree nuts are determined by the environment in which they are grown, harvested, shelled/hulled, cleaned, sorted, processed, packaged, and stored.

Hazards associated with processed products made from nuts and seeds are determined by the hazards associated with ingredients, lethality process associated with manufacture and risk for exposure to the process environment post-lethality treatment.

Four categories of raw and processed nut commodities or processed products made from nuts are considered in this evaluation of the utility and necessity for industry to test ready-to-eat (RTE) foods for pathogens and whether any microbiological testing is appropriate to verify pathogen control.

1. Ready-to-eat (RTE) nuts **not** processed for lethality (e.g., chopped untreated tree nuts)
2. RTE nuts processed for lethality (e.g., roasted tree nuts, roasted peanuts)
3. RTE nut products processed for lethality (e.g., almond milk, coconut milk, nut (cashew) cheese)
4. RTE nut/seed butters **not** processed for lethality beyond initial nut processing (e.g., peanut butter, sunflower butter)

1. Ready-to-eat nuts not processed for lethality

Some tree nuts are covered by FDA's rule "Standards for Growing, Harvesting, Packing, and Holding of Produce for Human Consumption" (21 CFR Part 112), which sets food safety standards for farms

to follow in an effort to minimize the risks of microbiological contamination that may occur during the production of covered produce *(39)*. Tree nuts that are covered by the Produce Safety rule (PSR) include pistachios, macadamia nuts, pine nuts, and walnuts. Other raw tree nuts (hazelnuts, pecans, cashews) and peanuts are excluded from the rule as they are considered "rarely consumed raw" (RCR) (21CFR Part 112.2(a)(40)). While almonds are not exempt from the PSR, the FDA has stated their intent to not enforce the PSR requirements for raw almonds *(38)*. Raw almonds have been associated with salmonellosis outbreaks *(13, 19)*; however, USDA regulation requires almonds for North America to be treated to mitigate the hazard prior to sale.

If nuts are subjected to manufacturing/processing activities not permitted under the farm definition, such as roasting, blanching, chopping, dicing and grinding, these activities are regulated by the FDA's rule "Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" (the CGMP & PC rule; 21 CFR Part 117) unless an exemption applies *(33)*.

Nuts that are classified as rarely consumed raw are those that FDA determined are almost always eaten only after being cooked and are included in an exhaustive list at 21 CFR 112.2(a)*(35)* Hazelnuts, cashews, pecans, and peanuts are exempt from the produce safety regulation because heat treatment in some form can adequately reduce the presence pathogens. These types of nuts are not considered in Category 1 of this assessment.

Category 1 of RTE raw nuts considers only chopped tree nuts and shelled whole nuts not processed for lethality that are manufactured, processed, packed, or held in a facility covered by the CGMP & PC rule unless an exemption applies. Although not chopped, some whole shelled nuts not processed for lethality would fall under the CGMP and PC rule. As an example, RTE whole shelled walnuts not processed for lethality packaged in a facility conducting other manufacturing/processing activities (e.g., roasting or glazing of walnuts) would be covered by the CGMP & PC rule. Producers of RTE chopped raw tree nuts and some types of whole RTE nuts will implement preventive controls to significantly

minimize or prevent hazards to provide assurances that the RTE nuts manufactured, processed, packed, or held in their facility will not be adulterated under section 402 of the Federal Food, Drug, and Cosmetic Act. These preventive controls include sanitation controls and a supply-chain program. As part of the supply-chain program, suppliers (i.e., farms growing, harvesting, packing, and holding the nuts) may be annually audited related to their compliance with the produce safety regulation.

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

A hazard analysis with implementation of appropriate controls is required, considering (1) possible microbial hazards, (2) likelihood of occurrence, (3) available processing control procedures such as a kill step or other reduction methods/controls, (4) potential for inherent contamination or recontamination after processing from handling or the environment, (5) survival (persistence) or growth on the product, (6) intended consumer, (7) shelf life of the product, and (8) steps in the process where testing would be appropriate to verify food safety controls.

1. Are pathogens associated with the food or ingredients?

Pathogens are associated with raw nuts not processed for lethality, including *Salmonella*, Shiga toxin-producing *E. coli* and *Listeria monocytogenes (19, 41)*. Contamination of outer shells begins at harvest where nuts may be shaken to the ground. Direct contact with contaminated soil during harvest provides an opportunity for introduction of foodborne pathogens, e.g., to walnuts *(1)*. *Salmonella* Enteritidis PT 30, *E. coli* O157:H7, and *L. monocytogenes* are capable of long-term survival on the surface of in-shell walnuts *(1, 14)*. *Salmonella* can persist on in-shell pistachios in storage silos for up to four months *(17)*.

In 2010, walnuts were recalled (without illness) by a company after *Salmonella* was detected in walnut halves and pieces sold to another nut company *(41)*. *Salmonella* was detected in pistachio nuts and walnuts *(11, 14)*, *E. coli* O157:H7 was found in walnuts for sale at retail markets in the U.S.*(42)*.

Outbreaks have been associated with pistachio nuts contaminated with *Salmonella* in 2009, 2016 and 2018 (42). The 2016 outbreak was linked to the consumption of roasted pistachios produced by one company *(11, 34)*. However, the outbreak strains of *Salmonella* Montevideo and *Salmonella* Senftenberg were also isolated from samples of raw pistachios from the farm where the pistachios were grown. Walnuts were implicated in a 2011 outbreak of *E. coli* O157:H7 in Canada *(41)*.

2. Are the ingredients likely to be contaminated?

Yes. There is a risk for microbiological contamination during the growing, harvesting, packing, and holding of raw nuts not processed for lethality *(21)*.

Nuts that are harvested off the ground without mats are more likely to be contaminated with pathogens inherent to the soil in which they lay. Persistence through storage, packing, and holding continues into the retail market *(42)*.

3. Are there robust processing control procedures such as a kill step or other reduction methods/controls?

No. These nut products are raw RTE foods that are not processed for lethality. Macadamia nuts, walnuts, and pistachios do not commonly receive a microbial reduction treatment prior to sale either whole or chopped.

Control is based on the expectation that processers beyond the grower are compliant with Sanitation and Supply Chain Programs under the Preventive Controls Rule (21 CFR Part 117) and that growers that supply the raw unprocessed nuts are compliant with the Produce Safety Rule (21 CFR Part 112) and GAPs.

4. Is there a potential for inherent contamination or recontamination after processing from handling or the environment?

Yes. The initial microbial flora of harvested nuts will include pathogens from the equipment/personnel used in harvesting, transportation, and storage. Shelled or unshelled dried raw nuts are stored refrigerated (4^{o}C) or frozen (-18^{o}C). However, pathogens such as *Salmonella* are not eliminated during refrigeration, freezing, or drying. Tree nuts may be submerged in water to remove debris, soften the shell (e.g., pecans), or remove floating/damaged nuts, then de-shelled physically, a process that may be facilitated by water sprayers *(16)*. Contaminated water may also be a source of pathogens contaminating nuts. Tree nuts that are de-shelled dry can produce dust that can spread pathogens *(16)*.

5. Does the product support survival or growth?

All nuts are artificially, or sun and air dried after harvesting. Immediate drying upon harvest restricts outgrowth of mold and vegetative pathogens but not their persistence.

6. Is this product meant for higher risk population?

In most instances, the product is made for consumption by the general population.

7. What is the shelf life of the product?

Months to years. Tree nuts can be stored for days to months before processing, making moisture control a necessity to prevent bacterial/fungal outgrowth *(16)*.

8. Would consumer handling and use be likely to increase or decrease risk?

(a) Heating for palatability (b) Holding a frozen food under refrigeration (c) Holding a refrigerated food beyond the use-by date?

If a thermal process was applied by the consumer for palatability, then the inherent pathogen risk posed by vegetative pathogens might be mitigated to some extent depending on the process (time/temperature), but the heating might not fully eliminate the pathogen, depending on the number present.

Example 1: Chopped walnuts shelled, sized and packaged in a facility that also roasts nuts.

Example 2: Whole shelled macadamia nuts packaged in a facility that also dices, roasts, and seasons macadamias.

Table D-1. Ready-to-eat nuts <u>not</u> processed for lethality – Examples.

Criterion/Factor	**Example 1: Chopped walnuts shelled, sized and packaged in a facility that also roasts nuts.**	**Example 2: Whole shelled macadamia nuts packaged in a facility that also dices, roasts, and seasons macadamias.**
	Nuts (in hulls) are grown on a farm (orchards) and mechanically harvested by shaking the trees. The nuts are pushed into windrows and mechanically picked up from the orchard floor. The nuts are passed through a huller (wet	*Macadamias are grown on a farm with an adjacent husking operation. Nuts fall naturally to the orchard floor and are either mechanically or hand-collected then husked. Nuts are then delivered to the processing facility*

Criterion/Factor	Example 1: Chopped walnuts shelled, sized and packaged in a facility that also roasts nuts.	Example 2: Whole shelled macadamia nuts packaged in a facility that also dices, roasts, and seasons macadamias.
	scrubber)/dryer, washed and dried to 8% moisture in a gas dryer. The in-shell raw walnuts are then delivered to the downstream processor used in this example. At the processor, whole walnuts are sized, cracked to remove the outer shell, kernels are sized, shell and foreign material is mechanically blown from the kernels, pieces are sized into small pieces, hand sorted and packaged.	*where they are dried in gas dryers, shelled, and packaged.*
A. Are pathogens associated with the food or ingredients?	Yes, an inherent risk due to the raw nature of the ingredient. *Salmonella* and *E. coli* O157:H7 were found in walnuts sold in retail markets in the U.S. *(41)*. Long-term survival of *L. monocytogenes* on the surface of in-shell walnuts can occur *(1, 14)*.	Yes, an inherent risk due to the raw nature of the ingredient. *Salmonella* can persist on in-shell tree nuts for extended periods of storage *(20)*. *Salmonella* prevalence in macadamia nuts collected at retail was 4.20% *(42)*.

Criterion/Factor	Example 1: Chopped walnuts shelled, sized and packaged in a facility that also roasts nuts.	Example 2: Whole shelled macadamia nuts packaged in a facility that also dices, roasts, and seasons macadamias.
B. Are the ingredients likely to be contaminated?	There is an inherent risk for microbiological contamination during the growing, harvesting, and holding of raw walnuts. Pathogens from the orchard floor, equipment used in harvesting, transportation, and storage are likely.	There is an inherent risk for microbiological contamination during the growing, harvesting, and holding of raw macadamia nuts. Pathogens from the orchard floor, equipment used in harvesting, transportation, and storage are likely.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	No Walnuts do not require a microbial reduction treatment prior to sale either whole or chopped. The facility packaging raw walnuts should establish and implement a supply-chain program that requires its suppliers (i.e., growers) to comply with the Produce Safety Rule (21 CFR Part 112)*(39)* to significantly	No Macadamias do not require microbial reduction treatment prior to sale either whole or chopped. The facility packaging raw macadamia nuts should establish and implement a supply-chain program that requires its suppliers (i.e., growers) to comply with the

Criterion/Factor	Example 1: Chopped walnuts shelled, sized and packaged in a facility that also roasts nuts.	Example 2: Whole shelled macadamia nuts packaged in a facility that also dices, roasts, and seasons macadamias.
	minimize pathogens on the incoming product.	Produce Safety Rule (21 CFR Part 112)*(39)* to significantly minimize pathogens on the incoming product.
D. Is there a potential for recontamination from the handling or the environment?	Yes Raw walnuts in shell, with an inherent potential for pathogen contamination, are received into the facility. The process area and process equipment are likely to be contaminated from the primary ingredient. In addition, the facility itself becomes a secondary source of contamination. Inadequate sanitation could lead to harborage issues with the potential to contaminate product as it is	Yes Once harvested, the outer hull is removed mechanically within 24 - 48 h. The nuts are dried to a stable moisture level and separated by size. Raw macadamia nuts entering the facility have an inherent potential for pathogen contamination. The husking, drying and packaging areas and equipment have the potential to be contaminated if adequate controls are not in place. The

Criterion/Factor	Example 1: Chopped walnuts shelled, sized and packaged in a facility that also roasts nuts.	Example 2: Whole shelled macadamia nuts packaged in a facility that also dices, roasts, and seasons macadamias.
	processed and packaged. Accordingly, the facility should establish a sanitation program to significantly minimize or prevent biological hazards in the areas in which RTE walnuts are exposed to the environment before packaging. Due to the increased risk of cross-contamination with the chopping equipment, the facility should establish and implement a robust environmental monitoring program to verify its sanitation program in those areas.	facility itself can be a secondary source of contamination; inadequate sanitation could lead to harborage issues with the potential to contaminate product as it is packaged. Accordingly, the facility should establish a sanitation program to significantly minimize or prevent biological hazards in the areas in which RTE macadamias are exposed to the environment before packaging.
E. Does the product support survival or growth?	The nuts are dried after harvesting and hulling. Immediate drying upon harvest restricts outgrowth of mold and vegetative pathogens but not their persistence.	The nuts are dried after harvesting and husking. Immediate drying upon harvest restricts outgrowth of mold and vegetative pathogens but not their persistence.

Criterion/Factor	Example 1: Chopped walnuts shelled, sized and packaged in a facility that also roasts nuts.	Example 2: Whole shelled macadamia nuts packaged in a facility that also dices, roasts, and seasons macadamias.
F. Is this product meant for higher risk population?	The product is made for consumption by the general population.	The product is made for consumption by the general population.
G. What is the shelf life of the product?	3 months at 20°C, 1 year at 0°C to 4°C. *(5)*.	-10°C /24 months, 0°C - 10°C/12 months, 20°C/5 months *(5)*.
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	The risk for outgrowth may be increased if the product is not kept dry during storage and condensate is allowed to form or if added to a product with a water activity that allows outgrowth. Pathogen risk is reduced if cooked or baked.	Risk may be increased if added to a product with a water activity that allows outgrowth. Pathogen risk is reduced if cooked or baked.

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

None known.

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

No. Verification testing for *Salmonella* and *Listeria* is routinely performed at some level by industry. Note: A hold, test and release program could be appropriate for this category of product and might be considered by some to be a type of "preventive control," but it is more appropriately considered verification of all the control measures applied to that point.

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

Finished product should be tested. Selection of pathogen targets is based on prevalence studies and recall/outbreak information and would include *Salmonella* and *Listeria*. Testing treenuts for generic *E. coli* is also a measure of adulteration with filth (Table D-2). Note: Because these are untreated nuts that are RTE, a greater reliance on verification testing would be expected and verification testing would occur at a greater frequency in comparison to treated nuts.

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?*

1. Level of implementation and adherence to the Produce Safety Rules/GAPs or Preventive Controls Rule.
2. Efficacy of Sanitation programs - including studies to determine frequency of sanitation and length of runs.
3. Environmental control - conduct environmental "deep dives" to assess where and how often pathogens are found and detect harborage sites.
4. Control of water and dust.

Greater adherence to effective programs such as these can reduce the amount of verification testing needed.

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

The entire process line has an elevated risk for pathogen contamination in the absence of process preventive controls. While sanitation controls can reduce the hazard at specific sites where cleaning and sanitation activities are conducted, contamination inherent to the raw nuts can persist and can amplify. The entire process is represented by end product testing of product in a final package or product sampled while filling bulk containers. Finished product samples are, in effect, one large "swab" of the entire process and are the most appropriate type of samples to verify the adequacy of any controls that are applied in the absence of a process preventive control. Finished product testing for *Salmonella, L. monocytogenes* and generic *E. coli* is recommended. Additional points of verification will not negate the need for finished product testing, however, testing product for generic *E. coli* and coliforms or Enterobacteriaceae at start-up and from samples taken along the process in husking, drying, chopping

and packaging areas could identify harborage sites (buildup of biofilms, water ingress, growth points) where pathogens could proliferate. Their removal would reduce the overall level of process contamination.

***Question 7**: What impact does environmental monitoring have on frequency and extent of product testing verification activities by companies?*

Environmental monitoring for pathogens will verify the effectiveness of sanitation/hygiene programs that control build up and harborage sites. However, environmental monitoring will not diminish the need for lot-by-lot finished product testing. Minimally, monitoring for *Salmonella* and *Listeria* spp. as an indicator for *L. monocytogenes* on Zone 2 and 3 surfaces should be conducted *(28)*.

Microbiological limits for hygiene verification testing of cleaned/sanitized product contact surfaces in a raw nut processing facility should be established and tracked. Recommended indicator organisms include aerobic plate counts, coliforms, generic *E. coli* and/or Enterobacteriaceae.

***Question 8**: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?*

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. This requires the development of measurable attributes that indicate whether a process maintains or surpasses an acceptable degree of hazard control or falls below that level (21).

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 Rule (21 CFR Part 117) and that growers that supply the raw unprocessed nuts are compliant with the Produce Safety Rule (21 CFR Part 112) and GAPs. Finished product testing is conducted to verify that sanitation controls are in place and effective within the manufacturing facility. Product testing for *Salmonella, L. monocytogenes* and generic *E. coli* provides highly relevant verification data and is appropriate for the level of risk associated with the raw nuts. Loss of control would be indicated by 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 reject the lot of raw nuts represented by the sample, especially when the food will not receive further processing using a validated kill step (29). Contaminated nuts may be reconditioned with a kill step.

The repeated finding of an indicator organism such as generic *E. coli* above a threshold level can also indicate a loss of sanitation control, although actions taken would follow a tiered approach based on numbers and frequency of occurrence (see Table D-2).

The types of actions that companies may take in the case of loss of control indicated by finished product verification results depending on the seriousness of the risk include:

1. Verification of the sanitation program
2. Increased verification testing frequency
3. Stopping the processing line until a root analysis is completed
4. Investigation of the source of contamination

Table D-2. Microbiological Limits for Ready-to-eat nuts not processed for lethality (29).

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 *(36)*
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

2. Ready-to-eat nuts and seeds processed for lethality

Examples: roasted tree nuts, roasted peanuts (whole or chopped), almonds treated by propylene oxide (PPO), blanched almonds, salted and roasted inshell sunflower seeds, salted and roasted pumpkin seeds, pistachios treated by steam, roasted pecans, roasted cashews.

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

1. Are pathogens associated with the food or ingredients?

Yes. *Salmonella* is a pathogen that can survive for long periods of time in low-moisture food products or ingredients. This pathogen is widely recognized as the pertinent pathogen in low moisture food such as nut and seed commodities because of outbreaks associated with these foods. Processed nut and seed commodities have been associated with pathogens such as *Salmonella*, shiga toxin-producing *E. coli* and *Listeria monocytogenes*. Since 2017, dry roasted macadamia nuts, dry roasted pistachios, and roasted cashews have been recalled due to known or suspected *L. monocytogenes*, *Salmonella,* and *L. monocytogenes* contamination, respectively (42). A multistate outbreak of *Salmonella* Montevideo and *Salmonella* Senftenberg infections was linked to the consumption of roasted pistachios in 2016 *(34)*. To date, there are no reported outbreaks of listeriosis or enterohemorrhagic *E. coli* infections linked to processed nut and seed commodities.

2. Are the ingredients likely to be contaminated? *(21)*

Extensive surveys have been conducted to determine prevalence and levels of pathogens on raw nuts, such as inshell walnuts *(14)*, inshell pistachios *(17)*, shelled almonds *(27)*, inshell pecans *(2)*, and shelled peanuts *(4)*. Facilities that are implementing a process preventive control for biological hazards on their RTE nuts generally would not be relying on supply-chain preventive controls for biological hazards such as *Salmonella*, since they are processing the nuts to control the hazards. Facilities that are not processing their nuts with a process that significantly minimizes biological hazards may have established a supply-chain program to control for biological hazards such as *Salmonella*. An example of this type of

facility is one that purchases bulk packed nuts that have previously been subject to a kill step for packaging in RTE form.

3. Are there robust processing control procedures such as a kill step or other reduction methods/controls?

Yes. There are several forms of processing that nuts can be subjected to as processes to significantly minimize or prevent biological hazards. Such processes would likely be process controls and include oil roasting, dry roasting, toasting, propylene oxide treatment, steam treatment, and blanching.

4. Is there a potential for inherent contamination or recontamination after processing from handling or the environment?

Yes. RTE processed nuts may be exposed to the environment after processing with a kill step. In these instances, finished product testing should be considered, particularly in operations that may operate for extended periods of time (e.g., a week or more) between cleaning and sanitizing activities. Facilities subject to the requirements of subpart C of the CGMP and Preventive Controls for Human Food rule (21 CFR part 117) must include in their hazard analysis an evaluation of environmental pathogens whenever a ready-to-eat food is exposed to the environment prior to packaging and the packaged food does not receive a treatment or otherwise include a control measure that would significantly minimize the pathogen.

5. Does the product support survival or growth?

Pathogens will survive but growth is prevented by low water activities. Numerous studies have evaluated the ability of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* to persist on various tree nuts and peanuts for months at various storage temperatures *(20)*. *Salmonella* is widely recognized as a pathogen that will persist in low moisture foods for extended periods of time *(30)*.

6. Is this product meant for higher risk population?

In most instances, the product is made for consumption by the general population.

7. What is the shelf life of the product?

Months to years

8. Would consumer handling and use be likely to increase or decrease risk?

a. Heating for palatability

b. Holding a frozen food under refrigeration

c. Holding a refrigerated food beyond the use-by date

If a thermal process was applied by the consumer for palatability, then the inherent pathogen risk relative to vegetative pathogens present due to contamination after processing might be mitigated to some extent depending on the process (time/temperature), but the heating might not fully eliminate the pathogen, depending on the number present.

Example 1: Roasted and salted inshell pistachios

Example 2: PPO-treated almond kernels

Criteria a facility can apply to determine whether and how often to test ready-to-eat nuts processed for lethality:

299 **Table D-3. Ready-to-eat nuts processed for lethality - Examples.**

Criterion/Factor	**Example 1: Roasted and salted inshell pistachios** Ingredients: pistachios, sea salt	**Example 2: PPO-treated almond kernels** Ingredients: almonds
Process Information	Raw pistachios are purchased directly from growers (suppliers) that may or may not be adhering to the produce safety regulation (21 CFR part 112). The roasting step is a process preventive control to significantly minimize *Salmonella.* Sanitation controls are implemented for the environment because the pistachios are exposed to the environment after roasting.	Packed almonds are purchased from a supplier that treats the almonds with propylene oxide, a process control to significantly minimize *Salmonella*. The supplier does not expose the almonds to the environment after treatment. The receiving facility packages the almonds; because this facility relies upon its supplier to significantly minimize *Salmonella* in the almonds, they have established and implemented a supply-chain program. As the almonds are exposed to the environment after receiving and prior to packaging, they have established and implemented sanitation controls for the environment.

Criterion/Factor	**Example 1: Roasted and salted inshell pistachios** Ingredients: pistachios, sea salt	**Example 2: PPO-treated almond kernels** Ingredients: almonds
A. Are pathogens associated with the food or ingredients?	Yes – roasted, inshell pistachios have been linked to an outbreak of salmonellosis *(34)*.	Yes – raw almonds have been linked to outbreaks of salmonellosis *(18, 23)*.
B. Are the ingredients likely to be contaminated?	Yes – a 2010-2012 survey of raw, inshell pistachios from storage silos found a *Salmonella* prevalence of 0.81% (32 positive of 3,968 samples) *(17)*	Yes – surveys of raw almond kernels at processor receiving found a *Salmonella* prevalence of 0.98% (146 positive of 14,949 samples) *(27)*.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes – roasting is expected to be a kill step that would be established as a process preventive control to significantly minimize pathogens such as *Salmonella*.	Yes – PPO treatment is expected to be a kill step that would be established as a process preventive control to significantly minimize pathogens such as *Salmonella*.
D. Is there a potential for recontamination from the handling or the environment?	Yes – roasted and salted pistachios are exposed to the production environment after roasting and prior to packaging.	Yes, PPO treated almonds are exposed to the production environment after treatment and prior to packaging.
E. Does the product support survival or growth?	*Salmonella* will survive for extended periods of time in low moisture foods, including pistachios *(26)*.	*Salmonella* will survive for extended periods of time in low moisture foods, including almonds *(26)*.

Criterion/Factor	Example 1: Roasted and salted inshell pistachios Ingredients: pistachios, sea salt	Example 2: PPO-treated almond kernels Ingredients: almonds
F. Is this product meant for higher risk population?	In most instances the product is being made for the general population	In most instances the product is being made for the general population
G. What is the shelf life of the product?	Months to years	Months to years
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	Risk may be increased due to consumer handling of the product as they remove shells, transferring contaminants (if present) from the shell to the nut kernel prior to consumption. Pathogen risk is reduced if cooked or baked.	Pathogen risk is reduced if cooked or baked.

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

No.

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

Generally, finished product verification testing should be performed for most products in this category, even if there is evidence that the appropriate treatment was applied. Quantitative assessments of the risk of human salmonellosis from the consumption of almonds *(32)*, pecans *(32)*, pistachios *(15)*, walnuts *(31)* (34), and peanuts *(6)* estimate the number of salmonellosis cases per year from the consumption of these nuts in the in the United States after various treatments (log reductions). These risk assessments include *Salmonella* dose-response models and U.S. consumption data. These risk assessments are appropriate sources of information facilities can use to establish target log reductions as part of the validation of their process controls, as required by subpart C of part 117; however, some atypical situations can occur that may result in pathogen prevalence and levels different from those in the dose-response models. Because of this, finished product testing as a verification of the effectiveness of a food safety plan and the facility's capability to consistently deliver against it should be conducted.

In addition to the finished product testing, a robust environmental monitoring program as a verification activity for sanitation preventive controls should be established, particularly if the treated nuts are exposed to these environments subsequent to their treatment. If there is a robust environmental monitoring program, verification testing of finished product could be less frequent. In-process indicator testing for hygiene monitoring, sanitation verification and evaluation of buildup on lines is necessary.

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

For processes that are not enclosed and for products that include post-lethality handling, finished product verification should be part of the preventive controls program. Selection of target organisms should be based on prevalence studies (cited above) and recall/outbreak information e.g., *Salmonella* and

L. monocytogenes (cited above). Quantitative indicator assays (Enterobacteriaceae, and/or coliforms) upon startup and in-process for process control (buildup of biofilms, water ingress, growth points) and sanitation verification may be used.

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?*

Base frequency of finished product testing on data from continuous programs that assess:

1. Process control - develop an indicator/pathogen baseline to demonstrate process control for line/product. Microbiological testing should be performed with GMPs and processes in control to determine what is achievable and the variability that is normal.
2. Environmental control - conduct extensive environmental monitoring to assess where and how often pathogens are found and detect harborage sites.
3. Hazard assessment of ingredients, e.g., epidemiological information and prevalence of target pathogens in ingredients.
4. Efficacy of sanitation programs, including studies conducted to determine frequency of sanitation and length of runs between cleaning and sanitizing activities.
5. Level of hygiene segregation.
6. Ability to determine in control/out of control quickly and see change when it occurs.

Finished product testing may not be warranted (or may be limited) for validated processes verified to be under control; however, it is incumbent upon the manufacturer of treated nuts to determine if finished product testing for pathogens or indicator organisms would provide information useful to assess process control in their facility. Factors to consider when deciding to conduct finished product testing include who is doing the treatment, how rigorous is the treatment, process validation information, confidence in the entity doing the treatment, historical information from the supplier, how much exposure to the

environment and other factors. It is not uncommon for treated nut manufacturers to conduct finished product testing and supply a certificate of analysis with the products they ship.

When finished product is screened for pathogens and/or coliforms or Enterobacteriaceae, testing a representative sample from a complete process run provides the most information about process control. A sampling plan and evaluation criteria should be developed based on the type of processed nut, the history of foodborne illness outbreaks associated with the processed nut, the storage and distribution chain, and the risk for cross contamination from the process environment. The sampling plan should clearly define what is considered the "lot". FDA guidance provides an example of a sampling plan (sample size and number) when testing for *Salmonella* in foods that would not normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption *(37)*. The sampling parameters are 30 analytical units/ 25-gram samples. The samples may be aggregated into 375-gram analytical units. ICMSF provides additional information about finished product sampling plans commensurate with risk *(22)*.

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

No, for processes that are not enclosed, finished product testing is recommended, although additional points of verification testing are also important including:

- Environmental monitoring –particularly in environments to which nuts are exposed after processing to reduce pathogens.
- Inbound ingredient testing – depends on processed state of ingredients. If a manufacturer is relying on a supplier to control the hazard (e.g., a supplier of PPO-treats almonds to be packaged by the manufacturer), the incoming nuts may be subjected to testing to verify the supplier's controls. If a

manufacturer is implementing a process control to significantly minimize the hazard (e.g., a manufacturer that roasts pistachios), the incoming nuts may not be subjected to testing.

- Sanitation/hygiene verification testing.
- Additional points of verification will not reduce the need for some level of finished product testing.
- In addition, we would expect that for those products subjected to a thermal process, there would be a record of the process meeting critical limits, as well as the appropriate process validation.

***Question 7**: What impact does environmental monitoring have on frequency and extent of product testing verification activities by companies?*

A robust environmental monitoring program (EMP) should be present or deployed targeting the post-lethality areas. Application of an EMP, however does not replace an active finished product verification testing program.

***Question 8**: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?*

Process control is based on delivery of a pathogen reduction treatment, either at the facility itself or by another entity. Control of the overall process is also based in part on effective sanitation as evidenced by environmental monitoring and finished product testing. Additionally, sanitation preventive controls and, in some cases, supply-chain preventive controls, and verification activities for these will contribute to the safety of the finished product. Note: Testing is predicated on established baselines for

the process and environment. This will require more frequent / intensive testing early on to establish a base line to demonstrate process control for this product / line.

The finding of a pathogen in finished product is an indication of loss of process control. In addition, finding levels of indicator organisms above an established limit could indicate loss of control (or a trend toward loss of control). The types of actions that companies may take in the case of loss of control indicated by finished product verification results depend on the seriousness of the risk include the following:

- Verification of process delivery (where a lethality process is delivered at the facility)
- Verification of process delivery by a supplier (where the supplier applies the process)
- Verification of the sanitation program
- Increase verification testing frequency
- Stop processing line until a root analysis is completed (unless running the process line is needed to help identify root cause)
- Investigate the source of contamination under reduced production

Table D-4. Microbiological limits for RTE nuts processed for lethality.

Target Microorganism	Microbiological Limit	Recommended Action if Limit is Exceeded	Comments
Salmonella	Negative in two 375 g samples	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Two 375 g analytical units derived from 30 25 g samples

Listeria monocytogenes	Negative in 25 g	Reject lot, conduct root cause analysis and implement corrective action	Investigative testing as response to EMP that suggests likely contamination of product
Coliforms or Enterobacteriaceae	≤100 CFU/g	Investigate, implement corrective action	Root cause analysis is recommended

Adapted from *(29)*

3. Ready-to-eat nut and seed products processed for lethality.

The largest group in this category are the nut beverages (almond "milk", macadamia "milk," coconut "milk," cashew "milk", etc.). All of these products are commercially sold as pasteurized. The second major group includes multiple nut and seed spreads, cheese-like and yogurt-like products.

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

1. Are pathogens associated with the food or ingredients?

Given the diversity of products in this group and the multiple ingredients that they may contain, often added post-processing (such as in the case of spreads and yoghurts), it is quite likely that pathogens may be associated. There is one documented outbreak case of cashew cheese, but it had not been subjected to a lethality step (CDC, 2014). To this date there has not been foodborne illness caused by pathogenic organisms associated with nut beverages.

2. Are the ingredients likely to be contaminated?

The ingredients may be contaminated but once the liquid "milk" mixes are pasteurized and handled aseptically, all vegetative pathogens are eliminated. Similarly, other nut and seed products may be free from pathogen contamination after pasteurization of the nut beverage used for fermentation, but depending on post-processing handling, they may be subject to recontamination.

3. Are there robust processing control procedures such as a kill step or other reduction methods/controls?

Yes. Formulated nut products are derived from processed nuts and incorporate a pasteurization step such as HTST and UHT for nut beverages or other products. Nut "milks" are often subjected to aseptic packaging, so most contamination is controlled. HTST pasteurized nut "milks" still require refrigeration for preservation. The liquid components of other nut products may be pasteurized before fermentation and cheese making steps, but because of the post-lethality processing steps that expose products to the environment, refrigeration is critical to inhibit microbial growth.

4. Is there a potential for inherent contamination or recontamination after processing from handling or the environment?

The recontamination of nut "milks" is relatively unlikely given the use of HTST and UHT technologies with aseptic packaging. In the case of other nut and seed products, there is the possibility that they may be contaminated during the post-lethality steps with environmental contaminants such as *Listeria monocytogenes, Salmonella* and *Staphylococcus aureus*.

5. Does the product support survival or growth?

Nut "milks" processed with HTST are not commercially sterile and spoilage will occur in unopened packages. UHT nut milks are considered shelf-stable, and it is very unlikely that un-opened packages will experience spoilage. If the product is recontaminated after opening, nut "milks" can support the growth

of a wide variety of microorganisms. Microbial growth and survival in other nut products are highly likely, depending on pH and refrigeration temperatures.

6. Is this product meant for higher risk population?

In most instances all the products in this category are intended for the general population.

7. What is the shelf life of the product?

UHT-treated nut "milks" have 8 to 10 months of shelf life in unopened packages. Refrigerated HTST nut "milks" have shelf lives between 2 to 3 months in unopened packages. Most commercial brands recommend no more than 10 days for consumption after opening the product.

The shelf life of other nut products is variable, depending on the intrinsic characteristics of the food product and ingredients. Fermented products with reduced water activity may have longer shelf lives. Additional ingredients such as nuts or spices may reduce the shelf life due to possible recontamination.

8. Would consumer handling and use be likely to increase or decrease risk?

Consumer handling will likely increase risk of any of the products in this category. Once nut beverages are opened, their shelf life decreases because of the potential for recontamination and the high susceptibility for microbial growth. Any of the products in this category may be subject to consumption beyond their expiration date. Cheese and yogurt-like products may be subjected to temperature abuse, which may increase their risk.

Example 1: Almond "milk"

Example 2: Cashew Cheddar cheese

Table D-5. Ready-to-eat nut products processed for lethality – Examples.

Criterion/Factor	Example 1: Almond "milk"	Example 2: Cashew Cheddar cheese
Ingredients	**Ingredients:** water, almonds, natural sweetener (sugar, cane syrup), salt, flavors (vanilla extract, other flavors), gelling agents (carrageenan, guar gum, gellan gum, xanthan gum, locust bean gum, starch), calcium salts (calcium carbonate, tri-calcium phosphate), sodium citrate, lecithin, vitamins (A, D2, E).	**Ingredients:** cashew base (water, cashews), coconut oil, modified food starch (modified potato starch, modified cornstarch), potato starch, salt, natural flavors, dried yeast, vitamins (B1, B3, B6, B12), folic acid, annatto extract (for color), lactic acid, yeast extract, cultures.
Process Information	Almonds are grown in orchards, mechanically harvested by shaking the trees and allowed to dry on the floor for a few days. The nuts are harvested mechanically from the orchard floor. Nuts are cleaned, sorted and fumigated in windrows. Almonds are then graded, shelled and separated from shells. Shelled	Cashew nuts are grown in evergreen tropical trees and almost all of them are imported. Cashews are harvested by manually separating them from the fruit. Inshell cashews are dried under sunlight for several days. Dry cashews are then sorted by size and treated with steam to loosen the shell. Shells are separated from nuts

Criterion/Factor	Example 1: Almond "milk"	Example 2: Cashew Cheddar cheese
	nuts are blanched, peeled and roasted. Roasted almonds are rinsed and soaked. Wet almonds are ground with water to obtain the almond "milk" paste. After this step, the "milk" paste is mixed with water and other ingredients, pasteurized using HTST or UHT processes and packaged aseptically.	manually and re-dried in ovens at 100 °C for 1 hour. Nuts are manually peeled, cleaned and sorted. Cashews are rinsed and soaked. Wet cashews are ground with water to obtain a cashew base. The cashew base is mixed with several ingredients and pasteurized. Starter cultures and gelling ingredients are added, and portions are molded. Cheese pieces are allowed to dry and mature.
A. Are pathogens associated with the food or ingredients?	Yes – raw almonds have been linked to outbreaks caused by *Salmonella* *(7, 23)*	Yes, a cashew cheese was linked to a salmonellosis outbreak in 2014 *(10)*. *Salmonella* has been detected in commercial cashew nuts *(42)*.
B. Are the ingredients likely to be contaminated?	Yes, pathogen contamination is considered reasonably likely for raw dry ingredients, in particular almonds. *Salmonella* prevalence has been reported to be 0.98% in raw almonds *(27)*.	Yes, pathogen contamination is considered reasonably likely for raw dry ingredients, in particular cashews.

Criterion/Factor	Example 1: Almond "milk"	Example 2: Cashew Cheddar cheese
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes, almond "milk" is subjected to very effective thermal processes that eliminate pathogenic microorganisms. UHT and HTST processing are typically subjected to strict process controls.	Cashew liquid base may be subjected to thermal treatment in order to fully cook starches that will serve as a kill step for controlling pathogens in the raw materials. In addition, the cheese mix is acidified and fermented with starter lactic acid cultures.
D. Is there a potential for recontamination from the handling or the environment?	No, recontamination potential is extremely low because of immediate post-lethality packaging under aseptic conditions.	Yes, handling of the product post-treatment may pose some risk of environmental recontamination
E. Does the product support survival or growth?	Almond "milk" processes deliver commercially sterile products (UHT) or with extremely low counts (HTST). HTST "milks" may support growth, especially if they are not refrigerated.	If the pH is maintained below 4.6, it will inhibit most pathogen growth, but spoilage by yeasts and molds can still occur.
F. Is this product meant for higher risk population?	In most instances the product is being made for the general population.	The product is intended for the general population.

Criterion/Factor	Example 1: Almond "milk"	Example 2: Cashew Cheddar cheese
G. What is the shelf life of the product?	UHT almond "milk" – 8 to 10 months. Refrigerated HTST almond "milk" - 2 to 3 months.	Depending on the handling, it could be up to 6 months.
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	Because the product is typically free from pathogens, it is unlikely that consumer handling would lead to pathogen recontamination. Temperature abuse may lead to growth of spoilage organisms previously present (HTST) or re-introduced with each serving.	Consumer handling will increase the likelihood of recontamination, survival, and growth of pathogenic microorganisms

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

There is no existing methodology that replaces pathogen and indicator microorganism testing.

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

Yes - if an enclosed pasteurization and filling process is used with the appropriate validation, verification testing may not be necessary. Batch processes are also used that would not be enclosed. In this case, verification testing would be needed. If there is a kill step, it would be designated a Process Preventive Control and a scientific validation study would be required.

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

For processes that are not enclosed and for products that include post-lethality handling, finished product verification testing should be part of the preventive controls program. The selection of target organisms should be based on prevalence studies and recall/outbreak information e.g., *Salmonella* and *L. monocytogenes.* Quantitative indicator assays (Enterobacteriaceae, and/or coliforms) upon startup and in-process for process control (buildup of biofilms, water ingress, growth points) and sanitation verification may be used.

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

For products that use a non-enclosed system and for which production involves post-lethality handling, the following criteria are recommended:

1. Base frequency of finished product testing on data from continuous programs that assess:
    - Process control - develop an indicator/pathogen baseline to demonstrate process control for line/product.
    - Environmental control - conduct extensive environmental monitoring to assess where and how often pathogens are found and detect harborage sites.
2. Hazard assessment of ingredients - epidemiological and historic prevalence of target pathogens in ingredients.

3. Efficacy of sanitation programs determined by including studies to determine frequency of sanitation and length of runs.

4. Level of hygiene segregation.

5. Ability to determine in control/out of control quickly and see change when it occurs.

For beverage products manufactured under enclosed systems, criteria 2, 3, 4 and 5 should be considered.

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

No, for processes that are not enclosed, finished product testing is recommended, although additional points of verification testing are also important including:

- Environmental monitoring
- Inbound raw material testing – depends on processed state of ingredients and COA data. Lot by lot testing may be needed if the supplier is deficient in pathogen mitigation interventions and hazards are not controlled by a process.
- Sanitation/hygiene verification testing.

Additional points of verification will not reduce the need for some level of finished product testing. In addition, we would expect that for those products subjected to a thermal process, there would be a record of the process meeting critical limits as well as the appropriate process validation.

For enclosed processes, using a validated microbial reduction process, periodic end-product testing could be useful for verification of process control, but monitoring of process delivery should minimize the need for finished product testing. Environmental monitoring would not be needed if product is not exposed to the environment.

***Question 7**: What impact does environmental monitoring have on frequency and extent of product testing verification activities by companies?*

For processes that are not enclosed, such as those used for other nut and seed products, a robust environmental monitoring program should be present or deployed targeting the post-lethality areas. Application of EMP, however does not replace active finished product verification.

For enclosed processes, such as those used for nut beverages, environmental monitoring would not be needed if product is not exposed to the environment.

***Question 8**: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?*

To determine a loss of control, companies ideally should have in place several food safety plan components that include internal microbiological criteria, data recording, data analysis, and process control studies or charts. The process control charts should incorporate the previously set microbiological criteria and baseline data that would involve operating limits (upper and lower limits) that may result in actions in response to results. Depending on the type of microbial data (quantitative or qualitative) and criteria, control charts determine the times where the microbial testing results indicate that the results are beyond the operating limits. Based on the hazard analysis part of the food safety plan, the level of risk of the off-limits results will determine the tolerance level and the extent of out of compliance that would lead to a determination of a loss of process control. The control charts will detect consistent out of operating limits or trends within limits that would indicate loss of control. For example, for pathogen analysis results, a single positive result may trigger a corrective action that mandates the complete

stoppage of the process. Indicator microorganism analyses may have a tolerance level for the number of out of operating limit results before a corrective action is taken.

The types of actions that companies may take in the case of loss of control indicated by finished product verification results, depending on the seriousness of the risk, involve:

1. Verification that process delivery was adequate
2. Verification of sanitation program
3. Increase verification testing frequency
4. Stop processing line until a root analysis is completed
5. Investigate the source of contamination under reduced production

Table D-6. Microbiological limits for nut and seed milks, cheeses and yogurts – RTE.

Target Microorganism	**Microbiological Limit**	**Recommended Action if Limit is Exceeded**	**Comment**
Coliforms or Enterobacteriaceae	≤ 10 CFU/g (milks) ≤100 CFU/g	Investigate, implement corrective action	Root cause analysis is recommended
Listeria monocytogenes	Negative in 25 g	Reject lot, conduct root cause analysis and implement corrective action	Investigative testing as response to EMP that suggests likely contamination of product

Salmonella	Negative in 375 g	Reject lot, conduct root cause analysis and implement corrective action	Two 375-g analytical units derived from 30 x 25-g samples

4. Ready-to-eat nut/seed butters <u>not processed for lethality beyond initial nut/seed processing</u>

This group of RTE products includes a variety of butters in which the main ingredient is a nut type and the level of moisture is relatively low. Commercially available butters are made of almonds, cashews, hazelnut, peanuts, pistachios, sesame seeds, sunflower seeds, walnuts, etc. In most cases, the whole nuts have been subjected to a thermal treatment such as roasting, which should have been validated. After roasting, butters are produced by grinding the nuts to obtain a paste that can be spread. Depending on the extent of grinding, their texture can be smoother. Other ingredients such as sugar, vegetable oils, and salt are often added.

***<u>Question 1</u>**. 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?*

1. Are pathogens associated with the food or ingredients?

Yes, nut and seed butters have been linked to outbreaks from *Salmonella* and *E. coli* O157:H7 *(8, 9, 12)*.

2. Are the ingredients likely to be contaminated?

Yes, raw nuts and seeds are likely to be contaminated; there are documented outbreaks and recalls linked to multiple nut types that may lead to contaminated butters.

3. Are there robust processing control procedures such as a kill step or other reduction methods/controls?

Yes, some manufacturers may apply several forms of processing to significantly minimize or prevent biological hazards in nuts and seeds before they are ground. Such processes would likely be process controls and include oil roasting, dry roasting, toasting, propylene oxide treatment, steam treatment, and blanching.

4. Is there a potential for inherent contamination or recontamination after processing from handling or the environment?

Yes, nut and seed butters may be inherently contaminated if the raw nuts or seeds had not been subjected to one of the processes described above and they may be susceptible to recontamination during mixing, grinding and packaging.

5. Does the product support survival or growth?

Salmonella, *E. coli* O157 and *Listeria monocytogenes* are capable of surviving in nut and seed butters for very long periods of time during storage, but since most nut/seed butters have relatively low water activity their growth is markedly inhibited*(3, 24, 25)*. Typically, none of the nut butters require refrigeration for storage because of their low water activity, although manufacturers may recommend refrigeration after opening to retard rancidity.

6. Is this product meant for higher risk population?

The product is intended for the general population.

7. What is the shelf life of the product?

Most nut and seed butters will have shelf-life periods 6 to 12 months. Their shelf life will be limited by how susceptible their fat is to rancidity; microbial growth is not a factor for shelf-life determination.

8. Would consumer handling and use be likely to increase or decrease risk?

The risk of shelf-stable nut and seed butters may not be affected by consumer handling because of their low water activity. Temperature abuse will not lead to microbial growth. Pathogen levels should slowly decline during ambient temperature storage.

Table D-7. Ready-to-eat nut/seed butters not processed for lethality beyond initial nut/seed processing – Examples.

Criterion/Factor	Example 1: Peanut butter	Example 2: Tahini
Ingredients	Ingredients: dry roasted peanuts, sugar, vegetable oil (hydrogenated or palm oil), salt, molasses	Ingredients: sesame seeds
Process Information	Peanuts are grown from a legume plant as row crops. Peanuts are mechanically harvested from the soil, allowed to dry in the field for 2 to 3 days, separated from the vine while being picked from the floor. Inshell peanuts will be heat dried to reach 8-10% moisture. Peanuts will be graded, shelled, and sorted. Peanuts are then roasted at more than 150°C, cooled, and dry blanched to remove the skins. The	Sesame seeds are grown in plants of the *Pedaliaceae* family. Mature sesame plants are harvested, bundled, and allowed to dry on soil. Seeds are collected and separated from the sickles when moisture level is less than 8%. Seeds are washed, dried by different methods, and roasted. Roasted seeds are cooled then milled to form a paste. Sesame paste is degassed and packaged.

Criterion/Factor	Example 1: Peanut butter	Example 2: Tahini
	nuts are ground, mixed with other ingredients, and packaged.	
A. Are pathogens associated with the food or ingredients?	Yes, there have been several salmonellosis outbreaks linked to peanut butter (CDC, 2007, 2009).	Yes, there have been several outbreaks caused by *Salmonella* linked to tahini (13, 35).
B. Are the ingredients likely to be contaminated?	Several surveys of raw shelled peanuts have reported *Salmonella* prevalence from 0.14 to 1.6% *(4, 28)*. If the blanching or roasting process is not validated to provide adequate microbial reduction, peanuts used in processing could be contaminated.	The prevalence of *Salmonella* in imported sesame seeds was determined to be almost 10% by the FDA *(40)*. If non-roasted seeds are used or if the roasting process is not validated to provide adequate microbial reduction, sesame seeds are likely to be contaminated with *Salmonella*.
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	A validated blanching, roasting, or other kill step may be used.	A validated roasting or other kill step may be used.

Criterion/Factor	Example 1: Peanut butter	Example 2: Tahini
D. Is there a potential for recontamination from the handling or the environment?	Yes, during grinding and packing the product can be recontaminated from equipment.	Yes, during grinding and packing the product can be recontaminated from equipment.
E. Does the product support survival or growth?	Its low water activity inhibits most microorganisms' growth, but pathogens such as *Salmonella* may survive for several months.	Tahini's low water activity inhibits all microbial growth, but *Salmonella* is capable of surviving for a long time.
F. Is this product meant for higher risk population?	While this product is intended for the general population, children are probably the largest segment that consumes peanut butter.	This product is intended for the general population.
G. What is the shelf life of the product?	Most commercial brands have 1-year shelf-life expectations	The shelf life of commercial products ranges from 1 to 2 years.
H. Will consumer handling and use increase or decrease likelihood of pathogen survival, growth, or toxin production and risk of consumer illness?	No, because of the low water activity, consumer handling would not influence survival or growth.	No, because of the low water activity, consumer handling would not influence survival or growth.

626

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

629 There are no existing assays/testing that replaces pathogen and indicator microorganism
630 testing.

631

632 ***Question 3***. *Are there situations where [microbial] verification testing would not be necessary if there is*
633 *evidence that the appropriate treatment was, in fact, applied?*

634 No. Finished product testing in addition to a robust environmental monitoring program (primarily
635 Zones 2 and 3) is recommended. In-process indicator testing for hygiene monitoring, sanitation
636 verification, and evaluation of buildup on lines is necessary.

637

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

642 Finished product should be screened. Microbiological limits should be set for target
643 microorganisms selected based on prevalence studies and recall/outbreak information e.g., *Salmonella*
644 and *L. monocytogenes*. Quantitative indicator assays (Enterobacteriaceae and/or coliforms) should be
645 conducted upon startup and in-process for process control (buildup of biofilms, water ingress, growth
646 points) and sanitation verification.

647

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

1. Base frequency of finished product testing on data from continuous programs that assess:
    - Process control - develop an indicator/pathogen baseline to demonstrate process control for line/product.
    - Environmental control – conduct extensive environmental testing to assess where and how often pathogens are found and detect harborage sites.
2. Hazard assessment of ingredients: Prevalence of target pathogens in ingredients.
3. Efficacy of sanitation programs - including studies to determine frequency of sanitation and length of runs.
4. Level of hygiene segregation.
5. Ability to determine in control/out of control quickly and see change when it occurs.

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

No. Finished product testing is needed, although additional points of verification testing are also important including:

- Environmental monitoring
- Inbound raw material testing for processed nuts sourced externally if a risk-based supplier program is not in place. A good supplier program involves regular audits to confirm the presence of a validated process, sanitation controls and a robust environmental monitoring program. Raw nuts sourced for internal roasting would not be tested, since the control is being applied in-house; hygiene segregation controls should be in place. Sanitation/hygiene verification testing should be conducted.

Additional points of verification will not negate need for some level of finished product testing.

***Question 7**: What impact does environmental monitoring have on frequency and extent of product testing verification activities by companies?*

Environmental monitoring should be an integral component of the testing program of companies manufacturing nut and seed butters. The deployment of environmental monitoring complements the application of finished product verification testing and along with implementation of effective process controls can reduce the amount of finished product testing needed.

***Question 8**: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?*

To determine a loss of control, companies ideally should have in place several food safety plan components that include internal microbiological criteria, data recording, data analysis and process control studies or charts. The process control charts should incorporate the previously set microbiological criteria and baseline data that would involve operating limits (upper and lower limits) that may result in actions in response to results. Depending on the type of microbial data (quantitative or qualitative) and criteria, control charts determine the times where the microbial testing results indicate that the results are beyond the operating limits. Based on the hazard analysis of the food safety plan, the level of risk of the off-limits results will determine the tolerance level and the extent of out of compliance that would lead to determine a loss of process control. The control charts will detect consistent out of operating limits or trends within limits that would indicate loss of control. For example, for pathogen analysis results, a single positive result may trigger a corrective action that mandates the complete stoppage of the process.

For indicator microorganisms, analyses may have a tolerance level of the number of out of operating limits results before a corrective action is taken.

The types of actions that companies may take in the case of loss of control indicated by finished product verification results depending on the seriousness of the risk involve:

1. Verification that process delivery was adequate
2. Verification of sanitation program
3. Increase verification testing frequency
4. Stop processing line until a root analysis is completed
5. Investigate the source of contamination under reduced production

Table D-8. Microbiological limits for nut and seed butters – RTE

Target Microorganism	**Microbiological Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Coliforms or Enterobacteriaceae	≤100 CFU/g	Investigate, implement corrective action	Root cause analysis is recommended
Listeria monocytogenes (product)	Negative in 25 g	Reject lot, conduct root cause analysis and implement corrective action	Investigative testing as response to EMP that suggests likely contamination of product
Salmonella (product)	Negative in two 375 g samples	Reject. Investigate and implement corrective action	Two 375g analytical units derived from 30 25 g samples

Adapted from *(29)*

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APPENDIX E - CATEGORY: FRUITS AND VEGETABLES

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

This category includes any fresh fruit or vegetable or combination that has been physically altered from its whole state after being harvested from the field (e.g., by chopping, dicing, peeling, ricing, shredding, slicing, spiralizing, or tearing) without additional processing (such as blanching or cooking). Fresh-cut produce may or may not undergo a wash or other treatment before being packaged for use by the consumer or a retail food establishment. Fresh-cut produce can be a single commodity or two or more mixed in the same package, such as garden salad kits, coleslaw, or fruit salads, and sometimes called "ready to use," "precut," or "value added" produce. Fresh-cut produce also does not include produce that has been processed by freezing, cooking, canning, or packing in a juice, syrup, or dressing. For the purposes of this document, only RTE fresh-cut fruits and vegetables to be consumed as such were considered; it does not apply to fresh-cut produce that require cooking (such as cut butternut squash).

Leafy greens have been most frequently associated with outbreaks of shiga-toxin producing *E. coli (7)*. The risk profile of leafy greens can be differentially linked to four categories 1) type of leafy greens; 2) growing locations; 3) harvesting practices and 4) processing practices. However, the pathogens associated with outbreaks (i.e., STEC) are widely acknowledged to have origins in very low levels of sporadic and diffuse contamination within the growing environment and during harvest. Investigative studies have yielded positive samples, primarily from water and sediments, in the implicated growing locations. These positive environmental samples have been recovered from water sources, including both irrigation canals and on-farm reservoirs generally near animal feeding operations or pasture-managed

animals. However, these may be more correlations of the environment than causative sources of the outbreak; significant knowledge gaps exist. Preventing contamination of leafy greens in the growing environment and subsequently prevention of its amplification during harvesting and processing is the key focus. In practical terms, testing can be applied in the growing environment as a monitoring tool for gross contamination, but direct pathogen testing of harvested commodity lettuces or bagged fresh cut leafy greens is not generally viewed as useful because of the extremely low level of contamination and the time it takes for to receive results (frequently minimum of 2-3 days on a 17-day shelf-life). Growers should consult the guidelines for production and harvest of leafy greens *(12)*; postharvest processing should rely on tight hygienic controls of the wash water, especially recirculated water, to lower contaminant residuals on product and prevent cross-contamination *(3)*.

Note: It has been well established that most microorganisms grow best at pH values around 7.0 (6.0–7.5), whereas few grow below 4.0 *(4)*. When reading this document please keep in mind that many fruits (e.g., citrus fruits) fall below the point at which bacteria normally grow because of their low pH (pH <4), whereas others (e.g., melons, bananas, and papaya) can support growth *(6)*. In contrast, most vegetables have pH values near neutrality (or slightly lower e.g., cucumber, carrots), therefore they are more supportive of bacterial growth.

1. RTE fresh-cut fruits

Examples include melon, sectioned grapefruit, sliced pineapple. These items are usually consumed raw, without a treatment that adequately reduces pathogens (i.e., a “kill step”). However, fruits with low pH (below 4) can help prevent the growth of certain pathogens such as *Listeria monocytogenes* and *Salmonella (5)*. However, fruits with high pH values, e.g., melon, bananas and papaya *(5)*, can support growth; therefore an important aspect of applying the information in this document is to consider the pH of RTE fresh-cut fruit products.

2. RTE fresh-cut vegetables, including packaged leafy greens

Examples include cut celery stalks, peeled baby carrots, sliced mushrooms, shredded cabbage and packaged leafy greens (e.g., chopped lettuce, spring mix). These items are usually consumed raw; the facility provides no microbial reduction step other than physical removal by cleaning, peeling, and washing. Most vegetables have pH values near neutrality; therefore, they are more subject to bacterial growth from pathogens such as *Listeria monocytogenes,* pathogenic *Escherichia coli* and *Salmonella*.

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

Principles that apply to finished product testing of fresh-cut fruits and vegetables:

a. Microbiological testing is most useful (1) if ingredients in a food have the potential to contain pathogens and there is no kill step (such as the case of RTE fresh-cut fruits) in the manufacture of the finished product, and/or (2) when finished products may be contaminated from the environment. Since these products contains raw ingredients that have not received a kill step and they are also exposed to the environment during preparation and filling of containers, they could be contaminated with *L. monocytogenes, Salmonella* spp. and pathogenic *Escherichia coli*, but parasites and viruses from growing and harvest could also be present.

b. Based on the above point, the outbreak history of the commodity plays a key role, as well as seasonality (especially for parasites), therefore looking at updated information on outbreaks from CDC *(2)* and FDA *(10)* is important in determining control measures to ensure product safety.

c. Use of microbiological testing as verification of control measures should consider risk to the consumer. Immunocompromised, infants, pregnant women, and elderly are more susceptible consumers, depending on the pathogen. Low pH (<4.0) fresh-cut fruits naturally inhibit microbial growth. The risk to consumers is higher when pH of final product is above 4.0 or if a product with pH

>4.4 has been kept at refrigerated temperature for extended periods, which supports the growth of *L. monocytogenes*.

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

Criteria a facility can apply to determine whether and how often to test ready-to-eat fresh-cut fruit and vegetable products:

Criterion/Factor	**RTE fresh-cut fruits**	**RTE fresh-cut vegetables**
A. Are pathogens associated with the food or ingredients?	Yes, fresh-cut fruits have been associated with pathogens, including viruses *(2)*.	Yes, fresh-cut vegetables have been associated with pathogens, parasites and viruses *(2)*
B. Are the ingredients likely to be contaminated?	Yes, and supplier verification programs are necessary for some ingredients. Each ingredient needs to be assessed depending on commodity type and its attributes (e.g., pH, water content, rind/peel)	Yes, and supplier verification programs are necessary for some ingredients. Each ingredient needs to be assessed depending on commodity type and its attributes (e.g., pH, water content, rind/peel)
C. Are there robust processing control procedures such as a kill step or other	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	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

Criterion/Factor	RTE fresh-cut fruits	RTE fresh-cut vegetables
reduction methods/controls?	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 sufficient heat to inactivate pathogens.	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 sufficient heat to inactivate pathogens.
D. Is there a potential for recontamination from the handling or the environment?	Yes, the product is exposed to the environment during ingredient preparation (e.g., chopping); however, sanitation controls and a robust environmental monitoring program (EMP) can reduce the potential for contamination with microbial pathogens.	Yes, the product is exposed to the environment during ingredient preparation (e.g., chopping); however, sanitation controls and a robust EMP can reduce the potential for contamination with microbial pathogens.
E. Does the product support survival or growth?	Pathogens will survive on fresh cut fruits.	Pathogens will survive on fresh cut vegetables

Criterion/Factor	RTE fresh-cut fruits	RTE fresh-cut vegetables
F. Is this product meant for higher risk population?	In most instances the product is being made for the general population.	In most instances the product is being made for the general population.
G. What is the shelf life of the product?	1 week	1 week
H. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Yes, if improper handling and temperature abuse occurs, the risk of pathogen growth increases in fruits with a pH supporting pathogen growth, if pathogens are present.	Yes, if improper handling and temperature abuse occurs.

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

Yes, monitoring the wash system parameters (e.g., washing time, water temperature, antimicrobial concentration, organic load as identified for a CCP in a food safety plan) may be used as appropriate verification activities for the process preventive control for biological hazards in wash water. However, these would not be an appropriate alternative to pathogen or indicator testing to verify supplier controls or sanitation controls in the processing facility.

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

RTE fresh-cut fruits and vegetables are not subjected to a kill step. However, antimicrobials should be used in wash water as a process preventive control for biological hazards to prevent cross-contamination during washing. Microbial testing of fresh-cut produce as a verification activity of this process control may not be necessary if other verification activities (e.g., a review of process logs for the sensor continuously monitoring the antimicrobial concentration in the wash water) indicate the process is being consistently implemented. However, pathogen or indicator testing may still be appropriate to verify supplier controls or sanitation controls in the processing facility.

Question 4. When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or specific indicator organism) and type of test (e.g., presence/absence or enumeration)?

What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

Considerations in selecting the test organism include outbreak and recall information for the ingredients or finished products, the lack of a process control to adequately reduce pathogens (i.e., a "kill step"), the types and adequacy of controls applied by suppliers (the growers), and the likelihood of recontamination in the production environment. Routine in-process or finished product testing for generic *E. coli* and environmental testing for *Listeria (11)* should be used to assess process control and insanitary conditions. Generic *E. coli* is a better indicator of process control and sanitation than pathogenic *E. coli*, because generic *E. coli* would be present in greater numbers.

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?

Preventive controls for raw ingredients should be established and implemented through a supply-chain program, as there is no kill step at any point in the production of fresh-cut fruits and vegetables. As

part of a supply-chain preventive controls program, annual onsite audits of each approved supplier (i.e., growers), including an assessment of the farm's procedures, processes, and practices during growing, harvesting, packing, and holding, as related to compliance with the Produce Safety Rule or GAPs are needed *(9)*. If the sanitation program, process controls for biological hazards (such as the use of antimicrobials in wash water to significantly minimize or prevent cross-contamination of pathogens), and the supply-chain program are all robust, the frequency of testing finished product may be less than when such programs are deficient. The frequency of such testing should be sufficient to demonstrate control. Testing frequency may increase (e.g., from weekly to daily) based on emerging issues (e.g., an on-going outbreak), EMP results, and seasonality considerations (particularly for parasites).

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

NACMCF *(8)* indicated that periodic testing by suppliers of in-process or finished products for *Salmonella* or *E. coli* O157:H7 (or other appropriate STEC) may be pertinent depending on the commodity, geographic location, and use of GAPs for various commodities (see https://www.ams.usda.gov/services/auditing/gap-ghp or other FDA or university guidance documents and training). Due to a relatively short shelf-life of fresh-cut produce, finished product testing that may require a "test and hold" could be a limitation to regular end-product testing as a verification activity. Accordingly, reliance on testing and other verification activities at other sites may decrease the frequency of finished product testing. Such testing or other activities include the following: annual onsite audits of suppliers and preharvest testing as part of a supply-chain program; environmental monitoring as a verification of a sanitation program; and robust process controls and associated verification activities for biological hazards in wash water (i.e., use of antimicrobials to significantly minimize or prevent the cross-contamination of biological hazards).

Question 7: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?

A robust EMP could reduce the amount of finished product testing, as some of the pathogens of concern (e.g., *L. monocytogenes, Salmonella*) can reside in the production environment and could result in environmental contamination. The source of these environmental pathogens is commonly raw produce; since pathogens associated with fresh-cut produces are likely to come in on the produce, product testing can be an important verification of control measures from farm to finished product, with environmental monitoring addressing the potential for contamination from the processing environment. There is an interrelatedness of the testing program with other controls such as supplier audits and process controls for wash water, along with GMPs in the plant, that should be used to determine the frequency and extent of finished produced verification testing.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

If generic *E. coli* exceeds a defined limit (e.g., 100 CFU/g) investigation into the cause is warranted. In some cases, product testing for pathogens such as STEC, *Salmonella* and *L. monocytogenes* may be warranted. Corrective actions should be taken for any finding of *L. monocytogenes* in the environment; corrective actions followed by repeat positives may indicate the need of more frequent product testing for *L. monocytogenes* (see FDA draft guidance on Control of *L. monocytogenes* in RTE Foods *(11)*) until the process is under control.

Recommendations: Based on the above, we recommend that for fresh-cut fruits and vegetables:

- Microbiological testing of finished product and the environment should play a role in the verification of control measures.
- Routine testing for *E. coli* should be conducted and action thresholds established
- Investigative pathogen (e.g., *Salmonella*, *E. coli* O157:H7 or other appropriate STEC, *L. monocytogenes*) testing when a problem is detected that indicates the potential for the food to be contaminated with a pathogen
- Depending on commodity, periodic testing of finished product for pathogens (e.g., *Salmonella*, *E. coli* O157:H7 or other appropriate STEC, *L. monocytogenes*) should be conducted to verify process control.
- Other activities include annual onsite audits of suppliers and preharvest testing as part of a supply-chain program; environmental monitoring as a verification of a sanitation program; and robust process controls and associated verification activities for biological hazards in wash water (i.e., use of antimicrobials to significantly minimize or prevent the cross-contamination of biological hazards).

Table E-1. Example of product testing for fresh-cut fruits and vegetables

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
E. coli	<100 CFU/g	Investigate reason for exceeding limit and correct. Determine if pathogen testing is warranted.	Routine testing

Target Microorganism	**Microbial Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Salmonella	Negative in 375 g	Destroy lot. Investigate cause of contamination. Determine if other lots involved. Determine steps to prevent reoccurrence.	Depending on commodity, geographical location and use of GAPs; sample size may vary, Can composite 15 25g samples into one 375 g analytical unit; sample numbers should increase for investigation sampling (e.g., 60 25 g samples tested individually or composited into 4 375 g analytical units)
E. coli O157:H7 (or other appropriate STEC)	Negative in 25g	Destroy lot. Investigate cause of contamination. Determine if other lots involved. Determine steps to prevent reoccurrence.	Depending on commodity, geographical location and use of GAPs; sample size may vary.
Listeria monocytogenes	Negative in 25g	Destroy lot. Investigate cause of contamination. Determine if other lots involved. Determine steps to prevent reoccurrence.	Depending on commodity, geographical location and use of GAPs; sample size may vary.

3. RTE dried/dehydrated fruits

Examples of dried or dehydrated fruits include cranberries, raisins, apricots, sliced apples, etc. The process for creating dehydrated fruits typically involves rinsing and trimming or cutting to the appropriate size. Industrial dryers can be used to dehydrate the fruits, or the fruits can be placed on trays and dried in the open air and sunlight. Most dried fruits are acidic with a low pH (< 4) and low water content.

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

The drying process is used to minimize the potential for the presence and growth of pathogens as it lowers the pH of the fruit and reduces the water content. The following are considerations to be applied in the design of a microbial testing program that includes finished product testing.

a. The pH of the fruit before drying (acidic fruit), the a_w and pH of fully dried fruit, as well as other properties that may be unique to a specific dried fruit, such as the antimicrobial properties of cranberries.
b. The quality of the fruit being used may contribute to the anticipated hazard profile; for some fruits, bruising or surface injuries may contribute to the microbiological risk of further processing. The process of preparing the fruit for drying, for example, whether it is washed, cored, sliced or cubed. These processes may also lead to potential for cross-contamination.
c. Supply chain considerations including growing location and practices, shipment and storage, as well as other information gathered under supplier verification activities, such as supplier history, supplier controls and testing data.

d. Finally, the end use of the dried fruit may contribute to the microbiological testing program, for example, dried fruit can be rehydrated and included in baked goods or other products, made into pastes or consumed as is- all of which may contribute to the types of data that would be expected.

Criteria a facility can apply to determine whether and how often to test ready-to-eat finished products:

Criterion/Factor	Response RTE dried/dehydrated fruits
A. Are pathogens associated with the food or ingredients?	Yes, but there is variability in the pathogens (hazard) and prevalence (likelihood of occurrence) that is dependent on a number of factors including commodity, farming system, region and other variable events (such as season or weather), poor worker hygiene, harvest or post-harvest practices. Pathogen (bacterial and parasitic) risk varies also depending on the processing and/or drying environment and effectiveness of associated process controls. Outside drying activities may have different risks than a facility-based dehydration process. Post-process contamination resulting from handling in bulk and prior to consumer packaging can also be a potential source for pathogens, specifically viruses, as dried fruits are often sold in bulk for dispensing at the store level and/or in mixtures sold in bulk.
B. Are the ingredients likely to be contaminated?	Depending on the fruit, its quality, growing practices, transportation and storage, the incoming commodities have potential for pathogen contamination. Fruits of lower pH are less likely to be contaminated.

Criterion/Factor	Response RTE dried/dehydrated fruits
C. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Information on the effect of drying on microbial inactivation is limited; drying/dehydration can result in microbial inactivation, but this is dependent on time, temperature, pathogen, drying technology, and type of fruit/vegetable, and more information is needed to validate these processes (Bourdoux, Li, Rajkovic, Devlieghere, & Uyttendaele, 2016). Drying/dehydration is adequate to control microbial growth, but pathogens may survive.
D. Is there a potential for recontamination from the handling or the environment?	Yes, the product is exposed to the environment during ingredient preparation; however, sanitation controls and a robust environmental monitoring program (EMP) can reduce the potential to be contaminated with microbial pathogens.
E. Does the product support survival or growth?	Product is not likely to support growth due to low water activity. The duration of survival depends on other stressors, such as acidity of the fruit and storage temperature; *Salmonella* can survive for 6-8 months at 4°C *(1)*.
F. Is this product meant for higher risk population?	In most instances the product is being made for the general population
G. What is the shelf life of the product?	Typically, several years; 1-2 years for select products

Criterion/Factor	Response RTE dried/dehydrated fruits
H. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Bulk containers for storage and dispensing in marketplaces can add risk for cross-contamination with viruses. If appropriately dried, the fruits are considered shelf-stable and robust for prolonged storage.

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

Analytical confirmation of preservatives (such as sulfites) water activity and pH may all be appropriate tests to be completed during processing and on finished product as a verification activity. The drying conditions and time should be supported by appropriate measures of control (such as water activity) that can be applied at several points to ensure final food safety parameters are achieved.

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

Yes, if supply chain is well-understood, pH and a_w of the finished product do not support pathogen growth and the monitoring activity of drying or de-hydrate step demonstrated control. However, sanitation and environmental controls must demonstrate hygienic control of the environment to ensure there is there no cross contamination with low infectious dose pathogens.

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

The appropriate microbial testing would vary based on the fruit, the processes for growing, harvesting and drying, as well as the final pH and a_w. Quantitative indicators of general hygiene may be considered appropriate at initiation, in-process or end, including APC, Enterobacteriaceae, and/or coliforms. Molds may also be appropriate for monitoring the general hygiene of the process as well as the finished good. Some shelf-life surveillance may also be appropriate, again for general indicators of hygiene. Such testing may also be appropriate if there was an important change in the process, such as incoming material changes or new equipment, or to support changes in sanitary controls.

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

Principles or criteria may include 1) commodity and/or growing/harvesting of a commodity; 2) if the dehydration or drying process is well-controlled and validated (for example, outdoor dehydrating process is less controlled than an indoor equipment-based dehydration process); 3) efficacy of sanitation programs as evaluated through environmental monitoring 4) or events, such an emerging supply chain concern, new equipment installation or changes in supplier.

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

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 at supplier. Lot acceptance 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.

Question 7: What impact does environmental monitoring have on frequency and extent of product testing verification activities by companies?

Environmental monitoring for pathogens of concern (likely *Salmonella* and *Listeria*) is warranted if the drying process is conducted in a closed environment and aided by equipment that can facilitate cross-contamination.

However, if the process is an 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.

Question 8: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control? What actions should a company take if test results indicate a loss of control? When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

For end products, microbiological testing is not considered a primary means of routinely assessing product safety and stability. Assessment of safety is best carried out through assurance that preventive controls have been established and executed, as necessary. Microbiological testing can provide a supporting role here, to verify hygiene and the drying process and can be reduced based on results demonstrating the process is well under control. If significant changes are introduced or if there is a failure in the process control, then testing can be intensified temporarily, including finished product testing, to verify that the process returns to control.

Environmental monitoring and sanitation controls provide key verification/monitoring data to support that cross-contamination during the drying and storage process are significantly minimized or prevented.

See Appendix D (Nuts and Nut/Seed Products) Tables D-2 and D-4 for microbiological limits for hygiene verification as these limits are appropriate as well for a dry processing facility supporting fruit dehydration.

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APPENDIX F - CATEGORY: SPICES AND HERBS

Introduction

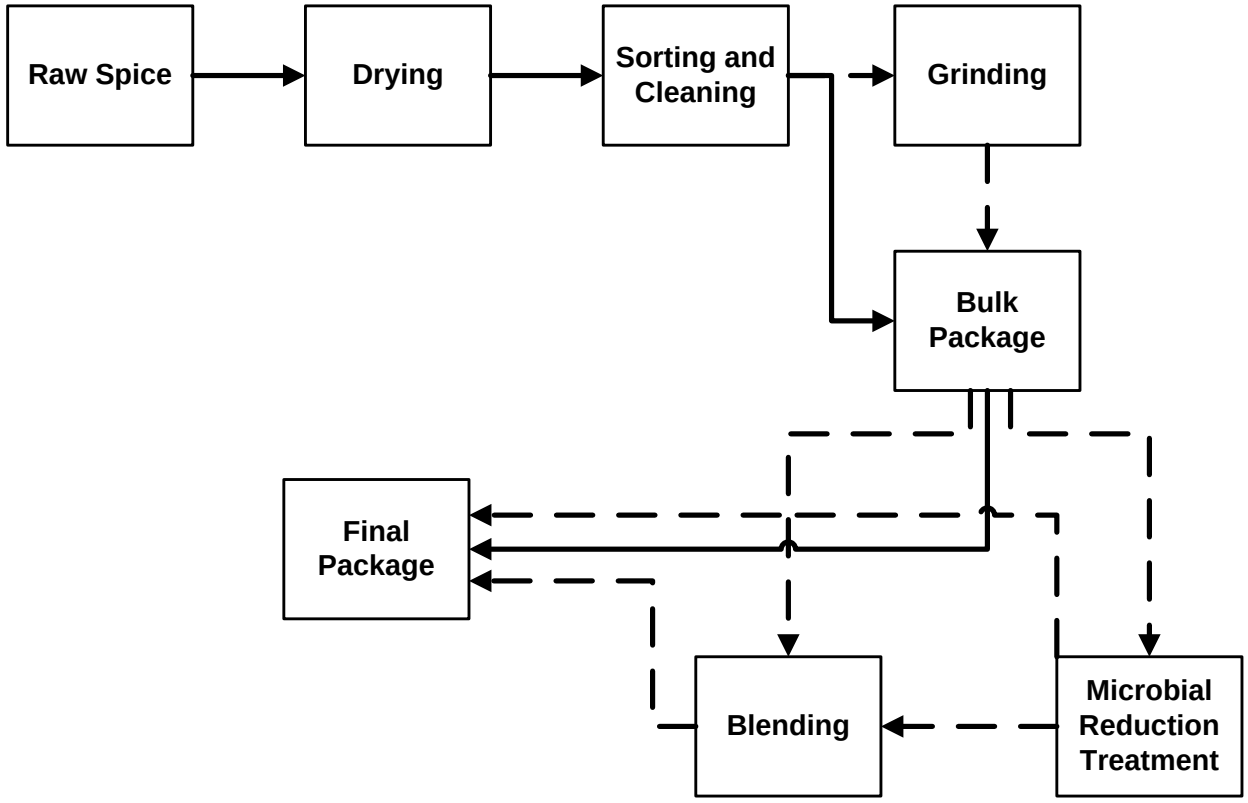
Spices and herbs are dried fragrant, aromatic or pungent edible plant substances in whole, broken, or ground form. They are differentiated based on the part of the plant from which they originate. Spices originate from seeds (e.g., cumin, sesame, poppy), leaves (e.g., basil, mint), roots (e.g., turmeric, ginger), bark (e.g., cinnamon), or flower/flower parts (e.g., saffron, cloves). Herbs, or culinary herbs, are defined as originating from non-woody plants, e.g., tarragon *(22, 23)*.

Spices, spice blends, and herbs have been implicated in foodborne illness outbreaks, despite having a low water activity (less than 0.85). The following biological hazards for both untreated/raw and treated herbs and spices are listed in FDA's draft Appendix 1: *Bacillus cereus, Clostridium botulinum, C. perfringens, pathogenic E. coli*, and *Salmonella* spp. *(21)*. *Salmonella* spp. and *B. cereus* are implicated in the majority of the outbreaks *(24, 25)*. Agricultural conditions play a significant role in product microbial contamination *(1)*. The process of drying spices and herbs allows some pathogens, such as *Salmonella* spp. and *B. cereus*, to survive for an extended period of time *(10, 19)*. When contaminated spices or herbs in untreated or treated form are added to ready-to-eat foods without further processing, the food has the potential to become a vehicle for foodborne illness.

Source plants for spices and herbs are grown worldwide. Agricultural practices vary widely by country and within a country. Farms may be basic or highly mechanized. The drying process may be rapid by mechanical means or by natural sunlight over several days *(8)*. The supply and processing chain can range from very simple within one processing facility to complex with numerous stages including outsourcing to a third party for pathogen reduction treatments (e.g., irradiation). The potential for contamination can occur at any stage: growing, harvesting, processing, packaging, storage, and distribution *(8)*.

Spices and spice blends may or may not be further processed for lethality. Microbial reduction treatments include steam treatments, ethylene oxide or other gas treatments, or irradiation.

Figure 1. Basic flow chart of spice processing.



Optional steps in the process are shown with broken lines. Some processes may include a microbial (pathogen) reduction treatment, some may include blending, and some may include both intervention and blending steps.

The intrinsic properties of certain spices and herbs such as allspice, cinnamon, cloves, and oregano can interfere with laboratory testing methodologies *(6)*. Microbial inhibition and the impact on detection is addressed in the FDA's Bacteriological Analytical Manual (BAM) *(4)*. The recommended approach to alleviating inhibition by spices containing inhibitory compounds is to dilute them with an initial 1:100 or

1:1000 dilution rather than the standard 1:10 dilution. However, as noted in the BAM, it is not possible to completely neutralize the toxicity of some spices, and this does affect the ability to recover pathogens contaminants, especially at low levels.

Spice Products

RTE spices and spice blends, not processed for lethality

RTE spices and spice blends, processed for lethality

Dried, chopped herbs

1. RTE Spices and Spice Blends, Not Processed for Lethality[1]

Raw RTE spices are more likely to be contaminated with *Salmonella (26)* than spices processed with microbial reduction treatments. Some spices/herbs are not processed with a pathogen reduction treatment because the available treatments can have an adverse impact on quality. Dehydrated onion and garlic are examples. Pathogen hazards can originate with the growers. Soil, organic fertilizers, compost and water are sources of microorganisms that remain with the spice after drying.

Manufacturers of RTE raw spices and spice blends must implement preventive controls to significantly minimize or prevent hazards and ensure that the RTE raw spices they manufacture will not be adulterated. These preventive controls include Good Agricultural Practices (GAPs) and sanitation controls on the part of their suppliers (i.e., farms growing, harvesting, packing, and holding the raw spices), supplier audits as part of their supply-chain program, and sanitation controls within their own facilities. While these interventions can reduce risk, they may not be sufficient, as process controls may not be adequate to completely eliminate the hazard. In 2019, the U.S was the top importer of spices

[1] Many spices that have pathogen hazards that are not processed to control such hazards may be destined for use in processed products that are subjected to processes such as cooking that will control the hazard; these are considered non-ready-to-eat (NRTE) spices and are not covered in this Appendix F. NRTE spices are required to bear a disclosure that they are not processed to control microbial pathogens.

globally *(7, 17)*. A robust supply chain control program is essential for imported spices where visibility to good harvesting and sanitary practices during handling and storage may be limited.

An example of an unprocessed imported spice sold as RTE is saffron derived from crocus flowers. Saffron is not thermally processed or irradiated in order to preserve the color, taste, and odor of the product *(9)*. Most saffron is used in cooked dishes and no recalls or outbreaks associated with saffron have been recorded to date. However, one study found high microbial loads in saffron grown in Iran. The study could not rule out the possibility that poor harvesting and lack of sanitary practices during storage contributed to elevated microbial levels *(18)*. How and where spices are stored have been related to the level of contamination. Spices held in bulk have higher concentrations of pathogens. Unpacked spices, stored in bulk open containers, can be contaminated through dust, wastewater and animal/human excreta *(18)*.

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

A hazard analysis is required considering (1) possible microbial hazards, (2) likelihood of occurrence, (3) available processing control procedures such as a kill step or other reduction methods/controls, (4) potential for inherent contamination or recontamination after processing from handling or the environment, (5) survival (persistence) or growth on the product, (6) intended consumer, (7) shelf life of the product, and (8) steps in the process where testing would be appropriate to verify food safety controls.

A testing program applied to a spice or spice blend not processed for lethality should focus on the potential presence of a pathogen. Note that testing alone will not provide adequate assurance of

82 safety for most untreated spices; when pathogens such as *Salmonella* have been associated with a RTE
83 spice, it should be treated to reduce the risk.
84

Criterion/Factor	Response
1. Are pathogens associated with the food or ingredients?	Yes – pathogenic bacteria are associated with raw unprocessed spices *(21, 24-27)*
2. Are the ingredients likely to be contaminated?	Yes. A supplier verification program is necessary but may not be sufficient to control the hazard. Spices sold at retail in the U.S are more likely to be treated to mitigate a hazard *(26)*.
3. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	No.
4. Is there a potential for recontamination from the handling or the environment?	Yes. Manufacturing environments in facilities that handle raw spices and blends may be a source of contamination.
5. Does the product support survival or growth?	Pathogens will survive but not grow.
6. Is this product meant for higher risk population?	The product is made for the general population including high risk consumers.

Criterion/Factor	Response
7. What is the shelf life of the product?	1 – 2 years
Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	The risk for outgrowth may be increased if the spice or blend is added to a product with a water activity that allows outgrowth. Pathogen risk is reduced if the spices or blends are added to a recipe that is cooked.

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

There are no situations where testing other than for pathogens or indicator organisms would be appropriate.

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

No. Raw RTE spices and spice blends are not processed for lethality by definition. Good Agricultural Practices are relied upon for limited control. Finished product testing for *Salmonella* in the absence of a pathogen mitigation process is used to screen for pathogen contamination.

Question 4. *When microbial testing is an appropriate verification activity [for finished product], what considerations should a company apply in selecting the test microorganism (e.g., specific pathogen or*

specific indicator organism) and type of test (e.g., presence/absence or enumeration)? What are appropriate indicator microorganisms for verifying processes adequately control pathogens?

The primary pathogen of concern in raw spices and spice blends is non-typhoidal *Salmonella*. The presence of this pathogen in ready-to-eat spices and spice blends is considered adulteration.

Manufacturers should test in-process and finished products routinely for Aerobic Plate Counts (APC) organisms, coliforms and *Salmonella;* lot by lot is recommended. Manufacturers should routinely test the environment for *Salmonella.* Nonroutine testing of finished products by manufacturers, when deemed necessary, includes *E. coli* and *E. coli* O157:H7 (or other STEC as appropriate) (NACMCF 2018).

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?*

A sampling plan and evaluation criteria should be developed based on the type of spice or spice blend, the history of foodborne illness outbreaks associated with the spice or spice blend, the source of the raw spices used in manufacture, the grower's history and GAP programs, the storage and distribution chain and the risk for cross contamination from the manufacturer's process environment. The sampling plan should clearly define what is considered the "lot".

Finished product testing for *Salmonella* using FDA category II sampling is recommended on a per lot basis to screen for pathogen contamination in the absence of a pathogen mitigation process. FDA Category II includes foods that would not normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption (FDA, 2018). The parameters of Category II are: 30 analytical units/ 25-gram samples. The samples may be aggregated into 375-gram analytical units.

A robust EMP that includes testing for *Salmonella* is essential. A weekly program is recommended.

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

Manufacturers of untreated RTE spices and spice blends do not apply a microbial reduction treatment to the bulk shipments of raw spices they receive and process. Testing at the end of the process is performed as a screen for the presence of *Salmonella* and does not verify the adequacy of microbial hazard controls that are not in place. An inbound testing program for the presence of *Salmonella* and populations of microbes on APC agar using a statistically significant sampling plan provides another level of screening for ingredient contamination prior to production.

Question 7: *What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?*

Although the primary concern might be the presence of pathogens in the actual spice, microbial contamination from the environment must also be considered. In a facility manufacturing product using raw spices, environmental pathogens such as *Salmonella* will be introduced to the plant environment from these raw ingredients. Sanitation controls will be needed to prevent *Salmonella* from gaining a harborage in the facility whereby it could contaminate products. A robust EMP that includes testing for *Salmonella* is essential.

Question 8: *What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?*

What actions should a company take if test results indicate a loss of control?

When verification testing indicates loss of process control, to what extent should verification testing be increased, how far upstream and downstream should it go, and when and how should it be scaled back?

Testing directly for the presence of *Salmonella* in inbound bulk ingredients and finished products does not verify an effective pathogen mitigation process in the case of untreated RTE spices and blends. The detection of *Salmonella* should cause inbound ingredients to be rejected and finished products to be destroyed or reprocessed using a validated pathogen mitigation treatment.

In the absence of positive pathogen results, out of specification indicator organism test results could also signal inherent contamination from the field or that the materials had been subjected to unhygienic conditions in the manufacturing facility (Table F-1). However, high populations enumerated on APC agar do not necessarily correlate with pathogen risk. Organic products, for example, may have higher plate counts due to agricultural methods used. Higher APC counts could trigger more robust pathogen testing and a review of controls. If warranted, a lethality treatment, if applicable, could be applied.

Of note, spices can contain high levels of sporeformers, which can result in high APC populations. These may include pathogenic sporeformers, which at levels $<10^4$ have not been associated with a safety risk when the spices are dry. However, the presence of microbial spores may be a concern for the foods in which the spices are used, and customers may request that treatments to reduce bacterial spores and/or that spices be tested for certain types of spores based on the intended use of the spice.

165 **Table F-1**. Microbial targets, limits, and recommended actions if limits are exceeded for RTE spices and
166 spice blends, not processed for lethality.

Target Microorganism in Finished Product	**Microbiological Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
Aerobic Plate Count (APC)	$\leq 10^6$ CFU/g	Depending on the specific spice and geographic source, exceeding this limit may require appropriate investigative and corrective actions.	
Coliforms	$\leq 10^4$ CFU/g	Investigate, implement corrective action	Option: Test for generic *E. coli* ($\leq$10 CFU/g) (Nonroutine)
Salmonella	Negative in 2 X 375-g samples	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Two 375-g analytical units derived from 30 x 25-g samples
E. coli (O157:H7 or other STEC)	Negative in 25 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Investigative testing if coliform or generic *E. coli* or environmental

Target Microorganism in Finished Product	**Microbiological Limit**	**Recommended Action if Limit is Exceeded**	**Comments**
			testing suggest contamation

Adapted from: Appendix J. Table J.40 *(16)*.

2. RTE spices and spice blends, processed for lethality

These spices and spice blends have undergone a microbial reduction treatment. Some lethality (intervention) processes are performed by third party contractors, while others are performed by the company.

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

A hazard analysis is required as detailed in the "Raw RTE Spices and Spice Blends Not Processed for Lethality" section of this Appendix F. However, the complexity of the manufacturing process for processed spices and blends must be considered when determining risk, assigning pathogen controls across the manufacturing continuum and designing a testing program to verify the effectiveness of controls implemented. There may be two or more entities taking part in the overall manufacturing process.

As an example, one entity could be a business that makes treated spices (they may treat them in-house or they may use a contract sterilizer). This entity could be a supplier to a business that makes

spice blends. A spice blend manufacturer could receive treated spices for blending or they could receive untreated spices and treat them before or after blending. While blending treated spices they receive from a supplier may be most common, this type of context is important in determining the testing that could apply at each stage of the process. As an example, a processor of an individual spice may test the spice after a microbial reduction treatment has been applied. The individual spice may be purchased by a spice blend manufacturer, who may test the individual spices upon receipt as a verification activity, and then test the finished blend of spices prior to shipment.

If a spice or spice blend is processed using a validated microbial reduction treatment, the primary focus of microbial testing at the finished product manufacturer should be verification that the process preventive control was successfully applied and that practices within their facility prevent cross contamination from the processing environment. The same verification program could be used at the contract sterilizer and verified by the manufacturer as part of their supplier assurance program.

Criterion/Factor	**Response**
1. Are pathogens associated with the food or ingredients?	Yes – pathogenic bacteria are associated with raw unprocessed spices *(24, 25)*
2. Are the ingredients likely to be contaminated?	Yes, and supplier verification program is necessary for some ingredients. Each ingredient needs to be assessed.
3. Are there robust processing control procedures such as a kill step or other reduction methods/controls?	Yes – spices and herbs may be subjected to lethal treatments such as with gas, steam, ionizing radiation, or other processes.

Criterion/Factor	Response
4. Is there a potential for recontamination from the handling or the environment?	Yes - unless the product is treated in package. Where spices and blends are treated in boxes or bags by ionizing radiation, ETO or steam, there is limited exposure (if any). If treated products are subsequently repackaged (e.g., in jars) then the potential for exposure exists.
5. Does the product support survival or growth?	Pathogens will survive but not grow.
6. Is this product meant for higher risk population?	In most instances the product is being made for the general population.
7. What is the shelf life of the product?	1 – 2 years
8. Will consumer handling and use increase or decrease risk of pathogen survival, growth, or toxin production?	Consumer handling may not have an impact on risk. In some cases, consumer handling may reduce risk if the spices are added to a recipe which is heated. However, the risk may be increased if spices containing pathogenic sporeformers are used in a food that is held under conditions that allow growth.

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

No. However, if the process intervention/ microbial reduction treatment is validated, and the process is monitored and verified, routine microbial testing of the finished product may not be necessary if the product is treated in an enclosed container and not exposed to the environment.

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

As noted above, if the process intervention/ microbial reduction treatment is validated and applied to a packaged product, routine microbial testing of the finished product for pathogens may not be necessary or may be limited. The process should conform to the appropriate ISO or ASTM standards (e.g., ISO 14470 *(13)* or ASTM F1885-4 *(5)* for irradiation or ISO 11135 *(14)* for ethylene oxide). Steam processes are typically proprietary.

However, if there is still a concern for post-process contamination from the environment, as would occur when the product is processed then subsequently packaged, microbial verification testing for *Salmonella* and Enterobacteriaceae is recommended.

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

Since these spices and herbs have been given a lethality treatment, the test organisms should be those that verify process delivery. Testing for Enterobacteriaceae or coliforms and *Salmonella* is useful to verify process control *(11)*. The surviving population of Enterobacteriaceae or coliforms should be determined quantitatively, using a method which has been demonstrated to recover injured microorganisms *(15)*. *Salmonella* should be absent.

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?*

Finished product testing may not be warranted (or may be limited) for validated processes verified to be under control; however, it is incumbent upon the manufacturer of treated spices and blends to determine if finished product testing for pathogens or indicator organisms would provide information useful to assess process control in their facility. Factors to consider when deciding to conduct finished product testing include who is doing the treatment, how rigorous is the treatment, process validation information, confidence in the entity doing the treatment, historical information from the supplier, how much exposure to the environment and other factors. It is not uncommon for treated spice manufacturers to conduct finished product testing and supply a certificate of analysis with the products they ship.

When finished product is screened for pathogens and/or coliforms or Enterobacteriaceae, testing a representative sample from a complete process run provides the most information about process control. A sampling plan and evaluation criteria should be risk based on the type of spice, the type of process, the geographic source of the spice and prior history. Sampling plans should be risk based and incorporate the fundamental principles of statistical process control and trend analysis. Upper control limits should be established for quantitative analyses, with action levels determined as some point less than the upper control limit. Sampling plans should follow the basic guidelines of investigative, routine and reduced sampling.

FDA guidance provides an example of a sampling plan (sample size and number) when testing for *Salmonella* in foods that would not normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption *(2, 3, 20)*. The sampling parameters are 30 analytical units/ 25-gram samples. The samples may be aggregated into 375-gram analytical units. ICMSF provides additional information about finished product sampling plans commensurate with risk *(12)*.

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

For treated RTE spices and herbs there would be no purpose in testing prior to a microbial reduction step. Testing after the treatment and prior to packaging could be appropriate when combined with effective GMPs and sanitation controls to prevent recontamination and with environmental monitoring.

***Question 7**: What impact should (does) environmental monitoring have on frequency and extent of product testing verification activities by companies?*

During and after treatment, spices and herbs are usually in a container that limits environmental exposure. If the spices and herbs are repackaged after treatment, then an environmental monitoring program may be appropriate. An environmental monitoring program may result in a temporary movement to investigational sampling, including testing for *Salmonella,* when an event in the environmental monitoring program indicates a potential for contamination of the spice or herb.

Question 8*: What criteria should a company apply in determining that microbial testing results indicate a loss of (systemic) process control?*

A loss of systemic process control is indicated when a pathogen is detected in finished product or indicator data repeatedly exceed the limits established for a stable process operating within predictable process variation or exceptionally high indicator levels are observed.

Producers of RTE spices/blends processed for lethality rely on preventive controls that include validated pathogen mitigation treatments, sanitation, and supply-chain programs. The finding of a positive pathogen result could indicate a loss in sanitation control. When a pathogen is detected from a sample taken at the end of the production line, the recommended action is to reject a lot of spices

represented by the sample unless reprocessing using a validated treatment can be conducted. The repeated finding of indicator organisms such as coliforms or Enterobacteriaceae above a threshold level can also indicate a loss of sanitation control although actions taken would follow a tiered approach based on numbers and frequency of occurrence.

Table F-2 details recommended specification limits for *Salmonella,* coliforms and Enterobacteriaceae that verify controls are in place and effective within the manufacturing facility. Note that treated spices can contain high levels of sporeformers which can result in high APC counts. As an example, black peppercorns treated with ethylene oxide can still have an APC of approximately 10^6 CFU/gram with no detectable surviving Gram-negative bacteria.

What actions should a company take if test results indicate a loss of control?

Depending on the seriousness of the hazard, the types of actions that companies may take when loss of control is indicated by microbial testing results include:

1. Verification of the sanitation program
2. Verification that processing parameters were met
3. Increase verification testing frequency
4. Stop processing line until a root analysis is completed
5. Investigate the source of contamination under reduced production

There is no acceptable incidence or population of *Salmonella* in ready-to-eat spices or herbs. The detection of *Salmonella* should cause finished products to be destroyed or reprocessed using a validated pathogen mitigation treatment. U.S. Regulations prohibit the irradiation of products which have been previously irradiated.

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?

Root cause analysis will determine whether verification testing should be increased and how long amplified testing should occur.

Table F-2. Microbial targets, limits, and recommended actions if limits are exceeded for RTE spices and spice blends, processed for lethality.

Target Microorganism in Finished Product	Microbiological Limit	Recommended Action if Limit is Exceeded	Comments
Aerobic Plate Count	$\leq 10^5$ CFU/g	Depending on the specific spice and geographic source, exceeding this limit may require appropriate investigative and corrective actions.	Spices can contain high levels of sporeformers, which can result in high APC counts even in treated spices.
Coliforms	$\leq$10 CFU/g	Investigate, implement corrective action	Option: Test for generic *E. coli* (<1 MPN/g) (Investigative)
Enterobacteriaceae	$\leq 10^2$ CFU/g	Investigate, implement corrective action	

Salmonella	Negative in two 375 g samples	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Two 375 g analytical units derived from 30 25 g samples
E. coli (O157:H7 or other STEC)	Negative	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Investigative testing, if EMP or coliform/Enterobacteriaceae exceed limits

303

304 **3. Dried, chopped herbs**

305 The responses and criteria for RTE spice and spice blends, not processed for lethality would be
306 applicable for dried, chopped herbs. Approved suppliers for source plants who follow Good Agricultural
307 Practices should be used and GMPs adhered to throughout the entire processing, storage, and
308 distribution chain. The same finished product testing criteria are recommended as shown in Table F1
309 and F2, depending on whether they have been treated for lethality.

310

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