

ORA OUTBREAK RESPONSE FIELD GUIDE #1
PATHOGENS: *E. coli*, *Listeria*, *Salmonella*
PRODUCT: SPROUTS

Subject: Commodity-specific inspectional reference for use in inspections and investigations of sprouting facilities, including those associated with outbreaks.

Effective Date: 01 March 2010

Target Audience: All personnel performing investigations or inspections of all parties involved in the production and distribution of sprouts, including seed and sprout producers, and those associated with outbreaks.

DFI prepared this field guide with the assistance of CFSAN. DFI also acknowledges the efforts of members of the California Food Emergency Response Team (CALFERT) and the 50 State Partnership for Food Protection Response Working Group for contributing to the information contained in this field guide.

Purpose:

This field guide is intended to be used as a tool to assist all field personnel conducting routine inspections and investigations in response to sprout-associated outbreaks or positive sample findings.

Conducting these types of comprehensive investigations in a timely manner is critical especially during outbreak investigations to identify distribution of the contaminated product and prevent additional illnesses. Inspections and investigations of sprout production facilities associated with outbreaks have several common goals, though the type and depth of information needed may vary for individual situations.

The primary goals for conducting these types of inspections or investigations are to:

- 1) Identify distribution of contaminated product and if appropriate, the seed lot used, as quickly as possible to prevent further exposure and additional illnesses.
- 2) Gather information, and observe and document practices that may have led to the pathogen specific contamination of the sprouts. Please keep in

mind that this information may be used to support enforcement actions, if appropriate.

- 3) Determine, through observation and documentation practices, if the current FDA sprout guidelines are being followed and if not, describe the failures.
- 4) Gather data to assist the development of recommendations on intervention and prevention strategies that will minimize microbial hazards associated with sprouts.

This field guide will assist field personnel in conducting sprout-specific inspections or investigations, including those with a sample collection component, by identifying the parameters, sample collection techniques, and methods to apply. It is not intended to be an exhaustive list of considerations nor does it replace any specific instructions that are in the work plan or assignment. Investigators should consult their supervisors if there are any uncertainties regarding the scope and use of this field guide.

Background:

Since late 2008 there has been an increase in reported outbreaks of foodborne illness associated with contaminated sprouts. This has raised concerns and reaffirms the need to determine whether or not sprout production facilities are consistent in applying current FDA sprout guidelines, practices and procedures to ensure the safety of sprouts. A thorough and consistent inspection of sprouting facilities will help to identify the deficiencies and allow the FDA to review current guidelines for further improvement.

In conducting this type of investigation, emphasis should be placed on observations and review of information to assess whether or not sprouting facilities have implemented appropriate practices to ensure that sprouts are not produced under insanitary conditions which may render food injurious to health. In addition, specific attention should be placed on whether the specific recommendations outlined in the two guidance documents (“Reducing Microbial Food Safety Hazards for Sprouted Seeds” and “Sampling and Microbial Testing of Spent Irrigation Water During Sprout Production”) are followed correctly.

Sprout-specific investigations require a detailed, systemic, science-based evaluation of environmental factors that may have contributed to contamination. This includes conditions and practices which could either exacerbate the problem or could have kept contaminated product from reaching consumers.

This field guide provides recommendations on specific areas for observation, examination, record review, sample collection, and analytical methods.

Inspectional Approach/Recommendations:

Sprouts have been identified as a particular problem because of the potential for pathogen growth during the sprouting process. If pathogens are present on or in the seed, sprouting conditions may favor their proliferation. There is no inherent step in the production of raw sprouts to reduce or eliminate pathogens once they are present in or on the sprouts.

To date, contaminated seed is the likely source for most reported sprout-associated outbreaks. The growing conditions for sprouts also favor the outgrowth of most pathogenic bacteria. Contamination present locally in seeds can multiply in numbers during sprouting and further spread throughout the production phases. In addition, practices and conditions at the sprouting facility may also impact the safety of the finished product (e.g., unsanitary conditions).

When conducting sprout-specific inspections, it is important that the recommendations outlined in the current FDA guidance for sprouts are taken into account. As sprouts are considered raw, agricultural products, be aware that sprout production operations are exempt from 21 CFR 110.19(a), but will continue to be regulated simply under the adulteration provision (Section 402) of the Federal Food, Drug, and Cosmetic Act (FD&C Act).

Detailed observations and thorough documentation focusing on critical areas (seed condition and storage, seed disinfection, seed lot(s), sprout production process, testing spent irrigation water, holding and distribution, and sample collection) are necessary to evaluate the factors that may have contributed to contamination. At a minimum, these areas should be covered:

A. Seeds:

As previously stated, seeds are the most likely source of contamination in reported sprout-associated outbreaks. Recent investigations have shown that a single contaminated seed lot can result in contamination of multiple production batches of sprouts at a single facility or even at multiple facilities that have received the same lot of seed. Consequently, when a batch of sprouts tests positive for the presence of a pathogen (e.g., *E. coli* O157 or *Salmonella*), the seed lot used for sprout production of the pathogen positive batch and any other batches of sprouts made from the same seed lot, are also deemed affected and that seed lot should no longer be used to produce sprouts. The same principle applies to seed testing. If seed is sampled and found to be positive for *E. coli* or *Salmonella*, the entire seed lot is deemed to be affected.

Note: Because contamination of seeds is generally low level and sporadic, it is possible for sprout production facilities to have been using seed from a given lot for some time before getting a positive finding. This scenario should trigger a review and evaluation of the level of confidence in a firm's spent irrigation water

testing protocol and an assessment of the safety of previous batches that may still be in commerce.

Seeds used for sprouting should be conditioned, stored, and transported in a manner that minimizes the likelihood that the seeds will be contaminated with pathogens. For example, seed should be inspected upon receipt and stored in closed or covered containers in a clean dry area dedicated to seed storage. Containers should be off the floor and away from walls to reduce the possibility of contamination.

1. Does the firm purchase seed from a supplier who obtains seed from growers following Good Agricultural Practices? Was the seed conditioned and stored under sanitary conditions?
2. Does the firm have written procedures for evaluation of seed shipments for signs of contamination (e.g., stain, rodent, insects, feces, urine, foreign material, etc) when seeds reach the sprouting facility? Are procedures written? Is a black light or visual exam included in the procedures?
 - a. If yes, collect copies of procedures and observe firm's practices.
3. Does the firm have written purchase specifications for microbiological and/or visual examination of seeds purchased from seed suppliers? Documentation of seed supplier testing results?
 - a. If yes, collect copies of specifications/documentation
 - b. Does the supplier of the seeds document if a disinfection process was used to reduce the levels of pathogens on the seeds?
4. Complete a visual examination of seed bags/seed storage area (visual examination for evidence of rodents and black light/UV examination of seed bags for evidence of rodents).
5. Does the firm have invoices from seed suppliers?
6. Does the firm maintain a seed log to track seed (and lot #) through receipt, use, or return to suppliers?
 - a. If yes, collect copies of log
7. How are seeds stored? (open or closed containers? Stored off the floor and away from walls? Identified with lot #?)
8. Are seed lots identified and maintained by the facility? What does a lot mean in terms of quantity of seed?
9. Are seed lots ever mixed?
 - a. If so, are records kept?
10. Does the firm sample seeds for microbiological testing? If yes:
 - a. Do they sample each bag?
 - b. How is sampling done (size and number of samples per bag)?
 - c. Who tests the seeds? In-house lab or contract lab?
 - d. Any past positive results? If so, what actions did the firm take?
 - e. Does the firm retain seed samples?
 - f. Does a certificate of analysis (COA) from the seed supplier exist for every seed lot received? If so, what/how was sampling and testing done?

- g. If sampling and testing procedures are available in writing, obtain a copy.

B. Seed disinfection/treatment:

A successful seed decontamination treatment must inactivate microbial pathogens while preserving seed viability, germination, and vigor. A number of treatments have been shown to reduce levels of pathogenic bacteria present on seeds, but none have totally eliminated pathogenic microorganisms.

The routine use of disinfection treatments is likely to reduce the level of contamination if present and, in turn, decrease the risk for foodborne disease with sprouted seeds.

Seeds for sprouting should receive one or more treatments (such as 20,000ppm calcium hypochlorite) that have been approved for reduction of pathogens in seeds or sprouts. Some treatments can be applied at the sprouting facility while others will have to be applied earlier in the seed production process. At least one approved antimicrobial treatment should be applied immediately before sprouting. Sprouters should carefully follow all label instructions when mixing and using antimicrobial chemicals.

1. Does the firm have written seed disinfection procedures?
 - a. If yes, collect copies of disinfection procedures
2. Does the firm have written seed disinfection logs?
 - a. If yes, collect copies of disinfection logs for the past 12 months
3. Does the firm have copies of invoices for disinfection chemicals purchased?
 - a. If yes, collect copies of invoices for past 6-12 months
4. Have the firm re-create an actual seed disinfection process from mixing of chemicals to actual disinfection of seeds while you observe and record data. Include process for mixing solution and testing concentration, if done.
 - a. Describe process and observations
 - b. Test the strength of the solution
 - c. What volume of seed to what volume of solution is used?
 - d. Is the seed/solution agitated during treatment?
 - e. How long is the treatment?
 - f. Is potable water being used?
 - g. How are the disinfected seeds transferred/transported to sprout production?
5. Is at least one antimicrobial treatment applied to seeds immediately before sprouting? (Note: Simply rinsing or soaking seeds in municipal water does not constitute a treatment).

6. Obtain the following information on antimicrobial treatment(s) used by the firm:
 - a. Type of seed
 - b. Biocide (include brand)
 - c. Concentration
 - d. Treatment method
 - e. Duration of treatment
 - f. Are seeds pre-rinsed, prior to any antimicrobial treatment?
 - g. Rinse after treatment? Is potable water being used?
7. How often does the firm check to verify that the concentration of antimicrobial treatment was prepared according to the firm's SOP and is within the target range established by the firm? (daily, weekly, etc)
8. What method and procedure are used by the firm to check the concentration (e.g., test strips)? Include brand, model and test range.

C. Known contamination: Sprout-associated outbreaks

In the event of a sprout-associated outbreak, considerable time and attention should be paid to determining and documenting the source of seeds and the identification of lot(s) used during the time period of interest. It is critical to identify as quickly as possible where the contaminated product was distributed and initiate a recall if appropriate (see section H of this field guide for guidance on collection of distribution information).

If contaminated seed lot #(s) are known:

1. Identify and document seed lots and the supplier(s) used during the time period of interest
2. Request firm and seed supplier to begin assembling distribution records (electronically, if possible) for all sprouts made from identified seed lots and time period in question
3. Identify any shipments of seeds from the contaminated lot(s) to other sprouters
4. Request that firm and seed supplier destroy contaminated seed or divert it to agricultural use only.

If contaminated seed lot #(s) are unknown, use the following information to identify potential lots of contaminated seed (this information is to be provided to the coordinating organizational unit at Headquarters; i.e., OEO, ORA or CFSAN, who will work with the District to analyze):

1. specific implicated product (alfalfa, clover, mixed sprouts, etc)
2. labeling information, if available
3. known points of service and/or exposure information
4. the earliest and latest exposure and/or illness onset dates

Identification of time period of interest:

1. Obtain appropriate time period of interest from lead office at HQ (i.e., OEO, ORA/DFI, CFSAN)
2. As a general guide, collect seed invoices for the last 6-12 months if possible

D. Sprout production:

Poor sanitation and inadequate hygiene can significantly increase the risk of contamination of sprouts. Sprouters should implement appropriate practices to ensure that sprouts are not adulterated under section 402(a)(4) of the Act [21 U.S.C. 342(a)(4)], where a food is deemed to be adulterated if it has been held under insanitary conditions whereby it may have been rendered injurious to health.

Sprouting facilities and equipment should be maintained in conditions that will protect against contamination. Good sanitation practices should be employed as a standard operating procedure to maintain control throughout all stages of sprout production.

If testing of spent irrigation water, seeds, or a batch of sprouts confirms presence of contamination with a pathogen, a thorough cleaning and sanitation process should be conducted to avoid contamination of subsequent batches of sprouts.

The thorough cleaning and sanitation process should include all affected areas of the sprouting facilities (such as floors, drains, tables) and all equipment that has come in direct contact with the contaminated seed lot, batches of sprouts, or water. Equipment may include drums, trays, bins, and other sprouting production and testing equipment.

Additionally, care must be taken in handling contaminated sprouts or water, equipment, and test materials to avoid accidental exposure to the pathogen(s).

The following should be considered:

1. Does the firm have a written SOP for sprout production?
 - a. If yes, collect a copy of the procedures
2. Does the firm have written logs to show what seed lots were used on what production days?
 - a. If yes, collect copies of logs
 - b. Identify the specific seed lots used during the time period of interest (include seed source, when seed lots were originally used, entire time period when relevant seeds were used, and last date of use)
 - c. Are any seeds from the lots of interest still available on-site?
 - d. Are any of these seed lots still potentially in production?

3. Were there any deviations from the production process during the time period when the seed lots of interest were being produced?
4. Are seed lots mixed during production (e.g., same type of seed but different seed lots)?
 - a. If yes, are there specifications or procedures for mixing seed lots?
5. Is the product produced with a mix of seed types (such as alfalfa and clover)?
 - a. If yes, is one seed lot for each type of seed used or are multiple lots of multiple seed types used in production?
6. What types of seeds are sprouted at the facility? (alfalfa, clover, radish, broccoli, mung bean, sprout mix, other?)
7. Are production lines and equipment dedicated to one seed type?
8. What is the source of water used for irrigation (e.g., municipal, well)?
 - a. Is the water potable (e.g. meets EPA Drinking Water Standards)?
 - b. Is the water chlorinated or otherwise disinfected by the firm?
 - c. If water disinfection is done, describe, e.g., method used and if applicable, determine what level of chlorine is used and how it is monitored.
 - d. Are other water treatment systems in place (softening, iron removal, filtration, etc)?
9. Does the firm capture and reuse spent irrigation water? If so, describe how it is handled and treated between uses.
10. How are sprouts handled and cooled after sprouting?
11. Does the firm have a written SOP for the dehulling, washing, dewatering, and packaging processes?
 - a. Is potable water used for finished product washing? Is the water chlorinated? What level of chlorine is used?
 - b. Is the wash water reused? Is the chlorine level tested prior to use?
 - c. Evaluate the cleanliness of the packaging area. Is the packaging area away from the sprouting area? Are gloves used by workers? What are the general worker health and hygiene practices?
12. Evaluate the overall cleanliness and condition of the sprouting area and food contact surfaces.
13. Does the firm have written sanitation standard operating procedure?
14. Does the firm have written procedures for routine verification of its cleaning and sanitation procedures?
 - a. If so, observe and verify implementation of sanitation standard operating procedures.

E. Spent Irrigation Water Testing:

Spent irrigation water is the water that has flowed over and through sprouts and the microflora in this water is fairly uniform. Spent irrigation water is a good indicator of the types of microorganisms in the sprouts themselves. Because currently approved antimicrobials have not been shown to be capable of eliminating all pathogens from seed, sprout producers should conduct

microbiological testing for each production batch to ensure that contaminated product is not distributed. FDA is recommending testing of spent irrigation water.

Salmonella and *Escherichia coli* O157:H7 have been the major causes of sprout-associated illness outbreaks and are the targets of the microbial testing of spent irrigation water. *Listeria* is generally considered an environmental contaminant and not associated with seeds. Environmental swabbing or product samples are the most reliable methods of detection for *Listeria*.

Testing spent irrigation water is the most reliable method for monitoring microbial levels and detecting pathogens that may be present during sprouting making it the strongest control to keep contaminated sprouts from entering commerce. FDA current recommendation is to test spent irrigation water collected from every production batch as early as 48 hours after the start of sprouting. A production batch is defined as sprouts from a single lot of seed that were started at the same time in a single growing unit (i.e., a single drum or rack of trays).

1. Does the firm have written procedures for collecting spent irrigation water for microbiological testing? If yes, describe the procedures.
 - a. When during the sprouting process are irrigation water samples collected by the firm? Within 48 hours of sprouting start?
 - b. What is the volume of spent irrigation water collected per sample?
 - c. What is the frequency with which spent irrigation water is collected for microbiological testing? (e.g., from every production batch of sprouts? Periodically (i.e., some batches tested, some are not)? When beginning to use a new seed lot?)
 - d. Does the firm sample/test every production batch individually or pool samples (i.e., combine irrigation water samples collected from multiple drums or bins)?
 - e. Is any neutralizing agent added to the sample container to neutralize residual chlorine in the spent irrigation water if present?
 - f. Are aseptic sampling procedures provided?
 - g. How are samples stored prior to testing? (Include temperature)
 - h. What are the minimum and maximum amounts of time these samples are stored before being sent to the lab?
 - i. How are the samples transported to the testing lab? Are samples shipped refrigerated? How long does it take from collection to delivery?
2. Have the firm re-create an actual spent irrigation water collection process while you observe and record data. Are the following aseptic sampling procedures followed?
 - a. The equipment (e.g., sampling tools and sample containers) used to collect samples should be clean and sterile.
 - b. Sample containers should be dry, leak-proof, wide mouthed and of a size suitable for the samples.

- c. Sample containers should be properly labeled prior to starting sample collection.
 - d. Sample collector should wear a clean lab coat, gloves and a hair net while performing sample collection.
 - e. Hand should be away from mouth, nose, eyes, and face while collecting samples.
 - f. Sampling instruments should be protected from contamination at all times before and during use.
 - g. The sterile sample container should be opened only sufficiently to admit the sample, then immediately closed and sealed.
 - h. Sample container should be filled no more than 3/4 full to prevent overflow.
 - i. Samples or sampling equipment should not be exposed to unfiltered air currents.
 - j. Samples should be delivered to the lab promptly and should be kept at an appropriate temperature, preferably at 0 – 4.4 °C (32 to 40 °F). Sealed coolant packs should be used.
3. Does the firm have written microbial testing logs?
 - a. If yes, collect copies of the logs for the last 12 months.
 4. Is spent irrigation water tested for:
 - a. *E. coli* generic
 - b. *E. coli* O157:H7
 - c. *Salmonella* spp.
 - d. Others? Specify.
 5. Is the initial microbial testing of spent irrigation water performed by a contract laboratory or the firm's in-house lab?
 6. Method and sample preparation used for initial testing? (FDA BAM method, rapid test kits, other?)
 7. Have the methods used been previously validated for detection of pathogens in sprouts?
 - a. If yes, collect records describing the method used and validation of method.
 8. Is a confirmation test done when a preliminary positive test result is received?
 - a. If yes, collect sufficient test results to verify
 - b. What is used for confirmation testing – spent irrigation water or enrichment media?
 9. Does the firm have written procedures to wait for results (either preliminary or confirmed results) before shipping product?
 - a. If yes, obtain copies of procedures
 10. Does the firm notify seed suppliers of positive results?

F. Testing sprouts:

Sprouts should not be tested in lieu of spent irrigation water (see section E) unless production methods make it impossible to test spent irrigation water. For example, spent irrigation water may not be available when sprouts are grown in soil. Testing of the sprouts themselves has several disadvantages. First, multiple sprout samples must be taken from different locations in the drum or trays to ensure that the sample collected is representative of the batch. Furthermore, additional preparation is required when testing sprouts. Each additional step in any procedure (sampling or testing) introduces a possibility for error. If testing sprouts in lieu of spent irrigation water, sprouters should have a sampling plan in place to ensure consistent collection of sprout product samples. Testing of finished product, if done, should be in addition to testing spent irrigation water.

For the purpose of this field guide, in-process sprouts refer to sprouts that have been grown for at least 48 hours inside a growing unit (e.g., drum, tray, and bin). Finished product refers to sprouts that have been harvested and have been subjected to the de-hulling, washing and dewatering processes. They may or may not have been packaged.

If testing in-process sprouts in lieu of (or in addition to) irrigation water:

1. Does the firm test in-process sprouts for pathogens? If yes:
 - a. Describe the procedure
 - b. When during the sprouting process are product samples collected by the firm?
 - c. How are samples stored? (Include temperature)
 - d. What are the minimum and maximum amounts of time these samples are stored before being sent to the lab?
 - e. How are samples transported to the lab for analysis?
 - f. Are samples collected from multiple lots, seed types, drums or bins?
2. Are in-process sprouts tested for:
 - a. *E. coli* generic
 - b. *E. coli* O157:H7
 - c. *Salmonella* spp.
 - d. *L. monocytogenes*
 - e. Others? Specify.
3. Review and collect copies of test results for the past 12 months.
4. Is the initial microbial testing of in-process sprouts performed by a contract laboratory or the firm's in-house lab?
5. Method and sample preparation used for initial testing? (FDA BAM method, rapid test kits, other?)

6. Have the methods used been previously validated for detection of pathogens in sprouts?
 - a. If yes, collect records describing the method used and validation of method.
7. Is a confirmation test done when a preliminary positive test result is received?
 - a. If yes, collect sufficient test results to verify
 - b. What is used for confirmation testing – in-process sprouts or enrichment media?
8. Does the firm have written procedures to wait for results (either preliminary or confirmed results) before shipping product?
 - a. If yes, obtain copies of procedures

If finished product testing is done:

1. Does the firm test finished product for pathogens? If yes:
 - a. Describe the procedure
 - b. When, during the entire sprout production process, are finished product samples collected by the firm?
 - c. How are samples stored? (Include temperature)
 - d. What are the minimum and maximum amounts of time these samples are stored before being sent to the lab?
 - e. Are samples collected from multiple lots, seed types, drums or bins?
2. Are finished sprout products tested for:
 - a. *E. coli* generic
 - b. *E. coli* O157:H7
 - c. *Salmonella spp.*
 - d. *L. monocytogenes*
 - e. Others? Specify.
3. Review and collect copies of test results for the past 12 months.
4. Is the initial microbial testing of finished product performed by a contract laboratory or the firm's in-house lab?
5. Method used for initial testing? (FDA BAM method, rapid test kits, other?)
6. Have the methods used been previously validated for detection of pathogens in sprouts?
 - a. If yes, collect records describing the method used and validation of method.
7. Is a confirmation test done when a preliminary positive test result is received?
 - a. If yes, collect sufficient test results to verify
8. What is used for confirmation testing – finished product or enrichment media?
9. Does the firm have written procedures to wait for results (either preliminary or confirmed results) before shipping product?
 - a. If yes, obtain copies of procedures

G. Environmental testing:

Because sprouts are a refrigerated food and can support the growth of *Listeria monocytogenes*, a *Listeria* control program may be appropriate for sprouting facilities. Typically, a *Listeria* control program includes environmental sampling for *Listeria spp* or *Listeria monocytogenes*, to identify sanitation failures and respond to positive results aggressively, with the objective of making continuous improvements toward the goal of controlling the pathogen in the processing facility.

1. Does the firm do any environmental testing? Any past positive results? If yes, obtain results and specific location where swab was taken.
2. Does the firm have written procedures for routine environmental testing?
If yes, obtain copies of procedures
 - a. Frequency of testing
 - b. Sample collection, how many and at which locations
3. Is the environmental testing for:
 - a. *E. coli* generic
 - b. *E. coli* O157:H7
 - c. *Salmonella spp.*
 - d. *Listeria spp.*
 - e. *L. monocytogenes*
 - f. Others? Specify.
4. Review and collect copies of test results for the past 12 months.
5. Are the samples tested by a contract laboratory or the firm's in-house lab?
6. Method and sample preparation used for initial testing? (FDA BAM method, rapid test kits, other?)
7. Have the methods used been previously validated for sprouts?
 - a. If yes, collect records describing the method used and validation of method.
8. Is a confirmation test done when a preliminary positive test result is received?
 - a. If yes, collect sufficient test results to verify
9. What is used for confirmation testing – separate samples or enrichment media?
10. Does the firm have written procedures to wait for results (either preliminary or confirmed results) before shipping product?
 - a. If yes, obtain copies of procedures

H. Storage and Distribution:

Distribution information should be requested and provided. In the case of a sprout-associated outbreak, dates of interest should be determined in order to minimize the likelihood of having to return to the firm for additional information

(see section C). The information collected is critical in determining information for recall of product if necessary. Knowing to whom and where the finished product has been sent can expedite the removal of product, thus decreasing the likelihood of further illness.

1. Does the firm cool their sprouts to 45 degrees Fahrenheit or below prior to distribution?
2. How are sprouts held while they are being cooled to 45 degrees Fahrenheit or below? (ex., shallow tray or bin for more efficient cooling, large bin or bag, bagged and cased, etc).
3. What is the core temperature of sprouts that were placed under refrigeration at the end of the previous days shift?
4. What is the core temperature of sprouts that were most recently placed in refrigeration?
5. How is product transported? Time? Distance? Type of vehicle?
6. Does the firm distribute sprouts under refrigeration?
7. Are production lots and dates identified on the finished product label?
8. Does the firm have written procedures for the recall of product?
 - a. If yes, collect copies of the procedure.
9. Does the firm have the ability to recall particular lots of finished product in the event of an outbreak?

If investigating a sprout-associated outbreak:

10. Obtain distribution list of any seed sold from the lots of interest. Ask the seed suppliers to identify which firms are sprouters; seed may also be sold for field production.
11. Obtain distribution list of customers who have received sprouts from these lots
12. Obtain distribution list of sprouts grown from these lots with production dates and ship dates
13. Obtain list of brand names and packaging type/sizes of sprouts produced during the time period of interest

I. Other considerations:

1. Has the firm been associated with previous sprout outbreaks?
2. Has the firm received any complaints or notification of illnesses from consumers?
3. Has the firm tested its traceback and recall system?
4. Describe any "unusual" events in the facility in the last 6 months (sewage/toilet overflow, rodent infestation, remodeling, broken equipment, leak in roof, extensive maintenance, etc)
5. Does the firm have a written SOP for cleaning and disinfecting facility and equipment in the event contamination is found?
 - a. If yes, collect copy of SOPs

6. Does the firm have written procedures to verify the effectiveness of their cleaning and sanitation procedures?
 - a. If yes, collect copies of the procedures.
7. Does the firm have written SOPs for corrective actions before resuming production after contamination is found?
 - a. If yes, collect copy of SOP and previous records, if available

Sample Collection

A. Equipment Needed:

1. Chlorine test strips (Free and Total Chlorine High Range) or colorimeter test kit for chlorine and pH test strips
2. Sampling kit:
 - a. 1-L sterile bottles (with and without sodium thiosulphate)
 - b. Whirlpak® bags – large
 - c. Aseptic sampling supplies (gloves, swabs, D/E broth if needed, disposable foot coverings)
 - d. Kits for collecting environmental samples (e.g., swabs and sponges)

B. Samples to collect: (See Table 1 for sampling instructions)

Previous research shows that contamination of seeds likely occurs at a very low level and those contaminants are not uniformly distributed throughout the entire seed lot. Therefore, a considerable number of seed samples are required to have a reasonable chance of finding pathogens in seeds. If seed, in-process sprouts, finished product, and spent irrigation water from implicated seed lots are available for sampling, see the instructions below. If implicated lots are no longer available, discuss with district management in consultation with DFI and CFSAN to determine if seed samples should be collected. Be sure to confirm if sample collection for non-implicated lots should be the same as collection from implicated lots.

Seeds, sprouts and spent irrigation water samples collected for the analysis of *E. coli* O157:H7 and *Salmonella* should be collected according to the following sampling instructions.

Seeds

A minimum of 30 subsamples should be collected throughout each seed lot. Subsamples are to consist of a minimum size of 454 grams (1 lb) collected from 3 or more different areas of the bag. If there are more than 30 bags (generally, a bag contains 50 lbs of seeds) to sample, then randomly select 30. If there are fewer than 30 bags to sample, then 30 random samples should be taken from existing bags. Collect one subsample from each bag of seed sampled. A seed

tryer can be used (with duct tape to patch the hole in the bag after using the tryer) to obtain samples from individual 50 lb bags. Disinfect the tryer by applying a 70% solution of isopropyl alcohol between bags. Be sure to label each 50 lb bag with a unique number in case bags need to be further sampled following positive test results.

Spent Irrigation Water

Collect one (1) sample of spent irrigation water from each type of sprouts being produced at the time of inspection, up to a maximum of four (4) samples per inspection. Each sample should represent one production lot or batch being processed at the time of inspection. A production lot or batch is defined as sprouts from a single lot of seed that was started at the same time in a single growing unit (e.g., a single drum, or rack of trays).

Samples should be collected as early as 48 hours after the start of sprouting. If seeds are presoaked (i.e., soaked in water for a short time and then transferred to growing units for sprouting), then presoak time should be included in the 48 hour minimum.

If sprouts are grown in drums, aseptically collect 1 liter (L) of water as the water leaves a drum during the irrigation cycle.

If sprouts are grown in trays, and all trays in a production lot have a common trough for collecting spent irrigation water, then a one (1) L sample may be collected at that point. If there is no common collection point for water from trays, then it may be necessary to collect water samples from individual trays and pool these samples. For example, collect about 100 ml of water from each of 10 trays to make 1 L of sample; about 125 ml from each of 8 trays, etc. When more than ten (10) trays make up a production batch, then ten (10) samples should be aseptically collected, approximately 100 ml each from different trays. Again, samples should be collected throughout the entire production lot (e.g., if there are 20 trays in a production lot, collect samples from every other tray in the rack moving from top to bottom, side to side, and front to back). Samples should be placed directly into a clean, sterile, pre-labeled container.

For each water sample collected, the following information should be recorded and reported on sample collection report:

- seed type (e.g., alfalfa, clover, etc.)
- seed lot number
- origin of seed
- type of growing units (e.g. drum, tray), and
- approximate time seeds have been sprouting, including the time seeds may have been presoaked

Sprouts

If testing in-process sprouts in lieu of irrigation water, thirty-two (32) sample units should be aseptically collected, approximately fifty (50) grams each, from different locations in the drum or growing trays. The total sprout sample will weigh approximately 1,600 g per production lot or batch. Sample units should be collected throughout the entire production lot (e.g., from top to bottom, side to side, and front to back of the drum or trays). Each 50 gram sample unit should be placed directly into individual clean, sterile, pre-labeled containers. (Keeping the thirty-two sample units separate will help the lab to select representative analytical units for microbial analysis, in contrast to pulling analytical units from a single 1,600 gram mass of sprouts.)

When testing finished products, thirty-two (32) sample units should be aseptically collected for each type of sprouts, approximately fifty (50) grams each. The total sprout sample will weigh approximately 1,600 g per production batch. Each 50 gram sample unit should be placed directly into individual clean, sterile, pre-labeled containers.

Environmental Sampling

When conducting environmental sampling for *Listeria*, swab hard to clean food contact surfaces, food contact surfaces that most frequently come into contact with sprouts, and cracks, crevices and other hard to clean areas in Zone 2 following the wet environment recommendations for *Listeria*, as per DFI bulletin # 30.

In each sprouting facility, collect approximately 20 - 30 environmental samples; 10 samples are to be collected from food contact surfaces. The remaining samples should be collected from non-food contact surfaces.

The following is a list of sprout-specific locations to also consider when collecting environmental samples:

- Pre-soak or pre-wash buckets
- Soaking tubs (unless these are the tubs used for disinfection with 20,000 ppm calcium hypochlorite)
- Buckets and screens used in rinsing seeds after soaking
- Rotating drums used for sprouting – interior divider walls, ventilation screen, exterior surfaces between the divider sections, edge or lip of drum where sprouts are removed
- Trays, bins, buckets used for germination and growth
- De-hulling and final rinse machinery, especially in seams and crevices
- Nozzles used to spray water during germination and growth
- Centrifuge used to spin-dry sprouts
- Growing racks – top and bottom of shelves

- Wheels and vertical/horizontal support on racks
- Knives used to harvest soil-grown sprouts
- Walls in sprouting area, especially where water splashes and where walls are cracked

Investigators should be assertive in requiring the firm to disassemble equipment for environmental sampling, especially those that are difficult to clean.

Table 1. Sample Collection

Samples to collect:	Location to sample:	Amount to sample:	Identify:
Seeds: Implicated lots	<ul style="list-style-type: none"> • 30 bags of each seed lot OR <ul style="list-style-type: none"> • If less than 30 bags available, collect 30 random samples from existing bags 	<ul style="list-style-type: none"> • Total of 1 lb (or 500 g) of seeds from the top, middle, and bottom of each bag 	<ul style="list-style-type: none"> • Seed lot(s) • Seed supplier • Country of Origin
Seeds: Non-implicated lots	<ul style="list-style-type: none"> • Consult with managers 		
In-process product: Sprouts from Implicated lots	<ul style="list-style-type: none"> • Different locations in the drum or growing trays • Sample units should be collected throughout the entire production batch (e.g., from top to bottom, side to side, and front to back of the drum or trays). 	<ul style="list-style-type: none"> • 32 sample units should be aseptically collected, approximately 50 grams each • Total sprout sample will be approximately 1,600 g (about 56.48 oz or 3.53 lb per production lot or batch) 	<ul style="list-style-type: none"> • Seed type • Seed lot(s) • Sprout lot #, if different than seed lot #
Finished product: Sprouts from Non-implicated lots	<ul style="list-style-type: none"> • Consult with managers 		
Spent irrigation water: Implicated lots, grown in drums	<ul style="list-style-type: none"> • As the water leaves a drum or trays during the irrigation cycle 	<ul style="list-style-type: none"> • 1 liter sample of water 	<ul style="list-style-type: none"> • Seed type • Seed lot(s)
Spent irrigation water: Implicated lots, grown in trays	<ul style="list-style-type: none"> • Common trough for collecting spent irrigation water • If there is no common collection point for water from trays, it may be necessary to collect water samples from individual trays and pool these samples. 	<ul style="list-style-type: none"> • 1 liter sample of water • If pooling from individual trays, a sampling plan should be devised to ensure collection of a sample that is representative of the production lot 	<ul style="list-style-type: none"> • Seed type • Seed lot(s)
Spent irrigation water: Non-implicated lots	<ul style="list-style-type: none"> • Consult with managers 		
Environmental	<p>See the above list of sprout-specific areas to sample</p>	<ul style="list-style-type: none"> • 20-30 samples may need to be collected from food processing facilities to have a reasonable chance of finding pathogens if present at low levels in the environment. 	<ul style="list-style-type: none"> • Specific location where environmental sample is collected

Table 1. Sample Collection

Documents to collect:

The following is a summary of documents to review and collect during the inspection, if available. This is not an all inclusive list, but represents the information needed to fully assess the firm's operations and determine next steps.

If a firm does not have or keep a particular document, please indicate.

Documents:

- Procedures for evaluation of seed shipments
- Specifications for seeds purchased from seed suppliers
- Procedures for visual examination of seed shipments
- Log to track seed through receipt, use, or return to suppliers
- Records of mixed seed lots
- Seed sampling and testing procedures
- Seed disinfection procedures
- Seed disinfection logs for past 12 months
- Disinfection chemical invoices for past 6-12 months
- SOPs for sprout production including postharvest washing, dehulling, dewatering and packaging.
- Production logs showing which seed lots were used on what production days
- Spent irrigation water sampling and testing procedures
- Spent irrigation water sample results for past 12 months
- In-process and/or finished product sampling and testing procedures
- In-process and/or finished product sample results for past 12 months
- Environmental sampling and testing procedures
- Environmental positive results and specific swabbing location
- Procedures on waiting for results (either preliminary or confirmed results) before shipping product
- SOP for cleaning and sanitation of production equipment and facility
- SOP for cleaning and sanitation of production equipment and facility in the event contamination is found.
- SOP for resuming production after cleaning of facility,
- Procedures for recall

Documents specific to time period of interest:

- Invoices showing supplier, lot #, date of receipt, and country of origin of seed
- Distribution list of any seed sold from the lots of interest
- Distribution list of customers who have received sprouts from the lots of interest
- Distribution list of sprouts grown from interested lots with production dates and ship dates
- List of brand names and packaging type/sizes of sprouts produced

References and Resources:

"Guidance for Industry: Reducing Microbial Food Safety Hazards For Sprouted Seeds" (1999)
<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm120244.htm>

"Guidance for Industry: Sampling and Microbial Testing of Spent Irrigation Water During Sprout Production" (1999)
<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm120246.htm>

"Microbiological Safety Evaluations and Recommendations on Sprouted Seed" (1999)
<http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/ucm078789.htm>

"Safer Processing of Sprouts" video training module, created by University of California Davis in collaboration with California Dept of Public Health and CFSAN
<http://postharvest.ucdavis.edu/Pubs/video-library.shtml#Sprouts>

"Guide to Traceback of Fresh Fruit and Vegetables Implicated in Epidemiological Investigations" (2006), <http://www.fda.gov/ICECI/Inspections/InspectionGuides/ucm075005.htm>

DFI Field Bulletin # 30
<http://inside.fda.gov:9003/downloads/PolicyProcedures/GuidanceRegulations/FieldInvestigations/UCM046269.doc>

DFI Field Bulletin #32:
<http://inside.fda.gov:9003/downloads/PolicyProcedures/GuidanceRegulations/FieldInvestigations/UCM094664.doc>