FDA FACT SHEET
Produce Safety Rule (21 CFR 112)

U.S. FOOD & DRUG ADMINISTRATION

Responding to Results Obtained from Testing Spent Sprout Irrigation Water (or In-Process Sprouts) for *E. coli* O157:H7 or *Salmonella* to Meet the Requirements of the Produce Safety Rule

The Produce Safety Rule (PSR) requires that sprout operations test spent sprout irrigation water (SSIW) or in-process sprouts from each production batch of sprouts for certain pathogens, including *E. coli* O157:H7 and *Salmonella*. For *E. coli* O157:H7 and *Salmonella* testing, the PSR requires you to use either the FDA reference method described in the PSR (Ref. 1) or an alternative method that is scientifically valid and at least equivalent to the FDA reference method in accuracy, precision, and sensitivity. The alternative methods that FDA has determined to be scientifically valid and equivalent to the FDA reference method are available on the FDA website (Ref. 2). A sprout operation's use of a method that is scientifically valid and equivalent to the FDA reference method to test SSIW (or in-process sprouts) for *E. coli* O157:H7 and *Salmonella* is critical to preventing adulterated food from entering commerce, and to protecting the public health, since a method that is not scientifically valid or equivalent to the FDA reference method might not identify such pathogens that are present in SSIW or sprouts.

What are the steps involved in testing?

The FDA reference method includes screening procedures and a confirmation step. If the result from the initial screening test for either *E. coli* O157:H7 or *Salmonella* is a presumptive positive, further confirmation is required to verify that the pathogen of concern is truly present. FDA is not aware of any method that is scientifically valid and equivalent to the FDA reference method that does not require a confirmation test.

What result will I get from testing of SSIW (or sprouts) for *E. coli* O157:H7 and *Salmonella*?

Under the FDA reference method and other methods that FDA has recognized as scientifically valid and equivalent to the FDA reference method, testing for *E. coli* O157:H7 and *Salmonella* in SSIW or sprouts can yield one of two results:

- A confirmed positive result, which is obtained when screening procedures yield a presumptive positive result that is followed by confirmatory steps demonstrating the presence of *E. coli* O157:H7 or *Salmonella*; or
- A negative result, which can be obtained if either:
    - Screening procedures do not yield a presumptive positive result; or
    - Confirmatory steps after a presumptive positive result do not show the presence of *E. coli* O157:H7 or *Salmonella*.

What should I do if my screening test is positive?

If you receive a presumptive positive result from screening procedures, the FDA reference method and alternative methods that FDA has determined to be scientifically valid and equivalent to the FDA reference method require you or your laboratory to conduct confirmatory steps. The methods do not allow you or your laboratory to stop the analysis at the presumptive positive result.

What should I consider regarding testing of additional samples?

If you or your laboratory test two or more samples from the same production batch of sprouts and one sample receives a presumptive positive result, and the other sample tests negative, these methods require you to consider the entire sample result to be presumptive positive. Even if the rest of your samples test negative, that does not negate the first positive result.

Why may additional samples from the same production batch that tested positive give negative results?

Because the pathogens may be heterogeneously distributed and/or present at a very low level in the production batch, the contamination may not be detected in additional SSIW or sprout samples collected from elsewhere in the same sprout production batch or in the leftover from the original (non-enriched) SSIW or sprout sample that had a presumptive positive result.

What sample is used for the confirmation test?

Under either the FDA reference method or the alternative methods that FDA has determined to be scientifically valid and equivalent to the FDA reference method, positive results from screening procedures are considered presumptive and must be confirmed by standard microbiological methods. These methods require you or your laboratory to use the same enrichment sample that resulted in the presumptive positive result to confirm a presumptive positive result from the screening test.

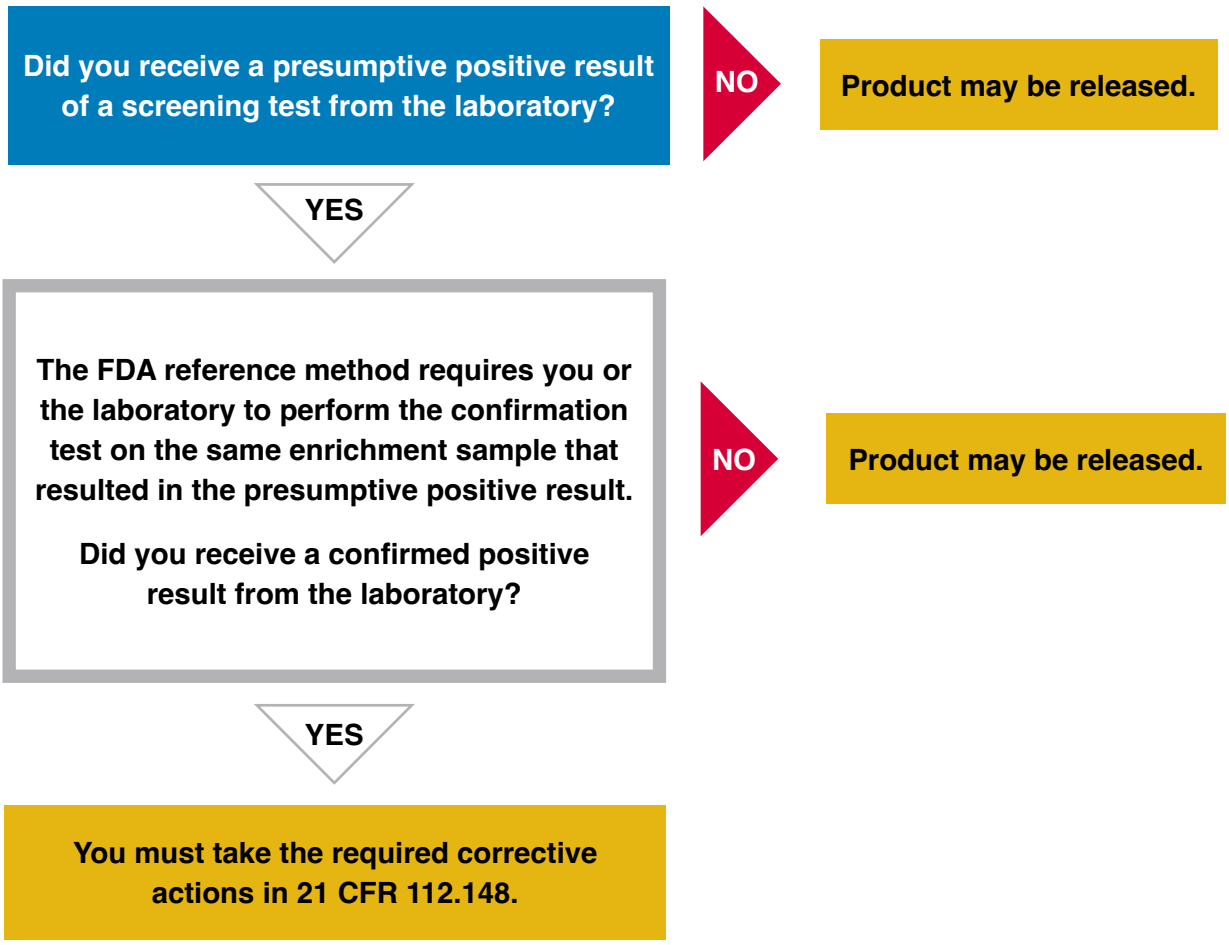
Real Consequences of Incorrect Testing

A sprout grower was testing SSIW and collected a sample from a growing production batch of sprouts. The test result was presumptive positive for *Salmonella*. Rather than conducting the confirmatory test on the same enrichment sample, the sprout grower conducted testing from a second sample from the same batch. The second test (not conducted on the original enrichment sample) was negative, and the sprout grower released the batch of sprouts. That batch of sprouts caused an outbreak of Salmonellosis which caused dozens of people to become ill. These events highlight the importance of conducting confirmatory testing on the same enrichment sample that was used for the screening test.

Where can I read more about the PSR requirements for SSIW (or in-process sprouts) testing?

You can find some of the requirements for testing of SSIW (or in-process sprouts) from each production batch of sprouts and sample collection in the Produce Safety Rule at 21 CFR 112.144(b) and 112.147. Further, the specific requirements for methods used to test SSIW (or sprouts) from each production batch of sprouts for *E. coli* O157:H7 and *Salmonella* can be found in the Produce Safety Rule at 21 CFR 112.153(a).

Below is a flow diagram for responding to results obtained from testing SSIW (or sprouts) for *E. coli* O157:H7 and *Salmonella* to meet the requirements of the Produce Safety Rule according to the FDA reference method. If the screening test returns negative results for *E. coli* O157:H7 and *Salmonella*, product may be released. If the result from the initial screening test for either *E. coli* O157:H7 or *Salmonella* is a presumptive positive, further confirmation is required to verify that the pathogen of concern is truly present. If the result is a confirmed positive, then certain required corrective actions (21 CFR 112.148) must be taken.



Below is a set of assessment questions for responding to results obtained from testing SSIW (or sprouts) for *E. coli* O157:H7 and *Salmonella* to meet the requirements of the Produce Safety Rule. If the screening test returns negative results for *E. coli* O157:H7 and *Salmonella*, product may be released. If the result from initial screening test for either *E. coli* O157:H7 or *Salmonella* is a presumptive positive, further confirmation is required to verify that the pathogen of concern is truly present. If the result is a confirmed positive, the required corrective actions (21 CFR 112.148) must be taken.

Text version

Did you receive a presumptive positive result of a screening test from the laboratory?

- If you say NO, you go to the box, “Product may be released.”
- If you say YES, you go to the box, “The FDA reference method requires you or the laboratory to perform the confirmation test on the same enrichment sample that resulted in the presumptive positive result. Did you receive a confirmed positive result from the laboratory?”
    - If you say NO, you go to the box, “Product may be released.”
    - If you say YES, you go to the box, “You must take the required corrective actions in 21 CFR 112.148.”

References

Ref. 1. Testing Methodologies for *E. coli* O157:H7 and *Salmonella* species in Spent Sprout Irrigation Water (or Sprouts), October 2015, Version 1, available at https://www.fda.gov/media/94349/download

Ref. 2. Equivalent Testing Methodologies for *E. coli* O157:H7 and *Salmonella* in Spent Sprout Irrigation Water or Sprouts Samples, Updated 03/16/2023, available at https://www.fda.gov/media/117047/download?attachment