Equivalent Testing Methodologies for *E. coli* O157:H7 and *Salmonella* in Spent Sprout Irrigation Water or Sprouts Samples

Updated 03/16/2023

FDA has determined that the following methods are "scientifically valid" and "at least equivalent to the method of analysis in § 112.153(a)(1) in accuracy, precision, and sensitivity"[1] in detecting *E. coli* O157:H7 and *Salmonella*. The method of analysis in § 112.153(a)(1) is "Testing Methodologies for *E. coli* O157:H7 and *Salmonella* species in Spent Sprout Irrigation Water (or Sprouts)" (October 2015, Version 1).

1. ***Salmonella* species***

    1) AOAC Official Method 999.08. Transia® AG *Salmonella* EIA in spent sprout irrigation water or sprouts.

    2) AOAC Official Method 999.09. VIP for *Salmonella* in spent sprout irrigation water or sprouts.

    3) AOAC Official Method 2011.03. VIDAS® *Salmonella* (SLM) Easy *Salmonella* in spent sprout irrigation water or sprouts.[2]

    4) AOAC Official Method 2016.01. 3M™ Molecular Detection Assay (MDA) 2 in spent sprout irrigation water.[3]

    5) Chapter 5 (*Salmonella*) of FDA's Bacteriological Analytical Manual (BAM, June 2021 Edition) in spent sprout irrigation water.

*Note: The sample size for testing in the listed methods above is 375 ml of spent sprout irrigation water or 375 g of sprouts.

[1] 21 CFR § 112.153(a)(2)
[2] The use of AOAC Official Method 2011.03 may be inadvisable when the total aerobic plate count in spent sprout irrigation water is more than 6 logs per ml. Using this method when spent sprout irrigation water has microorganism populations of more than 6 logs per ml may result in false negative results.
[3] The use of AOAC Official Method 2016.01 may be inadvisable when the total aerobic plate count in spent sprout irrigation water is more than 6 logs per ml. Using this method when spent sprout irrigation water has microorganism populations of more than 6 logs per ml may result in false negative results.

2. *E. coli* O157:H7

1) AOAC Official Method 996.09. VIP for EHEC Assay* in spent sprout irrigation water or sprouts.
2) AOAC Official Methods 2000.13 and 2000.14. Reveal for *E. coli* O157:H7* in spent sprout irrigation water or sprouts.
3) AOAC Official Method 2005.04. Assurance GDS® for *E. coli* O157:H7 in spent sprout irrigation water.

*** NOTE: The VIP for EHEC Assay and Reveal for *E. coli* O157:H7 are methods recommended in the 1999 FDA Guidance to the sprouts industry. The procedures for these methods have been modified to use the specific enrichment protocols listed below, therefore, these methods are a modification and no longer have the original status of AOAC Official Methods of Analysis. The procedures for VIP for EHEC Assay and Reveal for *E. coli* O157:H7 of *E. coli* O157:H7 in spent sprout irrigation water or sprouts are summarized below.**

I. Equipment and Materials

1. Mechanical blender (capable of 10,000 to 12,000 rpm) or Stomacher Model 400 (with required stomacher bags)
2. Sterile blender jars, with cover, resistant to autoclaving for 60 min at 121 °C
3. 1 Balance, with weights (2000 g capacity, sensitivity of 0.1 g)
4. 1 L Erlenmeyer flask
5. 2 Sterile graduated pipettes, 1.0 and 10.0 ml and pipette aids
6. Sterile instruments for use in taking and handling of samples (such as knives, tongs, scissors, spoons, etc.)
7. Sterile culture tubes, 16 x 150mm or 20 x 150mm
8. Incubator/shaker platform, 35 +/- 1 °C
9. pH meter or test strips
10. Fisher or Bunsen burner
11. Magnetic stirrer and stir bars
12. Sterile syringes
13. Sterile 0.2 m filters
14. Distilled water

II. Ingredients

1. Peptone
2. NaCl
3. Na2HPO4

4. KH2PO4
5. Casamino acid
6. Yeast extract
7. Lactose
8. Acriflavin (antibiotic)
9. Cefsulodin (antibiotic)
10. Vancomycin (antibiotic)

Preparation of antibiotic stock solutions

Prepare a stock solution of each antibiotic (acriflavin, cefsulodin, and vancomycin) by dissolving 1000 mg of each antibiotic in a separate tube containing 10.0 ml of distilled water. Filter-sterilize the solution using a 0.2 m filter and syringe. The stock solution may be stored for several months in foil wrapped tubes at 4°C (39.2°C).

Prepare the modified Buffered Peptone Water as described below, autoclave, cool, add antibiotic supplements. Instructions for sprouts are given in italics.

Modified Buffered Peptone Water (mBPW)

Step 1. To make 1000 ml of mBPW, mix the following constituents into distilled water, stirring to dissolve. For spent irrigation water, prepare double strength (2X) mBPW, as follows: *(If testing sprouts, use single strength (1X) enrichment broth base.)*

Modified Buffered Peptone Water(mBPW)		
Ingredient	Double strength (2X) (For use with spent irrigation water)	Single strength (1X) (For use with sprouts)
Peptone	20.0 g	10.0 g
NaCl	10.0 g	5.0 g
Na2HPO4	7.2 g	3.6 g
KH2PO4	3.0 g	1.5 g
Casamino acid	10.0 g	5.0 g
Yeast extract	12.0 g	6.0 g
Lactose	20.0 g	10.0 g
Distilled water*	1000 ml	1000 ml
*pH 7.2 +/- 0.2 (Test pH of distilled water BEFORE adding the ingredients above. If necessary, pH may be adjusted with 1N HCl or 1N NaOH.		

Step 2. Sterilize mBPW by autoclaving at 121°C (250°F) for 15 minutes. Remove from autoclave and allow to cool until cool to the touch.

Step 3. Once the medium is cooled and immediately prior to the addition of a subsample, add the following quantity of filter-sterilized antibiotics to 1000 ml of medium. For spent irrigation water, add the quantity of antibiotics listed in the column labeled double strength (2X) to the double strength mBPW. *(If testing sprouts, add the quantity of antibiotics listed in the column labeled single strength (1X) to the single strength mBPW.)*

Antibiotic supplements for mBPW		
Antibiotic Stock Solution	Double strength (2X) (For use with spent irrigation water)	Single strength (1X) (For use with sprouts)
Acriflavin (A)	0.2 ml	0.1 ml
Cefsulodin (C)	0.2 ml	0.1 ml
Vancomycin (V)	0.16 ml	0.08 ml

III. Testing

Step 4.

Spent Sprout Irrigation Water: Two (2) 100 ml subsamples of spent sprout irrigation water will be analyzed. From the 1000 ml sample of spent sprout irrigation water, aseptically transfer 100 ml of sample into a sterile 1L flask containing 100 ml of 2X mBPW+ACV. Repeat with second subsample.

Sprouts: Two (2) 50 g analytical units of sprouts will be analyzed. From two of the thirty-two 50 g analytical units collected, aseptically remove and weigh out a 25 g subsample of sprouts. Transfer each of the 25 gram subsamples of sprouts into separate sterile blender jars or sterile stomacher bags. Add 225 ml of single strength enrichment broth with added antibiotic supplements (1X mBPW+ACV) and blend at 10,000 to 12,000 rpm until homogenized (at least 60 seconds) or stomach for 2 minutes on medium setting in a Stomacher Model 400. Transfer sprout homogenate to a 1L Erlenmeyer flask.

Step 5. Incubate the enrichment broth/sample mixtures overnight at 42°C (107.6°F) with shaking at 140 RPM.

Step 6. Test each enrichment broth sample for the presence of *E. coli* O157:H7, using either the VIP EHEC device or the Reveal *E. coli* O157:H7 device. Use 0.1 ml from the inoculated and incubated mBPW +ACV to inoculate VIP or 0.12 ml for the Reveal. Follow the manufacturers instructions for the inoculation of test kits.

Step 7. Observe test results within 10 minutes to avoid possible fading of bands which could lead to false negative results. A band in both the test and control chambers is a positive test for contamination. A band in only the control chamber is a negative test. If a band does not appear in the control chamber, the test was not done correctly and must be repeated.

Update History

10/10/2018: Original publication

09/07/2021: Updated to add *Salmonella* method 5 (Chapter 5 (*Salmonella*) of FDA's Bacteriological Analytical Manual (BAM, June 2021 Edition) in spent sprout irrigation water)

03/16/2023: Updated to add note specific to *Salmonella* methods 3 (AOAC Official Method 2011.03) and 4 (AOAC Official Method 2016.01)