Preparation Recommendation for the Detection of *Salmonella* in basil, cabbage, cilantro, green onions, lettuce, parsley, spinach, and sprouts
Updated: 01/05/2018

Please note, this recommendation is intended to provide supplemental general information to private laboratories on how to perform the initial sample preparation for basil, cilantro, green onions, lettuce, parsley and spinach. This document **<u>does not</u> outline all of the analytical method or worksheet requirements for packages being submitted for FDA review. **

Please refer to the current FDA Private Laboratory Guidance for comprehensive information on private laboratory package requirements and the review process: http://www.fda.gov/downloads/ScienceResearch/FieldScience/LaboratoryManual/ UCM092191.pdf

Analytical Protocol

Samples should consist of 10 sub-samples for official analysis. An additional sub- sample of produce will be required if a matrix spike is being performed (refer to Matrix Validation/Spike section below).

Pre-sample preparation:

Do not rinse the produce, even if there is visible dirt. Examine the produce "as is".

Sub-sample soak preparation:

Combine 75 g from each of 5 individual sub-samples into a sterile flask (375 g composite), or other appropriate container, to make the first composite. Combine 75 g from the other each of 5 individual sub-samples to make the second composite. Add 3375 ml preenrichment media **(lactose broth for basil and green onions; Universal Preenrichment (UP) broth for cilantro, lettuce, parsley spinach, and sprouts; Modified Buffered Peptone Water (mBPW) for cabbage)** to each composite and swirl, so that all of the produce is completely wet. Loosely cap the flask. Adjust pH to 6.8 ± 0.2, if necessary. Allow the samples to remain in the pre-enrichment broth during incubation.

Sample preparation/method:

Incubate composites at 35 ± 2º C for 24 ± 2 h. After preenrichment, the selective enrichment strategy is dependent on whether the culture method or a rapid method is to be used.

For the *Salmonella* culture method (BAM and AOAC Official Method 995.20)

- Transfer **0.1** ml preenriched culture from composite 1 to 10 ml Rappaport-Vassiliadis (RV) medium; transfer **0.1** ml preenriched culture from composite 2 to 10 ml RV medium; Vortex
  - Incubate 2 RV medium for 24 ± 2 h at 42 ± 0.2°C in a circulating thermostatically controlled waterbath.
- Transfer 1 ml preenriched culture from composite 1 to 10 ml tetrathionate (TET) broth; Transfer 1 ml preenriched culture from composite 2 to 10 ml tetrathionate (TET) broth; Vortex
  - Incubate TET broth for 24 ± 2 h at 43 ± 0.2°C **(treat all of the above produce as high microbial load foods)** in a circulating thermostatically controlled waterbath.
- After incubation, follow the BAM Online, *Salmonella,* Chapter 5, section D, *Isolation of Salmonella*.

For rapid methods, selectively enrich as instructed by the kit manufacturer. For example:

- AOAC Official Method 2004.03: VIDAS *Salmonella* (SLM) Assay use RV medium and TET broth
  - o Analysis of RV and TET composites proceeds according to the AOAC Official Method 2004.03

- AOAC Official Methods 989.14, 990.13, 992.11 or 996.08 use selenite cysteine (SC) and TET broths.
  - o Analysis of SC and TET composites proceeds according to the AOAC Official Method 989.14, 990.13, 992.11 or 996.08

- AOAC Official Method 2011.03: VIDAS® *Salmonella* (SLM) Easy *Salmonella* Method use SX2 broth
  - o Transfer **0.1** ml preenriched culture from composite 1 to 10 ml SX2 broth; transfer **0.1** ml pre-enriched culture from composite 2 to 10 ml SX2 broth; Vortex

- Samples found positive are confirmed as directed in the BAM Online.

- After incubation of the TET and RV or SC, or SX2 composites, continue as directed in the BAM Online,

http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm

Matrix Validation/Spiking:

- Laboratories must demonstrate successful detection of *Salmonella* for the methodology being utilized by analyzing a spiked matrix concurrently with the sample through confirmation.

- Matrix spike should consist of an inoculum of 30 cells or less of *Salmonella* added to a matrix control sample. A negative matrix spike will invalidate the analysis.

- Matrix spike details (CFU or colonies/gram) must be included with the analytical package.

- Once a laboratory can demonstrate that matrix spikes have yielded at least seven positive and no negative matrix spikes or a >95% confidence level (19 of 20 samples positive), the matrix can be considered validated for the method being used. The laboratory can discontinue performing matrix spikes on subsequent samples analyzed with that method, **but must submit documentation of the matrix validation data with each subsequent sample analytical package submitted for FDA review.**

Quality Assurance:

- The Quality Assurance information for the media and reagents used in the analysis must be submitted with the analytical package (e.g. pH check, autoclave run time/temp documentation, performance and sterility of media, etc.).

- Laboratory must follow the methodology specified in the private laboratory package submission. Any method modifications or deviations to the cited method must be explained and validation must be documented.