Preparation Recommendation for the Detection of *Salmonella* in Cucumbers, Sweet peppers (bell), and Hot peppers (Jalapeno and Serrano) 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 cucumbers, sweet peppers (bell), and hot peppers (jalapeno and serrano). This document **does not 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 cucumber, sweet pepper, and hot pepper will be required if a matrix spike is being performed (refer to Matrix Validation/Spike section below).

Pre-sample preparation:

Do not rinse the cucumbers, sweet peppers (bell), and hot peppers (jalapeno and serrano), even if there is visible dirt. Examine the produce “as is”.

Sub-sample soak preparation:

For each individual sub-sample (e.g. approximately 454 g cucumbers, or bell peppers, or hot peppers), place contents into a sterile plastic bag (Biopro Sample

Bag, 12 x 18 inches, catalog number BP-41218, available from International Bioproducts, phone 800-729-7611 or equivalent). For sweet peppers (bell), crush or cut each pepper so that the interior of the pepper is exposed to the enrichment broth. For hot peppers and cucumbers, no need to cut or crush. Add enough **lactose broth** to fully immerse the peppers or cucumbers. This volume of lactose broth should be approximately 1.5 times the weight of the peppers or cucumbers. For example, peppers or cucumbers weighing 454 g will probably need a volume of approximately 681 ml lactose broth to be fully immersed. Add more lactose broth, if necessary. Clips may be used to keep the peppers fully immersed. Place the plastic bag, with peppers or cucumbers and lactose broth, into a non-sterile 5 liter beaker, or other appropriate container, for support during incubation. Let stand for 60 ± 5 min. Adjust pH to 6.8 ± 0.2, if necessary. Allow the open-end flap of the plastic bag to "fold over" to form a secure, but not air-tight, closure during incubation.

Sample preparation/method:

Incubate each, individual cantaloupe sub-sample at 35º±2º C for 24 ± 2 hours. After incubation, the sub-sample pre-enrichments are then to be "wet composited".

Example of a Wet Compositing Procedure for methods using RV/TT selective enrichments

- From each of 5 incubated sub-samples, remove **0.1** ml preenriched culture and place into a tube or flask containing 50 ml Rappaport-Vassiliadis (RV) medium (Composite 1). For the other 5 incubated sub-samples, remove **0.1** ml preenriched culture and place into a tube or flask containing 50 ml RV medium (Composite 2).

    - Incubate the 2 RV medium composites at 42 ± 0.2º C in a circulating, thermostatically controlled water bath for 24 h ± 2.

- In addition to sub-culturing the sub-sample pre-enrichments to RV medium, these sub-sample pre-enrichments are to be sub-cultured to tetrathionate (TET) broth. From each of 5 incubated sub-samples, remove **1.0** ml preenriched culture and place into a tube containing 50 ml TET broth (Composite 1). For the other 5 incubated sub-samples, remove **1.0** ml preenriched culture and place into a tube containing 50 ml TET broth (Composite 2).

- Incubate the 2 TET broth composites at 35±2°C in a circulating, thermostatically controlled water bath for 24 h ± 2.

- After incubation of the RV and TET composites, continue as directed in 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 uses RV medium and TET broth
  - Analysis of RV and TET composites proceeds according to the AOAC Official Method 2004.03

- AOAC Official Method 2011.03: VIDAS® *Salmonella* (SLM) Easy *Salmonella* Method uses SX2 broth
  - From each of 5 incubated sub-samples, remove 0.1 ml preenriched culture (total of 0.5ml) and place into a tube containing 50 ml SX2 broth. Incubate for 24±2 h at 42 ± 1°C. Repeat the procedure for the other 5 incubated sub-samples.

- Samples found positive are confirmed as directed in the BAM Online.
  - After incubation of the RV and TET or SX2 composites, continue as directed in the BAM Online, http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm

Note: Only the VIDAS *Salmonella* (SLM) Assay (AOAC Official Methods **996.08 and 2004.03**, **and 2011.03**) have been validated for use with **cantaloupes**.

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 their 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.