Papaya Composite Sample Preparation Recommendation for the Detection *Salmonella* Updated: 5/20/2016

Please note, this recommendation is intended to provide supplemental general information to private laboratories on how to perform the initial sample preparation for papayas. 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

Preparation Recommendation

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

Analytical Protocol:

Samples consist of 10 sub samples.

Preparation Modified Buffered Peptone Water

Peptone	10.0 g
Sodium Chloride	5.0 g
Disodium Phosphate (Na_2HPO_4)	7.0 g
Monopotassium Phosphate (KH_2PO_4)	3.0 g
Distilled water	1000 mL

pH to 7.2±0.2

Note: commercial Buffered Peptone Water (BPW; BD catalog number 218105) may be substituted for the above formulation. If a commercial preparation is used, then add 3.5 g of Na_2HPO_4 and 1.5 g of KH_2PO_4 to 20 g commercial BPW in 1000 ml distilled water.

Sterilize by autoclaving 15 min at 121°C.

Sub-sample soak preparation:

For each individual sub sample (e.g. one papaya), 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). Add a volume of modified buffered peptone water (mBPW) that is needed to allow the papayas to float. Normally this volume of mBPW is 1.5 times the weight of the papaya. Allow the open-end flap of the plastic bag to "fold over" so as to form a secure, but not airtight, closure during incubation.

Sample preparation/method:

Incubate each individual sub sample in mBPW for 24 hours at 42°C.

After incubation, the sub sample pre-enrichments are then to be "wet composited".

- **Example:** Wet Compositing Procedure for methods using RV/TT selective enrichments
    - From each of 5 incubated sub samples, remove **0.1** ml mBPW pre-enrichment 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 mBPW and place into a tube or flask containing 50 ml RV medium (Composite 2).
        - Incubate the 2 RV medium composites at 42 ± 0.2° C in a circulating, thermostatically controlled water bath for 18-24 h.
    - 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 mBPW pre-enrichment and place into a tube containing 50 ml TET broth (Composite 1). For the other 5 incubated sub samples, remove **1.0** ml mBPW and place into a tube containing 50 ml TET broth (Composite 2).

        - Incubate the 2 TET broth composites at 43 ± 0.2º C in a circulating, thermostatically controlled water bath for 18-24 h.

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

- **Example:** Wet Compositing Procedure for PCR-based methods :
    - From each of 5 incubated sub samples: remove 1 ml if each pre-enriched mBPW broth into one sterilized tube (total of 5 ml of 1 composite). Repeat with the other 5 subs samples for composite 2.
    - Analysis of pre-enrichment broth composites proceeds according method protocol being utilized.
    - Perform the qPCR from each individual sub-sample if the qPCR result is positive from composite samples.

        - Note: If lactose broth is utilized as the initial enrichment broth for any PCR based method, the sub samples **must** be analyzed individually.

    - It is acceptable for laboratories to use any AOAC Official Method for *Salmonella*.
      For rapid method kits, selectively enrich as instructed by the kit manufacturer.

- **Example**: In addition, the VIDAS® Easy method (OMA First Action Method 2011.03) may be used.

    - From each of 5 incubated sub samples, remove 0.1 ml mBPW pre-enrichment (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.

Confirmation:

- 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

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 papaya 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 papaya 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 papaya samples analyzed with that method, **but must submit documentation of the matrix validation data with each subsequent papaya 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.