Method Recommendations for Detecting *Salmonella* in Whole Mangos

Updated: 05/29/2026

Please note, this guidance is intended to provide supplemental general information to private laboratories on how to perform the initial sample preparation for mangos. This guidance document **does not outline all 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: Laboratory Procedure-Private Laboratory Analytical Packages

Analytical Protocol

1. **Sample Size**

   Collect 30 sub-samples for official analysis. One additional sub-sample may be required for matrix spike control (refer to Matrix Validation/Spike section below).

2. **Sample Preparation for Pre-enrichment**

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

   For each individual sub-sample (e.g. one large mango, or approximately 454g; 1lb), place contents into a sterile plastic bag (Nasco Whirl-Pak™ sterile bag, or equivalent). Add a volume of pre-warmed Buffered Peptone Water (BPW) at 35 °C that is needed to allow the mango to float. Normally this volume of BPW is 1.0 times the weight of mangos. For example, mangos weighing 454g will need a volume of 681 ml BPW. Place the plastic bag, with mangos and BPW, into a non-sterile 5-liter beaker for support during incubation. Allow the open-end flap of the plastic bag to "fold over" so as to form a secure, but not airtight, closure during incubation. Stand for 60 ± 5 min at room temperature. Adjust pH to 6.8 ± 0.2, if necessary. Incubate each individual sub-sample at 35 ± 2º C for 24 ± 2 h.

3. Wet Compositing for Selective Enrichment

Wet compositing depends on whether the culture method or a rapid method is to be used.

- **Wet Compositing for Culture Method**
  - **RV Composites:** Remove 0.1 mL of pre-enriched culture from each of five pre-enriched sub-samples (total 0.5 mL) and combine into a tube or flask containing 50 mL Rappaport-Vassiliadis (RV) broth to create one RV composite. For ten pre-enriched sub-samples, prepare two wet RV composites. Incubate the RV composites at 42 ± 0.2°C in a circulating, thermostatically controlled water bath for 24 ± 2 hours.

  - **TT Composites:** Remove 1.0 mL of pre-enriched culture from each of five pre-enriched sub-samples (total 5 mL) and combine in a tube containing 50 mL tetrathionate (TT) broth to create one TT composite. For ten pre-enriched sub-samples, prepare two wet TT composites. Incubate the TT composites at 43 ± 0.2°C **(treat mango as high microbial load food)** in a circulating, thermostatically controlled water bath for 24 ± 2 hours.

- **Wet Compositing for screening using FDA *Salmonella* qPCR method**
  - Mix 1ml of pre-enriched culture from each of 5 incubated sub-samples into a sterile tube to create a 5 ml wet composite
  - Take 1ml from the 5 ml wet composite for DNA extraction and qPCR following the FDA qPCR protocol (https://www.fda.gov/media/168834/download?attachment)
  - For presumptive positive wet composite, transfer 0.1 mL to 10 mL RV and 1 mL to 10 mL TT broth. Incubate the RV broth at 42 ± 0.2°C and the TT broth at 43 ± 0.2°C in a circulating, thermostatically controlled water bath for 24 ± 2 hours.

Note: Only the FDA *Salmonella* qPCR method has been validated and verified for detection of *Salmonella* in wet composited pre-enriched cultures of large fruits and vegetables. Using other validated molecular methods for wet composited pre-enriched cultures must be validated for FDA approval.

- **Wet Compositing for screening using AOAC VIDAS methods (2004.03, 2011.03)**

    - **AOAC Official Method 2004.03:** Use wet compositing of RV and TT as described above in section wet composting for culture method. Analyze RV and TT composites according to AOAC Official Method 2004.03.

    - **AOAC Official Method 2011.03:** Remove 0.1 mL of pre-enriched culture from each of five incubated sub-samples (total 0.5 mL) and combine in a tube containing 50 mL SX2 broth to create one SX2 composite. For ten incubated sub-samples, prepare two wet SX2 composites. Analyze the SX2 composites according to AOAC Official Method 2011.03.

4. Isolation, Identification and confirmation

Use methods detailed in BAM Chapter 5 *Salmonella*, Section E-F for isolation, identification and confirmation: https://www.fda.gov/media/178914/download?attachment

5. 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 or less *Salmonella* cells added to a matrix control sample. A negative matrix spike will invalidate the analysis.
- Matrix spike details (CFU/test portion) must be included with the analytical package.
- Once a laboratory can demonstrate that their matrix spikes have yielded at least seven consecutive positive and no negative matrix spikes or a >95% confidence level (19 of 20 samples positive) over time, the matrix can be considered validated for the method being used. The laboratory can discontinue performing matrix spikes on subsequent matrix samples analyzed with that method but **must submit documentation of the matrix validation data with each subsequent matrix sample analytical package submitted for FDA review.**

6. 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.