

Preparation Guidance for the detection of *Salmonella* in Cantaloupe **Updated: 11/02/2021**

Please note, this guidance is intended to provide supplemental general information to private laboratories on how to perform the initial sample preparation for cantaloupe. This guidance document **does not outline all of the analytical method or worksheet requirements for packages being submitted for FDA review.
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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 30 sub-samples for official analysis. An additional sub-sample of cantaloupe will be required if a matrix spike is being performed (refer to Matrix Validation/Spike section below).

Pre-sample preparation:

Do not rinse the cantaloupes, even if there is visible dirt. Examine the cantaloupe “as is”.

Sub-sample blend preparation for comminuted or cut fruit:

For comminuted or cut fruit, preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C.

Combine 25 g analytical unit from each of 15 individual sub-samples into a sterile blender jar (375 g composite), to make the first composite. Combine 25 g analytical unit from the other each of 15 individual sub-samples to make the second composite into a sterile blender jar. Add 3375 ml **Universal Pre-**

enrichment broth (UPB) to each composite and blend 2 min at 10,000 to 12,000 rpm, If blender jars do not exist that are capable of blending the entire sub-sample at a 1:9 sample-to-broth ratio, then the sub-sample should be divided into blendable portions and re-combined into a single container after blending. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/4 turn.

Sub-sample soak preparation for whole fruit:

For each individual sub-sample (e.g. one cantaloupe), place contents into a sterile plastic bag (Nasco Whirl-Pak™ sterile bag, or equivalent). Add a volume of **Universal Pre-enrichment broth (UPB)** that is needed to allow the cantaloupe to float. Normally this volume of UPB is 1.5 times the weight of the cantaloupe. For example, a cantaloupe weighing 1200 g will need a volume of 1800 ml UP broth. Place the plastic bag, with cantaloupe and UPB, 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.

Sample preparation/method for comminuted or cut fruit:

For comminuted or cut fruit, incubate composites at $35 \pm 2^\circ \text{C}$ for 24 ± 2 h. After pre-enrichment, the selective enrichment strategy is dependent on whether the culture method or a rapid method is to be used.

For the BAM *Salmonella* culture method:

- Transfer **0.1** ml pre-enriched culture from composite 1 to 10 ml Rappaport-Vassiliadis (RV) medium; transfer **0.1** ml pre-enriched culture from composite 2 to 10 ml RV medium; Vortex
 - Incubate 2 RV medium for 24 ± 2 h at $42 \pm 0.2^\circ \text{C}$ in a circulating thermostatically controlled water bath.
- Transfer 1 ml pre-enriched culture from composite 1 to 10 ml tetrathionate (TET) broth; Transfer 1 ml pre-enriched culture from composite 2 to 10 ml tetrathionate (TET) broth; Vortex

- Incubate TET broth for 24 ± 2 h at $35 \pm 2^\circ\text{C}$ in a circulating thermostatically controlled water bath.
- After incubation, follow the BAM Online, *Salmonella*, Chapter 5, section D, *Isolation of Salmonella*:
<https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm>

For rapid method kits, 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
 - Transfer **0.1** ml pre-enriched 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.
<http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm>

Sample preparation/method for whole fruit:

Incubate each, individual cantaloupe sub-sample at $35 \pm 2^\circ\text{C}$ for 24 ± 2 h. After incubation, the sub-sample pre-enrichments are then to be “**wet composited**”. Wet compositing strategy is dependent on whether the culture method or a rapid method is to be used.

For the BAM *Salmonella* culture method, Wet Compositing Procedure using RV/TET selective enrichments:

- From each of 5 incubated sub-samples, remove **0.1** ml pre-enriched culture and place into a tube or flask containing 50 ml Rappaport-Vassiliadis (RV) medium to make one RV medium composite. For 30 incubated sub-samples to make 6 wet RV medium composites.
 - Incubate the 6 RV medium composites at $42 \pm 0.2^\circ \text{C}$ in a circulating, thermostatically controlled water bath for 24 ± 2 h.
- From each of 5 incubated sub-samples, remove **1.0** ml pre-enriched culture and place into a tube containing 50 ml TET broth to make one TET medium composite. (composite 1). For 30 incubated sub-samples to make 6 wet TET medium composites.
 - Incubate the 6 TET broth composites at $43 \pm 0.2^\circ \text{C}$ in a circulating, thermostatically controlled water bath for 24 ± 2 h.
- After incubation, follow the BAM Online, *Salmonella*, Chapter 5, section D, *Isolation of Salmonella*:
<https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm>

**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
 - Wet compositing procedure using RV and TET as above
 - 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

- Wet compositing procedure using SX2
 - From each of 5 incubated sub-samples, remove 0.1 ml pre-enriched culture (total of 0.5ml) and place into a tube containing 50 ml SX2 broth make one SX2 medium composite. For 30 incubated sub-samples to make 6 wet RV medium composites.
 - Incubate at $42 \pm 1^\circ\text{C}$ for 24 ± 2 h
- Samples found positive are confirmed 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 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 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.**

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.