

Preparation recommendation for the detection of *Salmonella* in nut meats and nut seeds
Updated: 05/25/2021

Please note, this guidance is intended to provide supplemental general information to private laboratories on how to perform the initial sample preparation for nut meats and nut seeds. This guidance 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 30 sub-samples for official analysis. An additional sub-sample of nuts will be required if a matrix spike is being performed (refer to Matrix Validation/Spike section below).

Pre-sample preparation:

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

Sub-sample soak preparation:

Combine 25 g analytical unit from each of 15 individual sub-samples into a sterile flask (375 g composite), or another appropriate container, to make the first composite. Combine 25 g analytical unit from the other each of 15 individual sub-samples to make the second composite. Add 3375 ml **Universal Pre-enrichment broth (UPB)** to each composite and swirl, so that all of the nut meat or nut seeds are completely wet. Loosely cap the flask. Allow the samples to remain in the pre-enrichment broth during incubation.

Sample preparation/method:

Incubate composites at $35 \pm 2^\circ \text{C}$ for $24 \pm 2 \text{ h}$. 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 $24 \pm 2 \text{ h}$ 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 $24 \pm 2 \text{ h}$ at $35 \pm 0.2^\circ \text{C}$ (**treat all nut meats or nut seeds as low microbial load foods**) 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/laboratory-methods-food/bam-chapter-5-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

- 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 and incubate for 24 ± 2 h at $42 \pm 1^\circ\text{C}$.
- Samples found positive are confirmed as directed in the BAM Online: <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella>

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.