

**Sampling and Testing Method Guidance for the Detection of Norovirus and Hepatitis A
Virus in Soft Fruit
Date: 04/06/2020**

PART I – Introduction

PART II – Sampling

PART III – Verification recommendations

**PART IV – Reference Method: FDA’s Concentration, Extraction, and Detection of
Norovirus and Hepatitis A virus in Soft Fruit**

PART I - Introduction

1. Scope

This document is intended to provide supplemental general information to private laboratories on how to perform detection of Hepatitis A virus and/or Norovirus genogroups I and II from Soft Fruit. This document does not outline all the analytical method or worksheet requirements for packages being submitted for FDA review.

Please refer to the current FDA Laboratory Manual, Volume III, Section 7 for comprehensive information on private laboratory package requirements and the review process:

<https://www.fda.gov/media/73540/download>

2. Purpose

The purpose of this guidance document is to describe:

- Sampling methods.
- Reference method procedures.
- Validation requirements for methods other than the reference method that are utilized to ensure the alternate method is equivalent to the reference method (HAV LOD 0.1 PFU/g).
- Verification recommendations to ensure the:
 - Reference method functions (without any adaptation) in the user’s laboratory;
 - Reference method can detect and identify the analyte of interest; and
 - Performance of the reference method in the user’s laboratory meets the specifications of the method (HAV LOD 0.1 PFU/g).

3. Background

The “FDA’s Concentration, Extraction, and Detection of Norovirus and Hepatitis A virus in Soft Fruit”, described in Part IV, was developed by CFSAN’s Gulf Coast

Seafood Laboratory as a matrix and platform extension to BAM 26B. The method provides a rapid concentration of norovirus and HAV from soft fruit using murine norovirus as an extraction control. The method provides RT-qPCR assays for the detection of norovirus, HAV and murine norovirus with the inclusion of an internal amplification control (IAC) using the ABI 7500. **Valid norovirus and/or HAV sample results are contingent upon the successful detection of the extraction control from the sample being tested.**

The method “RT-qPCR Detection of Mengovirus Using ABI 7500 Platform” was developed by FDA’s GCSL to provide an alternate surrogate virus to use as an extraction control when using the reference method. Procedures for preparation of the ATCC mengovirus for use as an alternative extraction control for spiking of samples prior to the concentration and detection of target viruses is described. Procedures for the preparation of the mengovirus PCR positive control are also included in the work instruction. This method is intended to be used in conjunction with the reference method with mengovirus substituted for murine norovirus as the extraction control.

PART II – Sampling

- When possible, collect three, 400 gram (one pound is 454 grams) sub-samples of product per sample (LOT). Do not collect co-mingled lots.
- Three subs per sample should be collected. Each sub-sample will be tested individually.
- Document the lot code and any other unique identifiers on the product labeling for each sample.
- Provide photos of the entire labeling of the box sampled, including any identifier of the manufacturer.
- Collect all samples aseptically, per the current IOM. Sample temperature should be maintained throughout the collection and shipment process. Ample dry ice should be utilized for frozen samples and samples should be shipped for next day am delivery.

PART III- Verification and Spiking Recommendation

Method verification for FDA’s [Concentration, Extraction, and Detection of Norovirus and Hepatitis A virus in Soft Fruit](#) (HAV LOD 0.1PFU/g) data must be submitted prior to (or concurrently with) the first sample data package submission.

1. Verification scheme required for labs performing the FDA validated method or other validated approved method for the first time:

- 2 Uninoculated samples
- 2 low spikes (at or near LOD = 0.1 PFU HAV/g)
- 2 medium spikes (10 x LOD = 5 PFU HAV/g)
- All 50 g samples must include an extraction control

2. Validation Requirements for laboratories using a method not validated against the FDA reference method.

If the laboratory chooses to use a non-FDA approved method, the proposed alternative method must have been validated against the FDA reference method following the FDA's Guidelines for the Validation of Microbiological Methods for the FDA Foods Program, 3rd edition to demonstrate equivalence. The laboratory submitting information using the alternative method must also submit the validation package which demonstrates the same performance specifications as the FDA reference regulatory method.

PART IV- Reference Method

The protocol for the Concentration, Extraction, and Detection of Norovirus and Hepatitis A Virus in Soft Fruit is available on the FDA Compendium site:

<https://www.fda.gov/food/laboratory-methods-food/foods-program-compendium-analytical-laboratory-methods>

Instructions for preparing the surrogate extraction control virus (MNV) can be found in the FDA BAM on-line Chapter 26B: Detection of Hepatitis A Virus in Foods, Appendix G:
<https://www.fda.gov/food/laboratory-methods-food/bam-26b-detection-hepatitis-virus-foods>

The protocol "RT-qPCR Detection of Mengovirus Using ABI 7500 Platform" is included in attachment A.

Information on ordering Mengovirus from Spanish ATCC:

<https://www.uv.es/uvweb/spanish-type-culture-collection/en/news/cect-incorporates-mengovirus-vmco-public-catalogue-1285923246228/Novetat.html?id=1286062095701>