U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

July 22, 2022

Alison Bodor
President and CEO
American Frozen Food Institute
2345 Crystal Drive, Suite 801
Arlington, VA 22202

Re: Docket No. FDA-2022-P-0392

Dear Ms. Bodor:

This letter responds to your petition for a stay of action, which was submitted to the Food and Drug Administration (FDA or "we") on behalf of the American Frozen Food Institute (AFFI), Washington Red Raspberry Commission, and Oregon Raspberry and Blackberry Commission (collectively, "Petitioner"). Your petition requests that FDA stay the effective date of its planned resumption of the microbiological sampling assignment for frozen berries (see Petition for Stay of Action from Alison Bodor, President and CEO, AFFI, et al., dated March 18, 2022 ("petition")). Specifically, the petition requests that we "stay the sampling assignment until such time as FDA takes the following steps to ensure that the test methodology and interpretation of results as used in the study and that form the basis of the agency's decision to request voluntary product recalls when it detects Hepatitis A virus (HAV) or Norovirus (NoV) are grounded in an appropriate scientific basis:

1. FDA should perform a multi-laboratory validation of the FDA method for the detection of enteric viruses (HAV and NoV) in soft fruits, i.e., the reverse transcription quantitative polymerase chain reaction (RT-qPCR) detection assay, using independent laboratories outside the agency.
2. Once externally validated, the FDA method for the detection of enteric viruses in soft fruits, i.e., the RT-qPCR detection assay, should be published as a scientific manuscript in the peer-reviewed literature.
3. After steps 1 and 2 are complete, FDA should release protocols for the method in FDA's Bacteriological Analytical Manual (BAM) that are sufficiently detailed to enable the method to be evaluated and reliably reproduced by the relevant scientific community.
4. FDA should convene an international panel of experts (including members from within FDA such as MOD 1, the [Centers for Disease Control and Prevention (CDC)], [the United States Department of Agriculture's (USDA's)] Food Safety and Inspection Service, and academia) with expertise in virology and microbial risk assessments in

foods, tasked with establishing transparent and risk-based interpretive criteria, including sample positivity and negativity criteria, for interpreting RT-qPCR tests of enteric viruses in soft fruits, as well as interpretive criteria for results confirmed through sequencing. The panel's findings should be made publicly available, and the agency should provide an opportunity for public comment, before adopting the findings, as appropriate" (Petition at 1-2).

We have evaluated your petition in accordance with 21 CFR 10.35(e), and for the reasons described below, we are denying your petition. We have also provided background on the public health risks of hepatitis A and norovirus, the rationale for and evolution of FDA's frozen berry sampling assignment, and FDA's process for developing and validating analytical laboratory methods (including the RT-qPCR detection assay used in the frozen berry sampling assignment).

I. Background

A. Significant Public Health Risks Posed by Hepatitis A and Norovirus

Hepatitis A virus (HAV) is a very contagious foodborne virus that can spread via consumption of contaminated food or drink and through close personal contact with an infected person.[1] Food can be contaminated with HAV at any point during cultivation, harvesting, processing, or preparation.[2] HAV is very infectious, with small quantities capable of causing disease.[3,4] It has a long incubation and shedding period (peak shedding occurs in the two weeks prior to the onset of clinical symptoms and can continue after symptoms end) which can contribute to transmission while infected persons are asymptomatic.[5] Symptoms appear between 15 and 50 days after infection and can include yellow eyes or skin, abdominal pain, or pale stools.[6] Infection with HAV can result in liver inflammation and damage.[7] Illness can range in severity from a mild condition lasting a few weeks to a severe disease lasting several months and, in rare instances, death.[8] Although foodborne illnesses from HAV are less common than foodborne illnesses associated with norovirus, they are of concern due to the potential severity of illness.

Norovirus is a very contagious foodborne virus that can easily spread from an infected person through contaminated food or water, or by touching contaminated surfaces. Food can be

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[1] Centers for Disease Control and Prevention (CDC). 2020. Hepatitis A Questions and Answers for the Public. https://www.cdc.gov/hepatitis/hav/afaq.htm. Accessed June 29, 2022.
[2] Fiore, A. 2004. Hepatitis A Transmitted by Food. Clinical Infectious Diseases. 38:705-15.
[3] Bosch, A., Gkogka, E., Le Guyader, F.S., et al. 2018. Foodborne viruses: Detection, risk assessment, and control options in food processing. International Journal of Food Microbiology 285:110-128.
[4] Grabow, W. 1997. Hepatitis viruses in water: Update on risk and control. Water SA. 23:379-386.
[5] Food Standards Australia New Zealand (FSANZ). 2021. Imported food risk statement. Fresh and frozen ready-to-eat berries and hepatitis A virus. https://www.foodstandards.gov.au/consumer/importedfoods/Documents/Fresh%20and%20frozen%20berries%20and%20HAV%20OCT%2021.pdf. Accessed June 29, 2022.
[6] CDC, supra note 1.
[7] CDC, supra note 1.
[8] CDC, supra note 1.

contaminated with norovirus during cultivation, harvesting, processing, or preparation.[9] Norovirus is very infectious, with small quantities capable of causing disease.[10] Persons can develop gastrointestinal symptoms within 12 to 48 hours after being exposed to norovirus. Norovirus causes inflammation of the stomach or intestines, leading to diarrhea and/or vomiting and, in rare instances, death.[11] Norovirus causes 58% of foodborne illnesses acquired in the U.S., and is the leading cause of foodborne illness and outbreaks in the nation.[12]

B. FDA's Frozen Berries Sampling Assignment

1. Goals of the Sampling Assignment

The frozen berries sampling assignment is intended to fill an important gap in FDA's efforts to better understand and prevent viral foodborne illnesses. It will provide useful data that will inform policy, assist with the development of important guidance documents to support industry food safety activities, and facilitate compliance with FDA regulations, including Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food (21 CFR part 117) and Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (21 CFR part 112).

The frozen berries sampling assignment's objectives include:

- Estimating and comparing (when possible) the current prevalence of HAV and norovirus in domestic and imported frozen berries;
- Determining if there are common factors (such as country of origin) associated with positive findings and utilizing data to guide future programmatic sample planning; and
- Taking appropriate regulatory action when positive findings are observed.[13]

FDA will evaluate the data generated throughout the sample collection period and use the data to inform our short- and longer-term decision making. The data can help FDA identify potential vulnerabilities and ways to enhance the food safety system, such as by helping FDA determine if there are common factors among positive findings, e.g., origin, variety, or season.

FDA regularly uses sampling assignments to fill knowledge gaps and inform our regulatory approach. For example, since 2020, FDA has utilized sampling assignments as part of its Leafy Greens STEC (Shiga toxin-producing *E. coli*) Action Plan (LGAP), which is intended to help FDA address issues associated with leafy green STEC contamination. As part of the LGAP,

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[9] Bozkurt, H., Phan-Thien K.Y., van Ogtrop, F., Bell, T., McConchie, R. 2021. Outbreaks, Occurrence, and Control of Norovirus and Hepatitis A Virus contamination in berries. A review. Critical Reviews in Food Science and Nutrition. 61:1 116-138.
[10] Bokzurt et al., supra note 9.
[11] CDC. 2021. About Norovirus. https://www.cdc.gov/norovirus/about/index.html. Accessed June 29, 2022.
[12] CDC. 2021. Burden of Norovirus Illness in the U.S. https://www.cdc.gov/norovirus/trends-outbreaks/burden-US.html. Accessed June 29, 2022.
[13] Memorandum to FDA Industry & Regulatory Stakeholders. Summary of External Feedback and FDA Responses to the FY 19-20 Surveillance Sampling Program Assignment: Frozen Berry Assignment. (December 19, 2018).

FDA conducts sampling and reports results in an ongoing effort to identify trends and inform future activities to advance the safety of leafy greens.[14] In 2021, FDA sampled romaine lettuce from commercial coolers in Yuma County, Arizona, to test for STEC and *Salmonella* spp. Our findings led to the investigation of a specific farm to identify possible sources and routes of contamination.[15] In another example, we released our findings from a multi-year sampling assignment that collected and tested processed avocado and guacamole. The assignment data provided critical information on the prevalence of specific pathogens in these foods and underscored the need for processors and others in the supply chain to implement risk-based preventive controls.[16]

2. Selection of Frozen Berries

The frozen berries sampling assignment is part of FDA's broader Surveillance Sampling Program, which focuses resources on foods with data and knowledge gaps that are of interest to the Agency. In the summer of 2016, FDA shared the list of commodities, including berries, that were under consideration for future sampling with external stakeholders. The goal of that outreach was to elicit feedback from industry and regulatory partners to help inform our decision making. In 2018, FDA decided to focus additional resources on virology research to better prevent and control virus contamination, with specific focus on HAV and norovirus. To determine which commodities to focus resources on for the surveillance sampling program, we considered external feedback, data gaps, logistics, and available resources.[17] Below, we outline the data and considerations supporting FDA's decision to dedicate resources to the sampling of frozen berries.

HAV and norovirus are the most common causes of berry-linked viral disease and outbreaks around the world.[18] Berries can become contaminated with HAV or norovirus from contaminated water used for irrigation, contact with human feces during cultivation (e.g., waste that may be present in the soil), contact with contaminated harvesting equipment, and unhygienic practices of personnel in the production field, packing shed, or supply chain prior to consumption.[19] Frozen berries are often used as "ready to eat" foods and typically are not

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[14] FDA. 2022. Leafy Greens STEC Action Plan. https://www.fda.gov/food/foodborne-pathogens/leafy-greens-stec-action-plan. Accessed June 29, 2022.
[15] FDA. 2021. Microbiological Surveillance Sampling: FY21 Sample Collection and Analysis of Romaine Lettuce Obtained at Commercial Coolers in Yuma County, AZ. https://www.fda.gov/food/sampling-protect-food-supply/microbiological-surveillance-sampling-fy21-sample-collection-and-analysis-romaine-lettuce-obtained. Accessed June 29, 2022.
[16] FDA. 2022. FDA Releases Summary Report on Multi-year Processed Avocado and Guacamole Sampling Assignment. https://www.fda.gov/food/cfsan-constituent-updates/fda-releases-summary-report-multi-year-processed-avocado-and-guacamole-sampling-assignment. Accessed June 29, 2022.
[17] Memorandum from Erwin Miller, CFSAN, Office of Compliance, Division of Field Programs and Guidance, to FDA Industry & Regulatory Stakeholders. Summary of External Feedback and FDA Responses to the FY 19-20 Surveillance Sampling Program Assignment: Frozen Berry Assignment. (December 19, 2018) ("Memorandum to FDA Industry and Regulatory Stakeholders").
[18] Bozkurt et al., supra note 9.
[19] Bozkurt et al., supra note 9.

cooked prior to consumption.[20,21] Freezing does not significantly reduce infectivity of the viruses.[22] Therefore, consumers who eat contaminated frozen berries can become infected with HAV or norovirus.

Below, we summarize relevant outbreaks of HAV and norovirus; however, it is unlikely that the available data reflect all illnesses caused by the presence of these viruses in frozen berries. For example, for HAV, one article notes the following reasons that recognizing foodborne transmission using routine surveillance data may be difficult: (1) patients may have difficulty recalling food histories during the 2-6 weeks before illness, (2) cases may accrue gradually or not be reported, (3) a food item may be focally contaminated, (4) some exposed persons have unrecognized HAV infection, (5) some exposed persons have preexisting immunity (from a previous infection or previous vaccination), (6) persons who acquire infection through contaminated food are not recognized amid an ongoing high incidence in the community, and (7) cases are geographically scattered over several public health jurisdictions.[23]

Posing a separate data collection challenge, unlike HAV, infection with norovirus is not a "reportable" condition; state, local, and territorial health departments are not required to report individual cases of norovirus to a national surveillance system. Further, similar to HAV, many patients with norovirus may not seek medical care, and if they do, they are not tested for the virus, contributing to underdiagnosis.[24] Additionally, the low levels of HAV and norovirus in food and challenges with extracting the virus from food make detection difficult. Therefore, it is likely that foodborne illnesses attributable to these two viruses are underreported on both a national and global scale.[25]

The CDC estimates that annually, HAV causes 1,566 clinically diagnosed cases of domestically acquired foodborne illness in the U.S.[26] Surveillance data for 2019 indicated that there were 18,886 reported acute cases of HAV with an estimated 37,000 acute cases of infection. Additionally, 31.9% of cases did not have a specific risk behavior or exposure identified as the likely source of infection, and 32.9 % had risk data missing.[27] This could indicate an underreporting of cases associated with foods due to the relatively long period of incubation prior to onset of symptoms.[28]

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[20] Bozkurt et al., supra note 9.
[21] FSANZ, supra note 5.
[22] Butot, S., Putallaz, T., Sanchez, G. 2008. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. International Journal of Food Microbiology. 126(1-2):30-5.
[23] Fiore, supra note 2.
[24] Moore, M., Goulter, R., Jaykus, L. 2015. Human Norovirus as a Foodborne Pathogen: Challenges and Developments. The Annual Review of Food Science and Technology. 6:411-33.
[25] Nasheri, N., Vester, A., Petronella, N. 2019. Foodborne Viral outbreaks associated with frozen produce. Epidemiology and Infection. 147, e291, 1-8.
[26] Scallan, E., Hoekstra, R., Angulo, F., et al. 2011. Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Diseases 17(1):7-13.
[27] CDC. 2021. Figure 1.7. Availability of information regarding risk behaviors or exposures*† associated with reported cases of hepatitis A virus infection — United States, 2019. https://www.cdc.gov/hepatitis/statistics/2019surveillance/Figure1.7.htm. Accessed June 29, 2022.
[28] Acheson, D., Fiore, A. 2004. Hepatitis A Transmitted by Food, Clinical Infectious Diseases, 38(5): 705-715.

Regarding frozen berries specifically, between 1990 and 2016, there were four HAV outbreaks resulting in 594 illnesses.[29] In 1990, an outbreak of 28 HAV cases associated with domestically produced frozen strawberries was reported in two states.[30] In 1997, an outbreak of 258 HAV cases associated with frozen strawberries was reported in two states.[31] Similarly, in 2013, 165 cases associated with frozen berry mix were reported across 10 states and resulted in 69 hospitalizations and 1 liver transplant.[32] Also, in 2016, an outbreak of 143 HAV cases associated with frozen strawberries was reported across 9 states and resulted in 56 hospitalizations.[33]

Norovirus is responsible for approximately 5.5 million domestically acquired foodborne illnesses in the U.S. annually.[34] It accounts for about 15,000 hospitalizations and 150 deaths each year.[35] Three norovirus outbreaks related to frozen berries resulted in 157 illnesses between 1990 and 2016.[36] Specifically, in 2015, an outbreak of 123 cases was associated with frozen strawberries. In 2016, there were two outbreaks; one associated with frozen raspberries was linked to 15 cases of illness, and the other, associated with frozen berry mix, resulted in 19 illnesses.

Globally, there have been multiple HAV and norovirus outbreaks over the past five years with various types of frozen berries identified as likely vehicles.[37,38,39,40,41] Specifically, from 1983 to

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[29] Bozkurt et al., supra note 9.
[30] Niu, M., Polish, L., Robertson, B., et al. 1992. Multistate Outbreak of Hepatitis A Associated with Frozen Strawberries. The Journal of Infectious Diseases. 166:518-24.
[31] Hutin, Y., Pool, V., Cramer, E., et al. 1999. A Multistate, Foodborne Outbreak of Hepatitis A. The New England Journal of Medicine. 340:595-602.
[32] Collier, M., Khudyakov, Y., Selvage, D., et al. 2014. Outbreak of hepatitis A in the USA associated with frozen pomegranate arils imported from Turkey: an epidemiological case study. Lancet. 14:976-981.
[33] CDC. 2016. 2016 - Multistate outbreak of hepatitis A linked to frozen strawberries (Final Update). https://www.cdc.gov/hepatitis/outbreaks/2016/hav-strawberries.htm. Accessed June 29, 2022.
[34] CDC. 2018. Burden of Foodborne Illness: Findings. https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed June 29, 2022.
[35] Scallan et al., supra note 26.
[36] Bozkurt et al., supra note 9.
[37] Nordic outbreak investigation team collective. 2013. Joint analysis by the Nordic countries of a hepatitis A outbreak, October 2012 to June 2013: frozen strawberries suspected. Eurosurveillance. 18(27):20520. https://www.eurosurveillance.org/content/10.2807/1560-7917.ES2013.18.27.20520. Accessed June 29, 2022.
[38] Severi, E., Verhoef, L., Thornton, L., et al. 2015. Large and prolonged food-borne multistate hepatitis A outbreak in Europe associated with consumption of frozen berries, 2013 to 2014. Eurosurveillance. 20(29):21192. https://www.eurosurveillance.org/content/10.2807/1560-7917.ES2015.20.29.21192. Accessed June 29, 2022.
[39] New Zealand Food Safety. 2021. Making imported frozen berries safe to eat. https://www.mpi.govt.nz/food-safety/food-safety-for-consumers/is-it-safe-to-eat/frozen-imported-berries/. Accessed June 29, 2022.
[40] Public Health Agency of Canada. 2016. Public Health Notice: Outbreak of Hepatitis A infections; consumers advised not to eat Nature's Touch Organic Berry Cherry Blend frozen fruit. https://www.canada.ca/en/public-health/services/public-health-notices/2016/public-health-notice-outbreak-hepatitis-a-infections-consumers-advised-nature-s-touch-organic-berry-cherry-blend-frozen-fruit.html. Accessed June 29, 2022.
[41] Whitworth, J. 2018. Thirteen infected with Hepatitis A virus from frozen strawberries. Food Safety News. http://www.foodsafetynews.com/2018/07/thirteen-infected-with-hepatitis-a-virus-from-frozen-strawberries/#.W1Yy7IAvxEb. Accessed June 29, 2022.

2018, there were 68 berry outbreaks attributed to viral contamination.[42] Of the 50 outbreaks attributed to frozen berries, 36 were caused by norovirus and 14 were caused by HAV.[43] HAV and norovirus were the cause of more than 80% of berry-related outbreaks in the world from 1983 to 2018.[44]

In May 2022, FDA, along with CDC, the Public Health Agency of Canada, the Canadian Food Inspection Agency, and state and local partners, began investigating a multistate outbreak of HAV infections in the U.S. and Canada linked to fresh organic strawberries purchased between March 5 and April 15, 2022. As of June 29, 2022, there are 18 reported cases and 13 hospitalizations associated with the outbreak. Although this recent outbreak involved fresh berries, historical data illustrate that frozen berries can serve as a vehicle for HAV and norovirus infection, particularly as they are used in foods such as smoothie drinks that typically are not cooked prior to consumption.

Both historical and present outbreak data provide evidence of the presence of enteric viruses in berries and underscore the need to gather and evaluate prevalence data to identify potential vulnerabilities and inform decision making.

3. Engagement with Petitioners and Responsive Changes to Sampling Assignment

Before starting the sampling assignment, FDA conducted outreach to industry, associations representing domestic producers and importers, and state and regulatory partners, to obtain feedback on our plans.[45] In August 2018, AFFI provided comments to FDA on the planned sampling assignment.[46] After thorough consideration of AFFI's comments and before starting the sampling assignment, FDA: (1) limited the sampling to frozen berries in finished retail packaging to minimize cross contamination associated with loose or bulk sampling, (2) removed mixed frozen berries from the scope of the assignment to facilitate a greater understanding of prevalence for individual berries, and (3) prioritized early-in-the-supply-chain collection among the domestic samples to minimize the downstream effects if a sample is determined to be positive.[47]

After initiating the sampling assignment on November 16, 2018, we continued to communicate with AFFI, including meeting with AFFI multiple times.[48] As of July 2019, we made the following additional responsive changes to the sampling assignment: (1) collected samples at

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[42] Bozkurt et al., supra note 9.
[43] Bozkurt et al., supra note 9.
[44] Bozkurt et al., supra note 9.
[45] Memorandum to FDA Industry and Regulatory Stakeholders (December 19, 2018).
[46] Letter from Donna Garren, Executive Vice President, Science and Policy, AFFI, to FDA (August 31, 2018).
[47] FDA. 2022. Microbiological Surveillance Sampling: FY 19-20 Frozen Berries (Strawberries, Raspberries and Blackberries). https://www.fda.gov/food/sampling-protect-food-supply/microbiological-surveillance-sampling-fy-19-20-frozen-berries-strawberries-raspberries-and. Accessed June 29, 2022.
[48] FDA met with AFFI in-person or virtually on August 29, 2019; November 22, 2019; May 3, 2021; and February 11, 2022.

distribution centers rather than retail to minimize market disruption if product was found to contain virus, (2) implemented Sanger sequencing (a method for determining the nucleotide sequence of nucleic acid) for samples where HAV or norovirus was detected[49] and determined not to request initiation of a voluntary recall unless further characterization through sequencing was achieved, (3) made results from the initial sample analysis available within 5 business days following FDA laboratory receipt of the sample, and (4) made sequencing results available within 10 business days of a positive RT-qPCR result.[50] FDA also addressed AFFI's concerns in two letters.[51]

As a result of the COVID-19 pandemic, FDA paused the frozen berry sampling assignment on March 17, 2020. Before pausing the assignment, 56% (1,120 of 2000 planned samples) were collected with 1% (15 samples) testing positive for HAV or norovirus.[52] Samples where HAV or norovirus was detected resulted in a number of recalls,[53] increased import screening, and information that provided evidence for adding one firm to an import alert.[54]

On February 16, 2022, FDA notified AFFI of our intent to publish a revised BAM chapter incorporating the same analytical method that is available via FDA's Foods Program Compendium of Analytical Methods ("Compendium")[55] and then resume the frozen berry sampling assignment.[56]

C. Detecting HAV and Norovirus in Frozen Berries

1. How FDA Develops and Validates Test Methods

Once FDA identifies a need for an analytical laboratory method, a principal investigator (PI) with the appropriate expertise is responsible for working with other FDA scientists to develop a method and to have the method validated. The method validation process follows FDA's Foods Program Guidelines for the Validation of Analytical Methods for the Detection of Microbial

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[49] FDA. 2020. FDA Sampling Frozen Berries for Harmful Viruses. https://www.fda.gov/food/cfsan-constituent-updates/fda-sampling-frozen-berries-harmful-viruses. Accessed June 29, 2022.
[50] Letter from Frank Yiannas, Deputy Commissioner, FDA, to Donna Garren, Executive Vice President, Science and Policy, AFFI (July 24, 2019).
[51] Letter from Frank Yiannas, Deputy Commissioner, FDA, to Donna Garren, Executive Vice President, Science and Policy, AFFI (July 24, 2019); Letter from Frank Yiannas, Deputy Commissioner, FDA, to Donna Garren, Executive Vice President, Science and Policy, AFFI, (August 8, 2019).
[52] Any sample numbers published prior to issuing of the final report on the frozen berry assignment are considered interim figures.
[53] FDA. Recalls, Market Withdrawals, & Safety Alerts. https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts.
[54] FDA. Import Alerts. https://www.fda.gov/industry/actions-enforcement/import-alerts.
[55] FDA. 2019. Concentration, Extraction, and Detection of Norovirus and Hepatitis A virus in Soft Fruit. FDA's Foods Program Compendium of Analytical Methods. ("Compendium"). https://www.fda.gov/media/114183/download. Accessed June 29, 2022.
[56] Letter from Janet Woodcock, Acting Commissioner, FDA, to Alison Bodor, President and CEO, AFFI, (February 16, 2022).

Pathogens in Foods and Feeds ("Validation Guidelines").[57] The Validation Guidelines reflect FDA's understanding of the importance of validation to ensure the reliability of its methods.

The Validation Guidelines define validation as:

> a process by which a laboratory confirms by examination, and provides objective evidence, that the particular requirements for specific uses are fulfilled. It serves to demonstrate that the method can detect and identify an analyte or analytes:
> - In one or more matrices to be analyzed.
> - In one or more instruments or platforms.
> - With a demonstrated sensitivity, specificity, accuracy, trueness, reproducibility, ruggedness and precision to ensure that results are meaningful and appropriate to make a decision.
> - Reliably for its intended purpose. Intended purpose categories include, but may not be limited to, emergency/contingency operations; rapid screening and high throughput testing; and confirmatory analyses.
> - After the method developer has conducted experiments to determine or verify a number of specific performance characteristics that serve to define and/or quantify method performance (Validation Guidelines at 7).

After completing the appropriate type of validation study, the PI compiles and analyzes the study data, which is then submitted to FDA's Microbiology Methods Validation Subcommittee (MMVS) for review. The MMVS consists of a minimum of six voting members, with at least one representative from the Center for Food Safety and Applied Nutrition (CFSAN), the Center for Veterinary Medicine (CVM), and the Office of Regulatory Affairs (ORA). The MMVS includes representatives from diverse analytical disciplines of food and veterinary microbiology, including bacteriology, molecular biology, mycology, parasitology, virology, and statistics. Among other functions, the MMVS ensures that studies adhere to the Validation Guidelines and evaluates completed validation packages for microbiological methods submitted for approval.

If approved by the MMVS, methods can be included in the Compendium.[58] Part of the Compendium is dedicated to microbiological methods. The Compendium includes the BAM and validated microbiological methods not yet entered into the BAM. As these methods are already validated, there are no limitations on their use during the period before publication in the

[57] FDA. 2015. Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, 2nd ed. The matrix extension occurred in 2017 and 2018. Therefore, FDA will refer to the 2015 edition of the Validation Guidelines.
[58] MVS Charter, supra note **Error! Bookmark not defined.**, at 5.

BAM.[59] Methods in the Compendium are added to the BAM after approval by the BAM Council, which includes scientists from CFSAN, ORA, and CVM.[60]

FDA's microbiology laboratories are required to analyze a large variety of food matrices. When FDA wishes to extend a validated method for a specific food matrix (e.g., green onion) to another food matrix (e.g., soft fruit), the PI consults with the MMVS to determine what form of verification is required, including whether validation is needed. Verification of method performance with a new matrix is intended to ensure that the new matrix will produce neither high false positive rates (results that wrongly identify an analyte as present, when in fact it is absent) nor high false negative rates (results that wrongly identify an analyte as absent, when in fact it is present) (Validation Guidelines at 23).

The Validation Guidelines recognize that modifications to an existing validated method may be made for any number of reasons and may or may not affect the established validated performance parameters of the original method. For this reason, some modifications (e.g., ease-of-use capabilities, availability/substitution of reagents or instrumentation, sample handling/sample processing adaptations) may only necessitate verification against the original method whereas other modifications may require significant validation data to support their use (Validation Guidelines at 22). The MMVS determines what verification or validation is needed.

2. How FDA Developed and Validated a Test Method for Concentration, Extraction, and Detection of HAV and Norovirus in Soft Fruit

The method FDA uses for the frozen berries sampling assignment includes assays for concentration and extraction (sometimes collectively referred to as extraction) and for detection of HAV and norovirus. A concentration and extraction assay addresses how to isolate the target from the food and concentrate it so that it can be analyzed. As explained below, FDA's concentration and extraction assay for HAV and norovirus in soft fruit is based on a concentration and extraction assay originally validated for HAV in green onions. The same assay can be used for the concentration and extraction of both viruses because they are non-enveloped RNA viruses and are of similar size.[61,62]

Detection assays are used once the target has been extracted from the food and need not be specific to a particular food matrix. The preferred method for detection is molecular amplification, including RT-qPCR (Reverse Transcription-Real-time Polymerase Chain

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[59] FDA. Foods Program Compendium of Analytical Laboratory Methods: Microbiological Methods. https://www.fda.gov/food/laboratory-methods-food/foods-program-compendium-analytical-laboratory-methods. Accessed June 29, 2022.
[60] FDA. Charter - Bacteriological Analytical Manual Council (September 10, 2013).
[61] Bishop, R., Kirkwood, C. 2014. Enteric Viruses. Reference Module in Biomedical Research. http://dx.doi.org/10.1016/B978-0-12-801238-3.02566-6. Accessed June 29, 2022.
[62] Melnick, J. 1992. Properties and classification of hepatitis A virus. Vaccine. 10:24-26.

Reaction).[63] The objective of RT-qPCR, as used by FDA in the sampling assignment, is to allow monitoring of the amplification of nucleic acid for detection of HAV and norovirus. FDA developed and validated detection assays for HAV and norovirus for use with all food matrices.

In 2003, a large HAV outbreak associated with green onions resulted in over 555 illnesses.[64] At that time, FDA did not have a validated method for detecting HAV in food. Subsequently, FDA developed an RT-qPCR detection assay for HAV and protocols to extract the HAV pathogen from green onion. After FDA investigators, working with eight laboratories, completed a multi-laboratory validation study for the HAV RT-qPCR detection assay, they submitted a validation study report to the MMVS in January 2013. Separately, FDA investigators, working with eight labs, completed a multi-laboratory validation of an extraction assay for HAV from green onion. The validation study report for this assay was also sent to the MMVS for review in January 2013. The MMVS approved the validation study reports in September 2013. In October 2013, the BAM Council approved the validated method for incorporation into the BAM, and in January 2014, the method was published as BAM Chapter 26B: "Detection of Hepatitis A in Foods."[65]

In 2015, FDA investigators, working with 13 laboratories, completed a multi-laboratory validation for an RT-qPCR detection assay for norovirus. In April 2017, the investigators submitted a validation study report to the MMVS for review. The MMVS approved the study report in March 2018, and the validated detection method, which was applicable to all food, was published in the Compendium in June 2018 as "Concentration, Extraction, and Detection of Norovirus and Hepatitis A virus in Molluscan Shellfish."[66]

After the multi-laboratory validations for HAV and norovirus were completed, FDA investigators consulted with the MMVS regarding using the virus extraction assay for green onion published in the BAM for HAV and norovirus from soft fruit. The MMVS determined that the matrix extension for extraction of HAV and norovirus from soft fruit would require validation by a single laboratory, rather than verification or validation by multiple laboratories. We note that soft fruits (the subject of the matrix extension) are in the same food category as green onion (fruits and vegetables) (Validation Guidelines at 37). Further, the changes to the extraction assay involve only availability/substitution of reagents or instrumentation and sample handling/sample processing adaptations, which are considered the least likely types of changes to affect the validated performance parameters of the original method (Validation Guidelines at 22).

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[63] Moore et al., supra note 24.
[64] CDC. 2003. Morbidity and Mortality Weekly Report. Hepatitis A Outbreak Associated with Green Onions at a Restaurant – Monaca, Pennsylvania, 2003 52;1155-1157. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5247a5.htm. Accessed June 29, 2022.
[65] FDA. 2014. Bacteriological Analytical Manual. BAM Chapter 26B: Detection of Hepatitis A Virus in Foods. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-26b-detection-hepatitis-virus-foods. Accessed June 29, 2022.
[66] FDA. 2018. Concentration, Extraction, and Detection of Norovirus and Hepatitis A virus in Molluscan Shellfish. Compendium. https://www.fda.gov/media/114187/download. Accessed June 29, 2022.

The single laboratory validation study for the matrix extension for the concentration and extraction of HAV and norovirus from soft fruit was submitted to the MMVS in April 2017. The validation study report[67] for the matrix extension concluded in part as follows:

> The matrix extension of norovirus and [HAV] concentration and detection protocols for fresh and frozen soft fruit have demonstrated sensitivity and specificity for the detection of norovirus and [HAV]. The extraction control was detected in all replicates spiked, even those that exhibited inhibition. . . . The results show the assay is sensitive, reproducible and robust and has established the "Fitness of Purpose" for concentration and detection of norovirus GI, norovirus GII, and hepatitis A virus.
>
> Our conclusion is that this matrix extension can be used as a diagnostic method for the extraction and detection of norovirus and hepatitis A virus in fresh and frozen soft fruit. This protocol is ready to be incorporated as a matrix extension into the [BAM] and ongoing [ORA] Field Assignments.

On March 16, 2018, the MMVS approved the matrix extension. FDA published the matrix extension in the Compendium in June 2018 as part of "Concentration, Extraction, and Detection of Norovirus and Hepatitis A virus in Soft Fruit."[68]

On March 3, 2022, the BAM Council approved an updated Chapter 26 for incorporation into the BAM: "Concentration, Extraction and Detection of Enteric Viruses from Food." This BAM chapter includes methods for the concentration, extraction, and detection of HAV and norovirus from soft fruit as well as molluscan shellfish, scallops, finfish meat, green onion, and leafy greens. All concentration, extraction, and detection methods that are relevant to the sampling assignment are included in the revised Chapter 26 and were approved by the MMVS and publicly available since June 2018 or earlier in the FDA Compendium or the BAM. FDA posted the updated BAM chapter online simultaneously with responding to the petition on July 22, 2022.

3. How FDA Shared Test Method Details with Petitioner

The petition states, "Since August 2018, AFFI has repeatedly requested – including via a Freedom of Information Act (FOIA) request – but has *never* received, a copy of the test method being used as part of the surveillance program" (Petition at 7, emphasis in original). Also, on April 28, 2021, AFFI wrote to FDA in correspondence titled "Suspension of FDA's Microbiological Sampling Program for Frozen Berries."[69] As part of its discussion about the

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[67] FDA. 2018. Validation Study Report: BAM 26B Matrix Extension for the Extraction and Detection of Norovirus and Hepatitis A virus in Soft Fruit.

[68] FDA. 2018. Validation Study Report: BAM 26B Matrix Extension for the Extraction and Detection of Norovirus and Hepatitis A virus in Soft Fruit, supra note 67. See also Compendium, supra note 55.

[69] Letter from Alison Bodor, President and CEO, AFFI, to Frank Yiannas, Deputy Commissioner, FDA (April 28, 2021).

availability of the test method, AFFI states "Requests for details (e.g., control exclusion assay, sequencing protocol, redacted testing records) by FOIA were denied." In the same letter, AFFI questions whether the method to test frozen berries was subject to validation. AFFI states, "If scientific validation was done on the method, it was not released despite a FOIA request."

The petition does not provide the date of the referenced FOIA request. FDA received one FOIA request, dated November 18, 2019, that sought "all information related to the testing protocol and methodology used in the agency's sampling assignment for Hepatitis A and norovirus, specifically for analysis of FDA Sample Number 1109896…"[70] Although the requestor was not identified as AFFI, we presume this is the FOIA request AFFI references in the petition. On February 27, 2020, and April 17, 2020, FDA released responsive records in reply to the FOIA request.[71] Contrary to AFFI's assertion that it did not receive a copy of the test method, the responsive records included a copy of the Compendium chapter titled "Concentration, Extraction, and Detection of Norovirus and Hepatitis A Virus in Soft Fruit."[72] Further, the method provided in response to the FOIA request has been available online in the FDA Compendium since June 2018. In total, FDA provided 132 pages of records in response to the FOIA request. The records included: sample summary and collection reports, FDA emails, sample tracking charts, test records for sequence analysis of sample 1109896, description and reference to sequencing protocols, a peer-reviewed publication referenced in the sequencing protocol of test records, control exclusion assay test records for samples, and the Compendium method for detection, concentration, and extraction of HAV and norovirus from soft fruit. Where necessary, information was redacted in accordance with FOIA exemptions 4 (trade secrets or commercial or financial information) and 5 (deliberative process privilege).[73] The FOIA request did not specifically reference or request validation data, and therefore FDA did not provide the matrix extension validation data in the responsive records.[74]

Although Petitioner asserts that FDA denied "requests for details," the responsive records provided sufficient detail for Petitioner to ascertain the comprehensive sequencing protocol. Specifically, the response included details for the amplification of a second region of the viral genome (VP1-2A region for HAV,[75] and Region B or Region C for norovirus);[76] secondary amplification of one of these diagnostic regions, using M13 primers;[77] and sequencing of the

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[70] Letter from FOI Services, Inc, to FDA, Division of Freedom of Information (November 15, 2019).
[71] Letter (email) to FOI Services, Inc., from Sheila Wright, FOIA Officer, CFSAN (April 17, 2020); Letter to FOIA Services, Inc., from Shelley Petroski, Government Information Specialist, ORA (February 27, 2020) ("FOIA responsive records").
[72] FDA. FOIA responsive records at 78/127.
[73] 5 U.S.C. § 552 (b)(4) and (b)(5).
[74] We note that validation data not previously published will be included in the revised BAM chapter.
[75] FOIA Responsive records at 23/127. See also Shieh, Y., Khudyakov, Y., Xia, G., et al. 2007. Molecular Confirmation of Oysters as the Vector for Hepatitis A In a 2005 Multistate Outbreak. Journal of Food Protection. 70:145-150.
[76] FOIA responsive records at 23/127. See also Depaola, A., Jones, J., Woods, J., et al. 2010. Bacterial and viral pathogens in live oysters: 2007 United States Market Survey. Applied and Environmental Microbiology. 76:2754-2768 and Woods, J. W., K. R. Calci, J. G. Marchant, W. Burkhardt III. 2016. Detection and molecular characterization of norovirus from oysters implicated in outbreaks in the US. Food Microbiology. 59:76-84.
[77] FOIA responsive records at 23/127.

resultant product in both directions, following the sequencing instrument manufacturer's recommendations.[78] Also included were records referencing the use of default base callings and quality scores; the manual inspection and trimming of the chromatograms;[79] the alignment of forward and reverse reads, and; the verification of the sequence in BLAST.[80] The responsive records reference sequencing protocols that are consistent with published guidelines for Sanger sequencing.[81]

II. FDA's Method and Additional Testing Ensure the Accuracy of Test Results Obtained During the Sampling Assignment

A. FDA's Method Includes Sound Laboratory Practices

In its testing for HAV and norovirus as part of the berry sampling assignment, FDA uses multiple measures to ensure the accuracy of its test results. Consistent with the method published in the Compendium,[82] FDA's laboratory setup (physical separation of work areas) and unidirectional (i.e., one-way) workflow minimize the possibility of laboratory cross-contamination.[83] During sample processing, the concentration and extraction steps are conducted in an area separate from the subsequent RT-qPCR detection.[84] The master mix (a mixture containing agents and reagents used in the RT-qPCR technique) is prepared in a dedicated PCR hood or biological safety cabinet.[85] Then, addition of sample template (RNA) to the reaction tubes is done in a separate designated area, away from master mix preparation.[86]

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[78] FOIA responsive records at 125/127. See also Williams-Woods, J., Gonzalez-Escalona, N., Burkhardt, W. 2011. Direct sequencing of hepatitis A virus and norovirus RT-PCR products from environmentally contaminated oyster using M13-tailed primers. Journal of Virological Methods. 178; 253-257.

[79] FOIA responsive records at 125/127.

[80] FOIA responsive records at 125/127. Nucleotide sequences were analyzed using BLAST (www.ncbi.nlm.nih.gov/BLAST/).

[81] Crossley, B., Bai, J., Glaser, A., et al. 2020. Guidelines for Sanger sequencing and molecular assay monitoring. Journal of Veterinary Diagnostic Investigation. 32(6):767-775.

[82] FDA. 2019. FDA's Foods Program Compendium of Analytical Methods. https://www.fda.gov/food/laboratory-methods-food/foods-program-compendium-analytical-laboratory-methods. Accessed June 29, 2022. The relevant steps for laboratory set-up, sample processing, master-mix preparation, addition of the sample template, use of a negative control, data interpretation, proper personal protective equipment, cleansing, and decontamination of workspaces are listed in the Compendium for each assay.

[83] Compendium, supra note 55, at 8 (1.A. Outlined MNV RT-qPCR Assay for Smart Cycler, Sample Preparation), 11 (2.E. Outlined HAV RT-qPCR Assay for Smart Cycler, Sample Preparation) and 16 of 36 (3.I. Outlined NoV RT-qPCR Assay for Smart Cycler, Sample Preparation).

[84] Compendium, supra note 55, at 8 (1.A. Outlined MNV RT-qPCR Assay for Smart Cycler, Sample Preparation), 11 (2.E. Outlined HAV RT-qPCR Assay for Smart Cycler, Sample Preparation), and 16 of 36 (3.I. Outlined NoV RT-qPCR Assay for Smart Cycler, Sample Preparation).

[85] Compendium, supra note 55, at 8 (1.A. Outlined MNV RT-qPCR Assay for Smart Cycler, Sample Preparation), 11 (2.E. Outlined HAV RT-qPCR Assay for Smart Cycler, Sample Preparation), 16 (3.I. Outlined NoV RT-qPCR Assay for Smart Cycler, Sample Preparation), 20 (4.B.a. Outlined MNV RT-qPCR Assay for ABI 7500), 25 (5.2.a. Outlined HAV RT-qPCR Protocol ABI 7500), and 30 of 36 (6.2.a. Outlined NoV RT-qPCR Protocol ABI 7500).

[86] Compendium, supra note 55, at 9 (1.C.e Outlined MNV RT-qPCR Assay for Smart Cycler, Reaction Set-Up), 12 (2.G.f. Outlined HAV RT-qPCR Assay for Smart Cycler, Reaction Set-Up), 17 (3.K.g. Outlined NoV RT-qPCR Assay for Smart Cycler, Reaction Set-Up), 20 (4.B.d. Outlined MNV RT-qPCR Assay for ABI 7500, Reaction Set-

Appropriate PPE is worn, and all surfaces and equipment are cleaned and decontaminated prior to and immediately after work in each area.[87]

B. FDA's Method Includes Sufficient Controls

The petition also asserts that FDA's method is flawed because, unlike the approach used in the ISO 15216-1:2017 method,[88] "it does not have a fail-safe way of identifying cross-contamination, which is a common cause of false positive results" (Petition at 4). However, as indicated in its title (Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR — Part 1: Method for quantification), ISO 15216-1:2017 is relevant when RT-PCR is used to quantify the amount of a virus that is present (emphasis added). When RT-PCR is used to determine whether a virus is present (i.e., detection), which is how it is used in FDA's method, the relevant ISO standard for comparison is ISO 15216-2:2019 (Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR — Part 2: Method for detection) (emphasis added).

The relevant ISO method (ISO 15216-2:2019) does not require a procedure to identify cross-contamination. However, "informative" Annex G (this information is not part of the standard), which addresses generation of external control RNA, states that "[w]here contamination of samples with [an external control] is suspected," the contamination "can be checked" in either of two ways, one of which is "by sequencing of the PCR products."[89] As further explained below, in the sampling assignment, FDA is performing (Sanger) sequencing of RT-PCR products, one of two methods identified in the Annex to ensure that cross contamination has not occurred. Further, FDA is performing this sequencing every time HAV or norovirus is detected by RT-qPCR, not only "[w]here contamination of samples with [an external control] is suspected." Finally, we note that the RT-qPCR assays that may be used according to ISO 15216-2:2019 need not be validated; FDA uses only validated RT-qPCR assays in the sampling assignment.

The FDA method also includes a control in the detection process to address the possibility of false positives. Specifically, a negative control (i.e., water as template) is included with every RT-qPCR run.[90] If a negative control tests positive for HAV or norovirus, the sample results

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Up), 25 (5.2.d. Outlined HAV RT-qPCR Protocol ABI 7500, Reaction Set-Up), and 30 of 36 (6.2.d. Outlined NoV RT-qPCR Protocol ABI 7500, Reaction Set-Up).

[87] Compendium, supra note 55, at 8 (1.A. Outlined MNV RT-qPCR Assay for Smart Cycler, Sample Preparation), 11 (2.E. Outlined HAV RT-qPCR Assay for Smart Cycler, Sample Preparation) and 16 of 36 (3.I. Outlined NoV RT-qPCR Assay for Smart Cycler, Sample Preparation). Referenced provisions are for gloves and decontamination of biosafety cabinet hood. Remaining PPE is specified in the Biosafety Manual for each lab.

[88] ISO. 2017. Microbiology of the food chain – Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR – Part 1: Method for quantification. ISO 15216-1:2017. International Organization for Standardization.

[89] ISO. 2019. Microbiology of the food chain – Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR – Part 2: Method for detection. Annex G. ISO 15216-2:2019. International Organization for Standardization.

[90] Compendium, supra note 55, at 9 (1.C.e. Outlined MNV RT-qPCR Assay for Smart Cycler, Reaction Set-Up), 12 (2.G.e. Outlined HAV RT-qPCR Assay for Smart Cycler, Reaction Set-Up), 17 (3.K.f. Outlined NoV RT-qPCR

included in that run are considered invalid. This minimizes false positive reporting due to laboratory cross-contamination. Further, the method includes detailed data interpretation guidance to ensure accurate and consistent reporting of analytical results.[91]

Finally, as an additional control, FDA performs Sanger sequencing on a second, larger region of the genome. According to the petition, "[e]xperts recommend the use of alternative confirmation approaches such as . . . amplification of more than one viral genome region. . . ." (Petition at 4). FDA's approach in the sampling assignment is consistent with this recommendation. Specifically, FDA amplifies one region of the viral genome for the RT-qPCR testing and amplifies a second, larger region as part of Sanger sequencing.[92] Sanger sequencing is the gold standard method for enteric virus characterization.[93] Successful characterization of a HAV or norovirus sequence from a second region of the genome via Sanger sequencing supports the initial RT-qPCR detection and is further evidence of the presence of the virus in the sample. Further, detecting a second, larger, portion of the viral genome increases the probability that intact virus is present.[94] Finally, we note that the probability of detection by RT-qPCR is low because the virus is not uniformly distributed. Therefore, when a virus is detected, broader contamination is likely.[95]

C. FDA's Method Uses Appropriate Cycle Threshold (Ct) Values

The petition asserts that "positive RT-qPCR results that exceed the method's limit of detection (LOD) (typically around a Ct of 37-40) have a greater likelihood of being false positives" (Petition at 4). The LOD reflects the likelihood of detecting a specific analyte concentration when the analyte is present. Specifically, the LOD is the lowest amount of an analyte in a sample which can be detected with a certain probability. The LOD does not address the probability of a false positive, i.e., detecting an analyte when the analyte is not present. When an analyte is present below the LOD, the probability of detection decreases, but the validity of detection does not.[96] Therefore, Ct values below the calculated LOD are "absolutely valid in

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Assay for Smart Cycler, Reaction Set-Up), 20 (4.B.h. Outlined MNV RT-qPCR Assay for ABI 7500), 25 (5.2.h. Outlined HAV RT-qPCR Protocol ABI 7500), and 30 of 36 (6.2.h. Outlined NoV RT-qPCR Protocol ABI 7500).

[91] Compendium, supra note 55, at 11 (1.D.2 Outlined MNV RT-qPCR Assay for Smart Cycler, Data Interpretation Murine Norovirus Detection Assay), 14 (2.H Outlined HAV RT-qPCR Assay for Smart Cycler, Data Interpretation HAV Detection Assay), 19 (3.L. Outlined NoV RT-qPCR Assay for Smart Cycler, Data Interpretation for Detection NoV), 23 (4.C. Outlined MNV RT-qPCR Assay for ABI 7500, Data Interpretation), 28 (5.3 Outlined HAV RT-qPCR Protocol ABI 7500, Data Interpretation) and 33 of 36 (6.3 Outlined NoV RT-qPCR Protocol ABI 7500, Data Interpretation for Detection NoV).

[92] Woods et al., supra note 76.

[93] Majumdar, M., Celma, C., Pegg, E., Polra, K., Dunning, J., Martin, J. 2021. Detection and Typing of Human Enteroviruses from Clinical Samples by Entire-Capsid Next Generation Sequencing. Viruses. 13:641.

[94] Rodriguez, R., Pepper, I., Gerba, C. 2009. Application of PCR-Based Methods To Assess the Infectivity of Enteric Viruses in Environmental Samples. Applied and Environmental Microbiology. 75:297-307.

[95] Bosch et al., supra note 3.

[96] Burns, M., Valdivia, H., 2008. Modeling the limit of detection in real-time quantitative PCR. European Food Research and Technology. 226:1513–1524.

terms of microorganism presence,"[97] even if above the LOD for a quantitation.

In addressing Ct values, the petition cites articles that are not applicable to the Ct values generated by the method FDA uses during the sampling assignment. The Public Health Ontario article addresses testing clinical samples for SARS-CoV-2.[98] Clinical detection assays do not require the same level of optimization or sensitivity as food detection assays because clinical samples are anticipated to have much higher levels of the target virus than food samples.[99] Further, concerns about Ct values when performing quantitative testing are not directly applicable to qualitative testing (i.e., testing for detection) because more analyte is required for accurate quantitative testing than for accurate qualitative testing. Bustin et al., 2009 provides guidelines for quantitative testing, not qualitative testing.[100] The Burns & Valdivia article addresses using real-time PCR for quantitative testing, not qualitative testing.[101] Similarly, Stals et al., 2013 addresses the challenges of using RT-qPCR for quantitative, not qualitative testing.[102]

Further, the petition asserts that "[i]f high Ct values suggest questionable detection of a virus, it is challenging to conclude that identification has been confirmed through sequencing" (Petition at 4). For the reasons stated above, we disagree that FDA's method results in "questionable detection of a virus." Going beyond the method and identifying HAV and norovirus by sequencing a second region of the genome further ensures the reliability of FDA's testing. The petition does not explain how high Ct values provide any reason to question a confirmation made by Sanger sequencing.

As the table below illustrates, in many previous foodborne illness outbreaks, FDA detected HAV and norovirus in food remnants implicated in illness with Ct values at or above 37-40. In fact, when HAV and norovirus were detected as part of these outbreak investigations, the Ct values were at or above 37-40 16 out of 17 times.

Analytical Results of Seafood and Berries Associated with Illness (2009-2018)*

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[97] Kralik, P., Ricchi, M. 2017. A Basic Guide to Real Time PCR in Microbial Diagnostics: Definitions, Parameters, and Everything. Frontiers in Microbiology. 8:108.
[98] Public Health Ontario. 2020. An Overview of Cycle Threshold Values and their Role in SARS-CoV-2 Real-Time PCR Test Interpretation.
[99] Manuel, C., Suther, C., Moore, M., Jaykus, L. 2021. Comparison of a one-step real-time RT-PCR and a nested real-time RT-PCR for a genogroup II norovirus reveals differences in sensitivity depending upon assay design and visualization. PLoS ONE. 16(4):e0248581.
[100] Bustin, S., Benes, Vl., Garson, J., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M., Shipley, G., Vandesompele, J., Wittwer, C. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry. 55:611-622.
[101] Burns & Valdivia, supra note 967.
[102] Stals, A., Coillie, EV., Uyttendaele, M. 2013. Viral genes everywhere: public health implications of PCR-based testing of foods, Current Opinion in Virology. 3:69-73.

Source	Commodity	Analysis Year	RT-qPCR Result	Ct value (rounded)	Characterized Strains/ Genotypes
Outbreak Related	Oysters	2009	NoV GII	39, 42	NoV GII.7
Outbreak Related	Oysters	2009	NoV GII	49, 40	NoV GII.7
Outbreak Related	Oysters	2010	NoV GII	42	Nov GII.4 Minerva/Den Haag
Outbreak Related	Oysters	2011	NoV GI NoV GII	42 39	NoV GI.8, GII.3
Outbreak Related	Oysters	2012	NoV GII	37, 41	NoV GII
Outbreak Related	Oysters	2012	NoV GII	39, 40	NoV GII
Outbreak Related	Oysters	2013	NoV GII	42	NoV GII
Outbreak Related	Oysters	2013	NoV GI	41	NoV GI.4
Outbreak Related	Oysters	2014	NoV GII	40	NoV GII.12
Outbreak Related	Oysters	2014	NoV GII	43	NoV GII.21
Outbreak Related	Scallops	2016	HAV	36 to 49	HAV IA
Outbreak Related	Strawberries	2016	HAV	39 to 50	HAV IB
Outbreak Related	Oysters	2017	NoV GII	41	NoV GII.2 and NoV GIIP/GII.10
Outbreak Related	Oysters	2017	NoV GII	43	NoV GII.2
Outbreak Related	Raspberries	2017	NoV GI	42	NoV GI.3
Outbreak Related	Raspberries	2017	NoV GII	49	NoV GII.17B
Outbreak Associated[#]	Crabmeat	2018	HAV	47	HAV IA

***** Data compiled from: Woods, J. W., K. R. Calci, J. G. Marchant, W. Burkhardt III. 2016. Detection and molecular characterization of norovirus from oysters implicated in outbreaks in the US. Food Microbiology. 59:76-84; Viray, M. et al. 2018. Public health investigation and response to a hepatitis A outbreak from imported scallops consumed raw—Hawaii, 2016. Epidemiology and Infection. 147: E28: 1-8; Saupe A. et al. 2021. Outbreak of Norovirus Gastroenteritis Associated with Ice Cream Contaminated by Frozen Raspberries from China. Clinical Infectious Diseases, 73(11), 3701-e3707; and internal sources.

[#] Not meal remnant but associated lot.

The higher Ct values found in raspberries, oysters, and crabmeat linked to illnesses in the 17 outbreaks depicted in the table demonstrate that even when the levels of detected virus are low, the virus is capable of causing illness and thus presents a public health concern. These results are consistent with peer-reviewed literature showing detection of enteric viruses with Ct levels > 38 in foods implicated in outbreaks.[103]

[103] Raymond, P., Paul, S., Perron, A. Bellehumeur, C., Larocque, E., Charest, H. 2022. Detection and Sequencing of Multiple Human Norovirus Genotypes from Imported Frozen Raspberries Linked to Outbreaks in the Province of Quebec, Canada, in 2017. Food and Environmental Virology. 14:40-58. See also Bozkurt et al., supra note 9.

It is also noteworthy that when FDA used its method to detect HAV and norovirus in seafood and berries during these outbreaks, FDA did not have the detected genomic sequences in its lab prior to testing and was not aware of the specific sequences identified by CDC in the clinical strains. Nonetheless, using its validated method and with Ct values generally above 40, FDA characterized sequences that matched ill individuals, further supporting the reliability of FDA's testing. Finally, we note that the applicable ISO standard (15216-2:2019) does not contain a Ct value cut-off and requires a minimum of 45 PCR cycles; in contrast, the FDA RT-qPCR assay specifies 50 PCR cycles.

D. FDA's Testing During the Sampling Assignment Did Not Result in the "False Positive" Alleged by Petitioner

On July 10, 2019, AFFI wrote to FDA in correspondence titled "Discontinuation of FDA's Microbiological Sampling Program for Frozen Berries," asserting that an FDA RT-qPCR analysis on a blackberry sample (sample 1084927) resulting in a Ct value of 42 was a false positive and asserting that it was "highly likely that [the] finding [was] a consequence of cross contamination with a positive control strain used in the assay."[104] Further, AFFI stated that it "previously raised concerns that the current testing methodology has the potential to produce false positives. Indeed, it has done just that." AFFI, "[i]n light of this development," requested that "FDA reassess the entirety of the frozen berry sampling program to address the concerns we have previously identified and the additional issues and questions that arise now that our concerns have been manifested." In addition, AFFI requested "that if any recalls or withdrawals have occurred that are based on Ct values greater than 37, that FDA issue a statement acknowledging that the recalls were unwarranted."

FDA responded on August 8, 2019, conclusively demonstrating that AFFI's assertion that FDA's result was a "false positive" was incorrect. First, FDA explained the safeguards that were employed in its testing process:

> We have incorporated a series of positive and negative controls in analyzing samples associated with the berry assignment. The initial RT-qPCR assay incorporates a negative PCR control. If HAV is detected in a sample and the negative PCR control is negative, that sample is subjected to the Control Exclusion Assay (CEA). The purpose of the CEA is to ensure that the laboratory control strain was not responsible for the positive RT-qPCR result. The CEA was performed on FDA sample 1084927 and confirmed the laboratory control strain was not the cause of the positive result.[105]

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[104] Letter from Donna Garren, Executive Vice President, Science and Policy, AFFI to Frank Yiannas, Deputy Commissioner, FDA. (July 10, 2019).

[105] Letter from Frank Yiannas, Deputy Commissioner, FDA, to Donna Garren, Executive Vice President, Science and Policy, AFFI (July 24, 2019); Letter from Frank Yiannas, Deputy Commissioner, FDA, to Donna Garren, Executive Vice President, Science and Policy, AFFI, (August 8, 2019), supra note 51.

Next, FDA informed AFFI that we analyzed the sequence of the FDA sample and found that the sequence obtained was consistent with other HAV sequences in the National Center for Biotechnology Information database. FDA also informed AFFI that we submitted the HAV sequence from the sample to the CDC for comparison to CDC's HAV database of sequenced clinical specimens. CDC experts concluded that the HAV sequence from the sample was a 100% match to an HAV positive specimen detected during CDC's clinical surveillance sampling in 2002. This shows that FDA's detection was not the result of lab cross-contamination because that HAV positive specimen was never in FDA's possession. In addition, as further explained in the correspondence, FDA used the CDC sequence and performed its own additional analysis which confirmed that the HAV sequence from the sample is identical to the CDC clinical HAV specimen from 2002. FDA also determined that the sample sequence was not a 100% match to the laboratory control strain used by FDA during sample analysis; therefore, it could not have been introduced through cross-contamination.

III. The Petition's Requested Actions

A. FDA should perform a multi-laboratory validation of the FDA method for the detection of enteric viruses (HAV and NoV) in soft fruits using independent laboratories outside the agency.

The petition asserts that "the FDA method for HAV has only been subject to a published multi-laboratory validation for use in oysters and green onions, and it has not, to the best of our knowledge, been validated across external entities" (Petition at 12). Both the RT-qPCR detection assay for HAV and the assay for concentration and extraction of HAV from green onion were multi-laboratory validated using eight laboratories. The HAV concentration and extraction protocol for soft fruit that is used in the berry sampling assignment is a matrix extension of the assay for green onion that was multi-laboratory validated using eight labs. Consistent with FDA's Validation Guidelines, and as explained in section I.C.ii., the matrix extension was validated by one laboratory.

Similarly, the RT-qPCR detection assay for norovirus was validated by 13 laboratories. As with the HAV concentration and extraction protocol, the norovirus concentration and extraction protocol for soft fruit that is used in the berry sampling assignment is a matrix extension of the assay for green onion that was validated by multiple labs. Consistent with FDA's Validation Guidelines, and as explained in section I.C.ii., the matrix extension was single lab validated. For both HAV and norovirus, all of the validations were approved by FDA's MMVS and were approved by FDA's BAM Council for inclusion in the updated BAM chapter that we issued along with this response on July 22, 2022.

The petition also claims that FDA "relied upon only internal FDA laboratories at the district level and did not involve its own expert virologists at MOD1[106] within [CFSAN]" (Petition at 12). However, virologists who work in FDA's Office of Applied Research and Safety

[106] MOD1 is the name of a building located at 8401 Muirkirk Rd, Laurel, Maryland.

Assessment (OARSA), which is located in MOD1, participated in the multi-laboratory validations of the RT-qPCR detection assays for both viruses and the green onion extraction assay for HAV, and were involved in training analysts in use of the methods.

The petition further asserts that controls must be used to ensure RT-qPCR reactions are not inhibited by the food matrix (Petition at 3). FDA's method has such controls.[107] The extraction assay for HAV and norovirus includes a control to ensure the extraction process is not inhibited by the food matrix, and the method describes how to determine when the level of inhibition is not acceptable. In addition, the detection assays for HAV and norovirus both have an internal amplification control in the RT-qPCR assays.[108] As part of this control, RNA target is added to every reaction. If the RNA target is not detected, a level of inhibition is calculated. If the level is exceeded, the results are considered invalid.

The petition also asserts that FDA should perform a multi-laboratory validation of the RT-qPCR detection assay used for the detection of HAV and norovirus in soft fruits, using "independent laboratories outside the agency" (Petition at 1). The petition does not explain why method validation by FDA laboratories is not sufficient, and FDA's Validation Guidelines do not require the use of outside laboratories for method validation. FDA laboratories are located throughout the country, and they perform analyses independently of each other using the methods specified by the validation criteria. Further, each laboratory participating in the validation of a method has its own analysts, equipment, and instrumentation as specified by the validation criteria.

B. **Once externally validated, the FDA method for the detection of enteric viruses in soft fruits, should be published as a scientific manuscript in peer-reviewed literature.**

The petition states that "the frozen berry sampling assignment is the only FDA surveillance program we are aware of where the test method was not announced and published in a peer-reviewed journal" (Petition at 13). However, FDA does not routinely announce its test methods in journals. As explained in section I.B., FDA has a rigorous process for method validation that includes validation of an original method by multiple laboratories, review of the validation by numerous reviewers, and, if approved, publication of the method online (first in the Compendium and then in the BAM) where it is accessible to anyone. FDA's test methods are widely accepted in the scientific community. For example, FDA's methods for HAV and

[107] See Compendium, supra note 55.

[108] Compendium, supra note 55, at 9 (1.C.a[f]. Outlined MNV RT-qPCR Assay for Smart Cycler, Reaction Set-Up), 12 (2.G.g. Outlined HAV RT-qPCR Assay for Smart Cycler, Reaction Set-Up), 17 (3.K.h. Outlined NoV RT-qPCR Assay for Smart Cycler, Reaction Set-Up), 20 (4.B.f. Outlined MNV RT-qPCR Assay for ABI 7500), 25 (5.2.f. Outlined HAV RT-qPCR Protocol ABI 7500), and 30 of 36 (6.2.f. Outlined NoV RT-qPCR Protocol ABI 7500).

norovirus have been used for analysis in multiple articles published in peer-reviewed journals.[109,110,111]

C. FDA should release protocols for the method in FDA's BAM that are sufficiently detailed to enable the method to be evaluated and reliably reproduced by the relevant scientific community

The method for detection, concentration, and extraction of HAV and norovirus from soft fruit was made publicly available online in the Compendium in June 2018 before initiation of the sampling assignment and remains available today.[112] Further, the RT-qPCR detection assay for HAV has been available in Chapter 26B of the BAM since 2014, and the detection assay for norovirus has been available in the Compendium since 2018. These documents contain all the information necessary to perform the method that FDA is using to test berries during the sampling assignment. Therefore, contrary to the petition's assertion, "FDA has [] publicly shared adequate technical data and information on the method [so that it can] be used by external laboratories" (Petition at 2). Moreover, the validation data are included in the revised BAM chapter issued along with this response on July 22, 2022.

D. Convene an international panel of experts with expertise in virology and microbial risk assessments in foods, tasked with establishing transparent and risk-based interpretive criteria for results confirmed through sequencing and make the findings publicly available with an opportunity for public comment, before adopting the findings, as appropriate.

The petition asserts that "FDA has failed to establish that its findings present a potential public risk or that the product is adulterated, warranting a recall or other action" (Petition at 10). In doing so, the petition references section 402(a)(1) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)[113] (Petition at 10). We need not address section 402(a)(1) of the FD&C Act because another adulteration provision applies. Specifically, section 402(a)(4) of the FD&C Act provides that a food is adulterated "if it has been prepared, packed, or held under insanitary conditions [1] whereby it may have become contaminated with filth, or [2] whereby it may have been rendered injurious to health."[114] When FDA detects HAV or norovirus in berries as part of the sampling assignment, the berries are adulterated under both parts of this provision. Presence of either virus indicates that feces (most common) or vomitus (less common) has contaminated

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[109] Depaola et al., supra note 76.
[110] Saupe A., Rounds, J., Sorenson, A., et al. 2021. Outbreak of Norovirus Gastroenteritis Associated with Ice Cream Contaminated by Frozen Raspberries from China. Clinical Infectious Diseases, 73(11), 3701-e3707.
[111] Tian, P., Yang, D., Jiang, X., et al. 2010. Specificity and kinetics of norovirus binding to magnetic bead-conjugated histo-blood group antigens. Journal of Applied Microbiology. 109: 1753-62.
[112] Industry was notified no later than December of that year. Memorandum to FDA Industry & Regulatory Stakeholders. Summary of External Feedback and FDA Responses to the FY 19-20 Surveillance Sampling Program Assignment: Frozen Berry Assignment. (December 19, 2018).
[113] 21 U.S.C. § 342(a)(1).
[114] 21 U.S.C. § 342(a)(4).

the berries.[115] The presence of HAV or norovirus in berries is not the "mere possibility of contamination"; it is actual contamination (quoting Berger v. U.S., 200 F.2d 818, 821 (8th Cir. 1952)).[116] Further, the contamination occurred as the result of insanitary conditions (e.g., exposure to contaminated water or dirty hands) while the berries were prepared, packed, or held.[117] Otherwise, the berries would not be contaminated; HAV and norovirus do not naturally occur in berries.

In addition to being adulterated because the berries were prepared, packed, or held under insanitary conditions whereby they may have been contaminated with filth, the berries are adulterated because those same insanitary conditions also may have rendered the berries injurious to health. Infection with HAV or norovirus is unquestionably injurious to health. HAV, a type of liver infection, is associated with fever, jaundice, vomiting, stomach pain, diarrhea, joint pain, and in rare cases, liver failure, and death. Symptoms of norovirus include diarrhea, vomiting, nausea, stomach pain, fever, body aches, and, in rare cases, death.

Although RT-qPCR assays do not directly measure the infectivity of HAV and norovirus when detected in food, there are several reasons to conclude that it is reasonably possible that frozen berries with detected virus could make consumers ill. As explained above, FDA's validated method for detection and extraction of HAV and norovirus from soft fruits reliably detects the presence of these viruses. Any detected level of these viruses could cause human illness because of their extremely low infectious doses (i.e., contamination of foods with microscopic amounts of infected feces can cause outbreaks and illnesses).[118] For example, ingestion of 1 virus particle of norovirus results in a 72% chance of infection in susceptible persons.[119] An additional degree of assurance is provided by Sanger sequencing of a different and larger region of the genome. Successful Sanger sequencing increases the likelihood that the detected virus is intact, which means the virus is more likely to be infectious. Finally, using FDA's method, FDA has detected HAV or norovirus in food associated with 17 berry or seafood outbreaks over a recent ten-year period.

For similar reasons, we disagree with the Petitioner's assertion that "the state of the science dictates the agency must use epidemiology to support decisions regarding recalls" (Petition at 5). Under this constraint, FDA would not be able to act to prevent illnesses until illnesses had already occurred and been detected. This is particularly problematic given how many illnesses from these viruses likely go unreported.

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[115] Glass, R., Parashar, U., Estes, M. 2009. Norovirus Gastroenteritis. New England Journal of Medicine. 351:1776-85. See also Fiore, supra note 2. Less commonly, the viruses may have originated from vomitus, which is also filth. See Kotwal, G. and Cannon, J. 2014. Environmental persistence and transfer of enteric viruses. Current Opinion in Virology. 4;37-43.

[116] Note that "[i]t is not necessary that [the food] actually become contaminated." Berger, 200 F.2d at 821.

[117] The key routes of contamination are human sewage pollution and poor personal hygiene. Leggitt, P., Jaykus, L. 2000. Journal of Food Protection. 63(12):1738-1744.

[118] Nasheri et al., supra note 25.

[119] Messner, M., Berger, P., Napper, S. 2014. Fractional Poisson—A Simple Dose-Response Model for Human Norovirus. Risk Analysis. 34(10):1820-9.

The final action requested by the petition before the sampling assignment is resumed is for "FDA to convene and utilize an expert panel to evaluate the agency's current validation protocols; establish scientifically and statistically sound criteria for sample positivity and negativity; and establish reliable confirmatory methods that are able to rule out false positive results" (Petition at 14). As explained above, the petition has failed to identify any deficiencies with FDA's validation process or its test method. Nor has the Petition established the need for "risk-based interpretative criteria for results confirmed through sequencing."

IV. Discussion – Criteria for a Mandatory Stay

A. Legal Authority

FDA regulations at § 10.35 (21 CFR 10.35) outline the requirements for an administrative stay of action. In accordance with § 10.35(e), FDA must grant a stay in any proceeding if all of the following apply:

(1) The petitioner will otherwise suffer irreparable injury.
(2) The petitioner's case is not frivolous and is being pursued in good faith.
(3) The petitioner has demonstrated sound public policy grounds supporting the stay.
(4) The delay resulting from the stay is not outweighed by public health or other public interests.

Section 10.35(e) also provides for the discretionary implementation of a stay in any proceeding if it is in the public interest and in the interest of justice.

B. Criteria for a Mandatory Stay Are Not Demonstrated

There are four criteria that must be demonstrated for FDA to grant a mandatory stay. We find that the Petitioner did not demonstrate two of the four required criteria in § 10.35(e). As a result, we need not address the other two criteria in § 10.35(e).

1. Petitioner Does Not Demonstrate Sound Public Policy Grounds Supporting a Stay

The petition states that "[t]he frozen berry industry has raised serious concerns with FDA's test method" (Petition at 9) and indeed AFFI has previously claimed that a particular FDA sample was a "false positive."[120] As explained in Section II.D., in response to AFFI's claim, FDA established that the sample did not match FDA's control strain and was a 100% match to a clinical sample, proving AFFI's claim incorrect. Just as that specific claim was incorrect, the petition's broader criticisms of FDA's test method are misplaced.

[120] Letter from Donna Garren, Executive Vice President, Science and Policy, AFFI to Frank Yiannas, Deputy Commissioner, FDA. (July 10, 2019), supra note 104.

FDA's test method is properly validated, was published online in 2018, and was previously provided to AFFI. Moreover, all information needed for a laboratory to use the method is publicly available. In addition, the method has adequate controls to address the possibility of false positives, and FDA is performing Sanger sequencing of positive results found during routine surveillance. Sanger sequencing both provides additional confirmation of the presence of HAV or norovirus and increases the likelihood that intact, infectious virus is present.

HAV and norovirus do not occur naturally in berries. Detection of one of these viruses indicates that feces and/or vomitus is present on the berries. Such contamination occurs as a result of insanitary conditions at some point in the supply chain, can cause human illness and, in rare instances, death, and renders the food adulterated. Therefore, a recall is an appropriate action when HAV or norovirus is detected in berries.

2. Petitioner Does Not Demonstrate That the Delay is Not Outweighed by Public Health or Other Public Interests

The Petitioner has not demonstrated that any reasons for delaying the sampling assignment are not outweighed by public health or other public interests that support resuming the sampling assignment. Berries contaminated with HAV and norovirus have been linked to multiple outbreaks, and some illnesses have likely gone undetected. HAV and norovirus are very infectious; as few as 1-10 viral particles are sufficient to infect humans. To prevent human illness, it is important that FDA resume the berry sampling assignment so that we can continue to generate data to better understand the risk to the public and take appropriate immediate and longer-term steps to protect the public health, including taking appropriate regulatory action when positive findings are observed. As explained in section III, none of the four actions requested by the Petitioner merit delaying the resumption of the sampling assignment, let alone outweigh the public health interest in resuming the assignment.

C. Discretionary Stay is Declined

The petition maintains that "it is, indeed, in the public interest and the interest of justice for FDA to pause the sampling assignment until such time as the test methodology and evaluation criteria have been published peer-reviewed, and/or established with the input of independent experts" (Petition at 11). However, Petitioner offers no new or additional evidence in support of this position. We therefore disagree and decline to grant a discretionary stay in accordance with § 10.35(e).

V. Conclusion

For the reasons outlined above, we are denying your petition for a stay of action in accordance with the requirements of § 10.35(e). Resuming the frozen berry sampling assignment is an important part of our risk-based and preventive approach to food safety. We remain committed to sharing data from the sampling assignment and continuing to work with the Petitioner and

other stakeholders to determine the prevalence of these pathogens, prevent potential exposures, and protect the public health.

Sincerely,

Douglas W. Stearn -S

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Date: 2022.07.22 10:28:49 -04'00'

Douglas Stearn
Deputy Director for Regulatory Affairs
Center for Food Safety and Applied Nutrition