March 18, 2022

Division of Dockets Management
Food and Drug Administration
Department of Health and Human Services
5630 Fishers Lane, rm. 1061
Rockville, MD 20852

Petition for Stay of Action

The undersigned organizations submit this petition requesting that the Commissioner of Food and Drugs stay the effective date of the following matter.

A. Decision involved

A stay is requested for the Food and Drug Administration's (FDA's) planned resumption of its microbiological sampling assignment for frozen berries. FDA's decision to resume the sampling assignment is detailed in the attached February 16, 2022, letter from Acting Commissioner of Food and Drugs Janet Woodcock, M.D., to Alison Bodor, President and CEO of the American Frozen Food Institute (AFFI).[1]

B. Action requested

We request that the Commissioner 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 (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, 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

[1] See Appendix A1. We note that for purposes of timeliness of this petition, we consider the relevant date of the FDA decision involved to be February 16, 2022, the date of the above-referenced letter from Dr. Woodcock. We are submitting this petition within 30 days of the letter.

negativity criteria, for interpreting RT-qPCR tests of enteric viruses in soft fruits, as well as interpretative 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.

C. Statement of grounds

Executive Summary

The frozen berry industry strongly supports public health initiatives to better understand prevalence and risk of pathogens in foods. FDA's planned surveillance assignment for frozen berries, however, fails to satisfy basic principles of science, due diligence, and fairness. In particular, FDA intends to use a test method that is neither externally validated nor published in a peer-reviewed journal, and that is not reproducible outside of the agency as FDA has not publicly shared adequate technical data and information on the method to be used by external laboratories. FDA has dismissed serious concerns raised by the frozen berry industry related to the test method and the need to adopt scientifically and statistically sound sample positivity criteria and has failed to adopt or consider the recommendations of experts in the field. To ensure that the sampling assignment achieves its goals of enabling the agency, industry, and the public to better understand the prevalence of enteric viruses in frozen berries and accordingly to assess the public health risk, FDA must pause the sampling assignment until such time as the agency grounds its approach in transparency and scientific expertise. FDA should recognize that the accuracy of information derived from sampling assignments is critical, as it informs future improvements in industry practices and ways to reduce potential risks of contamination.

Background on Petitioner Organizations

As the voice of the U.S. frozen food industry, AFFI is the national trade association that represents the interests of all segments of the frozen food industry. AFFI members manufacture and distribute frozen foods throughout the United States and globally. AFFI represents a broad group of companies throughout the food distribution chain, including food producers, distributors, and retailers.

The Washington Red Raspberry Commission (WRRC) was established in 1976 to promote the general welfare of the state and for the purpose of maintaining existing markets or creating new or larger local, domestic, and foreign markets; increase production efficiency; ensure a fair regulatory environment; and increase per capita consumption of red raspberries grown in Washington state. Critical among the WRRC's objectives is to maintain high standards for food safety, both for domestic and imported berries.

Established in 1981, the Oregon Raspberry & Blackberry Commission (ORBC) consists of nine members who represent the interests of 300 Oregon caneberry growers. The ORBC focuses on promoting caneberries to multiple audiences, and supports Oregon berry farmers by fostering plant research and farming education initiatives. The mission of the ORBC is to enhance the image of the blackberry and raspberry industry and to increase opportunities for profitability through promotion, education, research, and food safety.

The petitioners and their members are committed to ensuring that our industry provides the safest and most nutritious foods to consumers. As part of that commitment, we support efforts to improve public health and reduce food safety risks, including science-based microbiological sampling assignments to better understand the prevalence of enteric viruses such as HAV and NoV in foods. In its current state

and use, however, the FDA surveillance program for frozen berries violates basic tenets of science and transparency and has resulted in regulatory action in the absence of a defined public health risk.

Background on Testing for Hepatitis A and Norovirus

Both HAV and NoV are for all practical purposes considered "non-cultivable" pathogens. Unlike bacterial pathogens such as *Salmonella* or *Listeria monocytogenes*, for which the organism could be cultivated and grown using laboratory techniques,[2] and such growth would confirm the bacteria is viable, this cannot be done for HAV or NoV.[3] For non-cultivable pathogens, the scientific community relies on nucleic acid-based testing (RT-qPCR) to detect their presence in foods. Because cultivation is not possible, HAV and NoV must be concentrated and purified directly from the food matrix sample, the nucleic acid extracted, and then amplified by RT-cPCR. Controls must be used to ensure the efficacy of the concentration process and the efficiency of ensuing nucleic acid extraction and amplifications.

Importantly, the RT-qPCR method is designed merely to detect the presence of a small fragment of the viral ribonucleic acid (RNA); it cannot confirm the presence of intact viral particles, nor can it distinguish between infectious and noninfectious viral particles.[4] *A "positive" RT-qPCR result, therefore, is not synonymous with the presence of intact or infective virus. In fact, given the nature of this test, sample "positivity" only demonstrates the presence of a portion of the viral genome that is amplified and detected, and certainly does not establish the presence of infective virus.*[5]

In the RT-qPCR method used for the detection of HAV and NoV, a key indicator of the detectable level of the virus is determined by the Cycling threshold (Ct) value.[6] Although RT-qPCR can signal that viral nucleic acid is detectable, it does not establish the presence of infectious material. In contrast, for bacterial pathogens, cultural enrichment establishes viability and thus infectivity of the detected organism. This distinction is a vital one. Quite simply, any RT-qPCR signal or cycling threshold (Ct) value detected does not establish sample contamination or potential public health risk.

Furthermore, there are other limits to testing for these viruses. Controls must be used to ensure RT-qPCR reactions are not inhibited by the food matrix, ensure no cross contamination has occurred, and ensure that all the RT-qPCR steps are working properly. Further, expert guidelines on PCR testing

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2 This process is called cultural enrichment.

3 We note that culture-based detection of bacterial pathogens in foods represents the gold standard, and even this testing is typically followed with confirmatory biochemical, serological, or PCR-based assays.

4 Stals A. et al. 2013. Viral Genes Everywhere: Public Health Implications of PCR-Based Testing of Foods. Current Opinion in Virology 3(1):69-73.

5 Diez-Valcarce M. et al. Nov. 2011. Virus Genome Quantification Does Not Predict Norovirus Infectivity After Application of Food Inactivation Processing Technologies. Food and Environmental Virology 3:141-146.

6 In a RT-qPCR assay, a positive reaction is detected by accumulation of a fluorescent signal. The Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e., exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the Ct level the greater the amount of target nucleic acid or viral genetic material in the sample). The following document provides an explanation of the significance of Ct values and production of false positives in a RT-qPCR assay. Public Health Ontario, An Overview of Cycle Threshold Values and their Role in SARS-CoV-2 Real-Time PCR Test Interpretation, Sept. 17, 2020, available at https://www.publichealthontario.ca/-/media/documents/ncov/main/2020/09/cycle-threshold-values-sars-cov2-pcr.pdf?la=en.

indicate that high Ct values (greater than 40) are suspect and generally should not be reported. [7] The reason for this is 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. False positives can occur due to laboratory cross-contamination[8] or due to non-specific signals that increase in frequency during later rounds of amplification.[9] Another limitation to the testing method is that a minimum number of replicates with a positive signal may be needed to establish a high probability of positivity, particularly where high Ct values are involved. Again, one must be cautious when interpreting RT-qPCR results because they only indicate the presence of a small fragment of RNA, not the entire genome or an intact viral particle. Therefore, it can be very difficult to accurately identify the presence of the virus.

Given these challenges, a confirmation step is necessary to establish sample positivity. As of August 2019, at AFFI's request, FDA has included routine follow-up Sanger sequencing to its RT-qPCR testing method to further characterize any viral sequences present in RT-qPCR–positive samples. If sequencing is used for confirmation, though, criteria need to be set to define adequate sequence quality, particularly when sequencing amplicons from samples with high Ct values. It is unclear if FDA has done so.

In addition, there must be an assured way to identify laboratory cross-contamination. This is done by distinguishing between an amplicon generated from the positive control (the strain developed and used in laboratories) and from a wild-type strain (a strain that would be found in the environment and thus could potentially be found in food). Critically, the FDA method does not have a fail-safe way of identifying cross-contamination, which is a common cause of false positive results.[10] A recommended approach to control for this is to use positive controls that contain inserts replacing some of the wild-type virus sequences, which provides a means by which to readily identify cross-contamination. This is the approach used in the ISO 15216-1:2017 method.[11] In contrast, it does not appear that the FDA controls contain such inserts, though these sorts of hybrid molecules are easy to construct.

Though the use of confirmatory steps such as sequencing to establish amplicon identity is a welcomed approach, there are challenges in obtaining interpretable, high quality sequences on samples with Ct values greater than 40.[12] Experts recommend the use of alternative confirmation approaches such as re-extraction of the food sample and repeat of the Rt-qPCR assays or amplification of more than one viral genome region (which is commonly done for SARS-Cov-2 testing).

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[7] Bustin S.A. et al. Apr. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 55(4):611–622.

[8] *See, for example,* FDA BAM Chapter 26B, Appendix C, Tables 1 and 3, available at https://www.fda.gov/food/laboratory-methods-food/bam-26b-appendices-multi-laboratory-validation-hepatitis-virus-concentration-and-detection-protocols.

[9] These are referred to as mis-priming.

[10] We understand that FDA's method uses positive controls that are commercially available through the American Type Culture Collection (ATCC) and are described as synthetic GI and GII NoV RNA, and HAV. However, RNA sequences for these controls are not publicly available.

[11] ISO Method 15216-1:2017. 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, available at https://www.iso.org/standard/65681.html.

[12] The logic here is that detection through RT-PCR testing precedes identification performed through sequencing. If high Ct values suggest questionable detection of a virus, it is challenging to conclude that identification has been confirmed through sequencing.

Moreover, in addition to conducting sequencing, it is critical to establish appropriate sample positivity criteria. When RT-qPCR testing is conducted using a Ct value below 38, most virologists would agree the sample should be considered positive. In contrast, higher Ct values are typically considered unreliable.[13] FDA, however, has relied upon Ct values of 40 or greater to support its determinations that samples collected during the sampling assignment pose a public health risk and its decisions to request product recalls. By way of comparison, in clinical practice, high Ct values (usually those greater than 35) do not constitute a diagnosis for norovirus in humans. The same can be said for SARS-CoV-2 testing. In addition, the international scientific community has implemented a variety of criteria that can be used in establishing interpretive guidelines for sample positivity, including requirements for replicate positive amplifications; repeat testing; Ct value cut-offs; amplification of two or more targets (e.g., as is done in SARS CoV-2 testing); and/or establishment of genome copy 'tolerances' below which public health risk is minimal.

As a final piece of the puzzle, because of the inherent limitations in testing non-cultivable pathogens like HAV and NoV, to consider a sample infectious, epidemiological data is critical. As explained, detecting the presence of a piece of viral nucleic acid does not mean that the virus has been identified, there is infectious material, or it poses a public health risk. We recognize that FDA cannot resolve questions regarding infectivity in the short term (and we are not asking the agency to do so before resuming the sampling assignment). Given the limitations discussed above, however, the state of the science dictates the agency must use epidemiology to support decisions regarding recalls. For instance, in 2013, there was an outbreak of HAV in the U.S. associated with frozen pomegranate arils imported from Turkey. In this case, the agency used RT-qPCR sequences from clinical samples – rather than food samples – to establish strain relatedness between patients and to identify the potential source. 165 cases of HAV were reported across 10 states and there was significant data available.[14]

As we hope is apparent from the complex and technical nature of this background information, testing and interpretation of such test results for non-cultivable pathogens is laden with challenges and uncertainty. Such testing should not be undertaken without a clear plan for how these challenges will be addressed.

Background on Safety History of Frozen Berries

Frozen berries have a long history of safe consumption. *Critically, domestic frozen berries have never been associated with foodborne illness.* In support of this sampling assignment, between 1997 and 2016 – a nearly twenty-year period – FDA reported three HAV outbreaks and one NoV outbreak linked to frozen berries in the U.S.[15] *Each of these, however, involved imported product.* More recently, FDA cited a 2019 article regarding frozen fruits and foodborne illnesses.[16] The article documented 12 HAV outbreaks, of which only two (previously reference by FDA), occurred in the U.S. These include

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[13] Stals et al. (2013), *supra* note 4. *See also* Bosch A. et al. 2018. Foodborne viruses: Detection, risk assessment, and control options in food processing. International Journal of Food Microbiology 285:110-128.

[14] Collier M.G. et al. 2014. Outbreak of hepatitis A in the USA associated with frozen pomegranate arils imported from Turkey: an epidemiological case study. Lancet Infectious Diseases 14:976-81. *See also* Outbreak of Hepatitis A, Linked to Pomegranate Seeds | CDC.

[15] *See* Microbiological Surveillance Sampling: FY 19-20 Frozen Berries (Strawberries, Raspberries and Blackberries) | FDA (current as of Feb. 25, 2022).

[16] Nasheri N. et al. 2019. Foodborne viral outbreaks associated with frozen produce. Epidemiology and Infection 147, e291, 1–8.

pomegranate arils from Turkey that went into frozen berry blend (2013), and frozen strawberries from Egypt (2016).[17] The article also documented 40 NoV outbreaks, of which only one occurred in the U.S. (frozen raspberries from China, 2016). Further, there have been multiple NoV outbreaks on cruise ships sailing under U.S. jurisdiction in September-October 2019, with the investigation concluding that raspberries from China were the cause of the outbreaks. NoV outbreaks have never been associated with domestic berries. Again, domestic frozen berries have never been linked to foodborne illness, reflecting a long history of safe consumption.

FDA has explained that strawberries, raspberries, and blackberries are delicate and may become contaminated with bacteria or viruses if handled by an infected worker who does not use appropriate hand hygiene, or if exposed to contaminated agricultural water or a contaminated surface, like a harvesting tote.[18] As an initial matter, berries that are intended to be frozen are mechanically harvested in the United States. As FDA notes, the transmission of enteric viruses to berries would occur if an infected worker failed to wash his or her hands and then handles the berries. Because mechanical harvesting is most commonly used in the United States for berries intending to be frozen, there is reason to be skeptical of findings of enteric viruses in frozen domestic berries. We recognize that there are instances where berries are intended to be sold fresh, and therefore are handpicked, but that may ultimately be sold as frozen due to marketplace conditions. But the FDA's produce safety rule provides a layer of protection in that it establishes requirements for worker hand hygiene and for preventing contamination from communicable diseases that present a public health risk.[19] Food safety audits are routinely conducted for compliance with these requirements.

More importantly, if infectious virus were being transmitted to frozen berries, one would expect to see cases of HAV or NoV across the country. HAV is a reportable disease that must be reported to state or local health departments or the Centers for Disease Control and Prevention (CDC) when diagnosed by doctors or laboratories. To date, there have not been any norovirus or hepatitis A illnesses traced to recalled products from the berry sampling program. This data suggests that costly recalls have been undertaken even in situations where the actual risk of disease is not present – in other words, the test protocol used in the surveillance assignment effectively gives a false positive result.

We recognize the risks associated with imported berries and believe the opportunity to identify and mitigate risks should be focused on imported product. Further, given the nature of farming and harvesting practices among small and marginal farming communities that are the predominant suppliers in the exporting nations, we believe that efforts toward risk assessment and risk management practices are critical to ensuring the safety of imported frozen berries.

Background on FDA Sampling Assignment for Frozen Berries and Exchanges with Industry

In August 2018, FDA solicited feedback from stakeholders on the agency's planned sampling assignment for frozen berries. The solicitation did not include details on the test methodology to be used. AFFI submitted comments at that time, supporting the sampling assignment, but requesting

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17 Unreported in this paper are hepatitis A outbreaks associated with frozen strawberries from Mexico (1997); and with fresh blackberries (2019), which may have been manually harvested.

18 *See* Microbiological Surveillance Sampling: FY 19-20 Frozen Berries (Strawberries, Raspberries and Blackberries), available at: https://www.fda.gov/food/sampling-protect-food-supply/microbiological-surveillance-sampling-fy-19-20-frozen-berries-strawberries-raspberries-and (current as of Feb. 25, 2022).

19 21 C.F.R. §§ 112.31; 112.32(b)(3).

additional information about the analytical methods that would be used. 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.

FDA began collecting and testing frozen berries (strawberries, raspberries, and blackberries) under the sampling assignment in November 2018. Following initial positive results for Hepatitis A in a small but unexpected number of samples tested under the assignment, AFFI asked FDA to sequence these results due to the high Ct values detected, as well as the fact that the positive results were never associated with food-transmitted cases of HAV. The agency then conducted such sequencing, but relied on RT-qPCR Ct values of 40 or more and based on its conclusion that the samples represented positives, requested that companies conduct recalls in June 2019.[20]

AFFI submitted letters to FDA dated June 13, and July 2, 2019 related to the sampling assignment, FDA regulatory actions, and the release and reporting of results. The letters raised and reiterated serious concerns with the testing methodology, including the recommendation that FDA establish a Ct cut-off value for determining negative results, when amplification should be repeated, and when sequencing is appropriate. AFFI noted that international experts are suspicious of Ct values above 38 as being a false positive due to limits in the method and potential for cross contamination. AFFI recommended that FDA establish several different Ct thresholds, including (1) a value above which a result would be considered a false positive, (2) a range within which the PCR test would be repeated, and (3) a value below which a result would be sequenced for confirmation. AFFI noted that international experts have concluded that at a higher Ct value, it is increasingly difficult to assess public health risk. Based on expert advisement, AFFI suggested that samples yielding Ct values of 42 or higher do not represent a demonstrated public health risk.[21]

FDA responded in two separate letters, dated July 24, and August 9, 2019. The agency explained that the RT-qPCR method has undergone multi-lab validation by FDA for the detection of HAV and NoV in soft fruit and will remain the primary test under this assignment. This validation, however, has never to our knowledge been peer-reviewed or published, nor was it conducted by independent laboratories outside the agency. FDA disagreed with the concerns raised about high Ct values, pointing to its ability to sequence samples with high Ct values.[22] The agency, however, agreed to implement Sanger sequencing as a routine component of the sampling assignment to characterize viruses present in any RT-qPCR positive samples. Some of the challenges associated with sequencing high Ct RT-qPCR positives discussed above were apparent in reviewing the agency's sequencing results. Thus, the agency's confirmation step, namely sequencing, did not provide the industry with added confidence in the agency's approach.

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[20] *See* Public Health Alert Concerning Heptatitis A Virus Contamination of Kroger Brand Frozen Blackberries and Costco Kirkland Signature Brand Three Berry Blend, available at: https://www.fda.gov/food/alerts-advisories-safety-information/public-health-alert-concerning-hepatitis-virus-contamination-kroger-brand-frozen-blackberries-and.

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

[22] We question the agency's claimed ability to sequence samples with high Ct values, noting that international experts routinely agree that sequencing quality is compromised at high Ct values due to lower sensitivity, which is why sequencing criteria is critical. Further, the agency's ability to sequence samples with high Ct values has primarily been in the case of foodborne illness outbreaks. In the absence of corresponding epidemiological data, public health risk cannot be ascribed to high Ct values.

Following these exchanges, as well as in-person meetings held in August and November 2019, the sampling assignment was paused in 2020 due to the COVID-19 pandemic, at which time it was 56% complete, with an approximate 1% violation rate based on 1,120 samples collected and analyzed. In the meantime, AFFI convened its own panel of international experts in enteric viruses to review the state of the science around testing for enteric viruses in berries and included these experts in several meetings with the agency to share their insights. AFFI sent another letter to FDA in April 2021, held a virtual meeting with the agency in May 2021, and sent follow-up correspondence in June 2021 detailing the conclusions reached by the expert panel. AFFI sent a follow up letter to FDA dated December 10, 2021, reiterating its significant concerns with the testing method and sample positivity criteria. Another virtual meeting was held in February 2022. In its February 16, 2022, response letter to AFFI, FDA announced the planned resumption of the sampling assignment and the planned continuation of FDA's policy to request a recall when FDA detects a positive using RT-qPCR and Sanger sequencing further characterizes a virus.[23] Many of the serious concerns raised by the frozen berry industry have gone unaddressed. It is for this reason that we are petitioning the Commissioner for a stay of the sampling assignment.

Basis for Staying the Surveillance Program

Our request meets the criteria under which the Commissioner *must* grant a stay. A stay must be granted if all the following four factors are met.[24] As discussed below, each of the factors are satisfied here.

(1) The petitioner will otherwise suffer irreparable injury.

Irreparable injury to the frozen berry industry would result from FDA's continued implementation of the sampling assignment and surveillance program in its current state. This injury includes: reputational harm to the frozen berry industry caused by product recalls and associated press released absent a real public health risk, and economic costs related to unnecessary recalls and loss of sales.

AFFI has sponsored an economic analysis of the FDA's enteric virus surveillance program for frozen berries, conducted by the Policy Navigation Group, attached to this petition as Appendix C. The analysis estimates the total social costs and economic impact for the expected 21 false positives that could result from the remainder of FDA's testing program. The social costs are nearly $40 million, depending on the growers' costs. The economic impact is much larger, approaching $800 million. Market losses comprise most of both cost categories. For consumers, the social costs are the loss of enjoyment from frozen berry products. For producers, the lost sales are distributed along the supply chain. Direct costs include the costs to take back and manage the affected products; respond to customer, supplier, and government inquiries; retain experts; and review and, if necessary, change

[23] Microbiological Surveillance Sampling: FY 19-20 Frozen Berries (Strawberries, Raspberries and Blackberries), available at: https://www.fda.gov/food/sampling-protect-food-supply/microbiological-surveillance-sampling-fy-19-20-frozen-berries-strawberries-raspberries-and (current as of Feb. 25, 2022). ("If the FDA detects hepatitis A virus or norovirus RNA in a sample, the agency will notify the firm of the findings and work with the firm to take appropriate action to protect the public health. Enforcement activities include actions to correct and prevent violations and to remove violative food from the market, as appropriate. Since July, the FDA began notifying firms of positive RT-qPCR positive samples and is awaiting the results of the Sanger sequencing to affirm the findings before asking the affected firms to conduct an immediate voluntary recall of the product."). *See also* Woodcock Letter, Feb. 16, 2022.

[24] 21 C.F.R. § 10.35(e).

operating procedures. Indirect costs may include the loss of brand reputation, increases in litigation, and increases in insurance premia.

Given the number of small growers and small processors, FDA's inaccurate testing results and associated conclusions regarding positivity and public health risk will almost certainly have a significant economic impact on small businesses. If, as expected, growers bear a disproportionate share of the market disruptions and loss of revenue, thousands of smaller farms would lose more than three percent of their revenue – the threshold of concern under the Small Business Administration's guidance for the Regulatory Flexibility Act.

Therefore, the social cost and industry impact of FDA's testing protocol are substantial. In its current form, the sampling assignment will lead to inefficient spending of real resources and market dislocations that harm consumers and growers, processors, and retailers of frozen berry products. These market disruptions almost certainly will have significant impacts on thousands of small businesses. Given these high social costs, FDA should spend a small fraction of these social costs to verify and to improve the specificity of its testing protocol for enteric viruses.

(2) The petitioner's case is not frivolous and is being pursued in good faith.

The frozen berry industries need to have confidence in the scientific approaches and regulatory precedents being set, especially where public health impact is asserted by the agency. In the past three years, we have communicated earnestly with the agency and brought attention to key and pragmatic concerns related to sampling, testing and interpretation of results associated with enteric viruses in berries, yet these scientific concerns, including those raised by a panel of international experts, have been dismissed by the agency. The concerns identified have very serious consequences to the berry category and merit consideration and a response by the agency. These are not frivolous concerns or ones raised in bad faith. To the contrary, since 2018, the frozen berry industry has been supportive of the sampling assignment, provided it is undertaken with the scientific rigor it merits. Accordingly, we are not requesting that FDA discontinue the sampling assignment entirely. Rather, we are requesting that the agency delay the assignment until it takes the steps outlined above to ensure it is on sound scientific footing.

(3) The petitioner has demonstrated sound public policy grounds supporting the stay.

As explained below, sound public policy grounds, as well as legal requirements, dictate that the stay must be granted. To proceed with the sampling assignment without a validated and published test method, and to conclude samples may present a public health risk without a scientific basis to do so, would be arbitrary and capricious and contrary to law under the Administrative Procedure Act. The frozen berry industry has raised serious concerns with FDA's test method, and the agency should take the further steps recommended below to ensure the test method is scientifically sound. Simply, the FDA method has never been made public; it is not used around the world; private laboratories cannot replicate it because FDA has not published sufficient details to allow this, meaning that industry cannot use the method to do its own testing to assess risks; all method validation to date has been internal within FDA; and FDA will not accept the ISO methods that experts in enteric virus detection recommend.[25] Taken together, FDA's failure to validate and publish details on its analytical methodology violates basic principles of science, due diligence, and fairness.

[25] ISO standards are reference methods for food microbiological regulations and are widely used for food microbiological analysis across the world. ISO methods form the basis for laboratory accreditation globally. They are internationally agreed upon by experts. *See* www.iso.org/standards.

Further, even assuming the test method is valid and FDA had been appropriately transparent about the method and its validation, FDA has failed to establish that its findings present a potential public health risk or that the product is adulterated, warranting a recall or other action. FDA has concluded that a snippet of RNA present in berries renders a product adulterated and capable of presenting a public health risk – without having scientifically and statistically sound criteria for sample positivity. This snippet of RNA is not sufficient information to support a determination that the samples present a public health risk.

The FDA policy to request a recall is contrary to law. Product is only recalled if it violates the laws the agency administers and is one against which the agency would initiate legal action such as a seizure.[26] The current state of science for testing or enteric viruses in frozen berries does not sufficiently establish that berries with detectable viral nucleic acid are adulterated within the meaning of the Federal Food, Drug, and Cosmetic Act or violate the agency's laws. In particular, FDA has not established that a piece of nucleic acid that is detected and sequenced is a poisonous or deleterious substance, nor that it may render the food injurious to health.[27]

Without a better understanding of when a piece of nucleic acid is infectious, FDA cannot conclude that it is indeed poisonous or deleterious, let alone whether it "may render" the food injurious to health. The courts have made clear that the term "may" requires a "reasonable possibility."[28] FDA has not provided sufficient evidence and information on which to support a conclusion that simply by detecting a snippet of RNA, there is a reasonable possibility of harm. In fact, the courts have also made clear that Congress did not intend to prohibit a food that contains any amount of a poisonous or deleterious substance.[29] That is what FDA has done here. While we are not asking to FDA to establish principles for infectivity before resuming the sampling assignment, the agency should at the least have greater confidence in the test results by implementing sound sample positivity criteria.

In addition to being contrary to law, the FDA policy on recalls in the context of the sampling assignment is also arbitrary and capricious because the agency has not taken the time needed to develop appropriate assessment criteria for the test methodology. It is arbitrary and capricious to conclude,

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Two ISO methods exist for determination of HAV and NoV in food using real-time RT-PCR. These are ISO 15216-1: 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 available at https://www.iso.org/standard/65681.html; and ISO 15216-2:2019 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, available at https://www.iso.org/standard/74263.html.

[26] 21 C.F.R. § 7.3(g).

[27] 21 U.S.C. § 342(a)(1) (stating "a food shall be deemed to be adulterated . . . if it bears or contains any poisonous substance which may render it injurious to health").

[28] *U.S. v. Anderson Seafoods, Inc.*, 622 F.2d 157 (5th Cir. 1980) (stating "The 'may render' standard has been interpreted to mean that there is a reasonable possibility of injury to the consumer.) *See also U.S. v. Lexington Mill & Elevator Co.*, 232 U.S. 399 (1914); *Berger v. U.S.*, 200 F.2d 818, 821 (8th Cir. 1952) (explaining that the "'mere possibility' of contamination" is not sufficient).

[29] *U.S. v. Lexington Mill & Elevator Co.*, 232 U.S. 399, 411 (1914) (explaining that if Congress had intended to deem food adulterated if it contains any poisonous or deleterious substance, it would not have used the words "may render … injurious to health"). The Court also cited a member of Congress stating "let me state that everything which contains poison is not poison. It depends on the quantity and the combination." *Id*. at 412.

based only on a piece of viral nucleic acid, particularly one detected using a high Ct value, that a sample should be considered positive and to ascribe public health risk to such a food. This would be akin to finding a strand of human hair and concluding that the source of hair is a living human being based only on that single piece of information.

> (4) The delay resulting from the stay is not outweighed by public health or other public interests.

Based on the long history of safe consumption of frozen berries in the U.S. and the lack of evidence of food-transmitted cases of enteric viruses in domestic frozen berries, there is not a sufficient public health risk that warrants the agency's deviation from the established scientific procedures FDA has used *for any other sampling assignment*. The surveillance assignment is designed to better understand potential prevalence of viruses in frozen berries. It was not issued in response to known instances of contamination or foodborne illness outbreaks with domestic berries, nor is there an active outbreak related to this commodity, as has been the case with other FDA sampling assignments (e.g., Romaine lettuce). To the contrary, and as discussed above, frozen berries have a long history of safe consumption in the U.S. Indeed, we are not aware of any confirmed illnesses associated with frozen berries since this assignment began. If the agency's goal in undertaking the sampling assignment is to better understand the prevalence of enteric viruses in frozen berries and the corresponding public health risk, proceeding at this stage with a flawed testing method and without appropriate sample positivity criteria will not accomplish the agency's goal.

Given the low public health risk here, and the harm that could result to the berry industry through the appearance that its products are harmful to health when that has not been sufficiently established, it would be arbitrary and capricious for the agency not to take the time to get the test method right and shore up the criteria used to determine whether a sample is positive. Indeed, FDA has already paused the sampling assignment for two years due to the COVID-19 pandemic. We fail to see why FDA cannot now take the time needed to ground its work in basic scientific principles.

Our request therefore meets the criteria for the Commissioner to grant a mandatory stay. Our request also meets the conditions under which the Commissioner *may* grant a stay.[30] A stay *may* be granted if it is in the public interest and the interest of justice. 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. The interests of both the public and of justice are served where the agency acts in a manner that is transparent and in keeping with the same scientific principles FDA has historically applied in sampling assignments. Indeed, these are the same tenets FDA would require regulated industry to follow for analytical methodology validation and application. The interests of the public are *not* served where FDA requests product recalls in the absence of scientifically sound testing methodology and assessment criteria. As detailed in the economic analysis of the sampling assignment, there are significant economic and social costs to such regulatory actions.

Basis for Additional Requested Steps Before Resumption of Sampling Assignment

AFFI requests that the Commissioner stay the sampling assignment until such time as the agency has completed the four requested steps related to the detection method and sample positivity criteria. Sampling and testing foods for enteric virus contamination is complex and fraught with unresolved

[30] 21 C.F.R. § 10.35(e).

scientific issues, including but not limited to the inability to culture them outside the human body and the relationship between detection and infectivity. It is therefore critical to apply scientifically valid, vetted, and robust methods and testing criteria.

Confidence in the agency's capability to utilize an appropriate method and analysis is the direct result of the agency's transparency with its protocols and use of methods. *Neither the soft fruit test method nor its validation have been published in the peer-reviewed literature or in the FDA BAM.* Peer-review and publication is necessary to provide transparency and establish credibility in the agency's methods. Further, a thorough, vetted validation can be used to develop scientifically justified criteria for interpretation of test results and application for regulatory purposes. It also allows for reproducibility by industry in private labs, which can support the industry's prevention practices. As such, the agency should establish, submit to peer-review, and publish its current validation protocols and confirmatory methods, including criteria for data quality and interpretation, particularly where samples with high Ct values are subjected to sequence confirmation. The agency should also recruit the views of independent experts in virology and microbial risk assessments, to establish credible sample positivity criteria.

Below we provide greater detail on the four specific steps we are asking FDA to take before resuming the sampling assignment for frozen berries.

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 RT-qPCR detection assay, using independent laboratories outside the agency.

To date, 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.[31] We understand from one of our virtual meetings with the agency that a validation of FDA's matrix extension protocol for soft fruits has been completed and was to be submitted for publication. However, to date, no such validation has been peer-reviewed and published, nor does it appear that the FDA validation work involved external laboratories; instead, it relied upon only internal FDA laboratories at the district level and did not involve its own expert virologists at MOD1 within the Center for Food Safety and Applied Nutrition. We ask that FDA perform a validation of its test method that (1) addresses its use in soft fruits, (2) utilizes independent laboratories external to the agency.

As part of the validation, it is critical that FDA address how, using the method and subsequent sequencing step, the agency is able to delineate the positive control strain from wild-type virus sequences. We request detailed scientific information supporting how this delineation can be made and establishing that the agency is applying high quality sequencing and sound methods of bioinformatics.

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.

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[31] FDA BAM Chapter 26B Appendices: Multi-laboratory Validation of Hepatitis A Virus Concentration and Detection Protocols, available at https://www.fda.gov/food/laboratory-methods-food/bam-chapter-26b-appendices-multi-laboratory-validation-hepatitis-virus-concentration-and-detection.

Put simply, the frozen berries 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. We are disappointed to see that the agency has not yet taken this step and believe it is critical for FDA to do so before resuming the sampling assignment. Peer-review and publication is necessary to provide transparency and establish credibility in the agency's methods. Further, a thorough, vetted validation can be used to develop scientifically justified criteria for interpretation of test results and application for regulatory purposes.

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.

We understand from FDA's February 16, 2022, letter to AFFI that FDA intends to release a new chapter of the BAM focused on the detection of viruses in berries and to resume the sampling assignment two weeks after its publication. This is critical, as the agency's failure to release a detailed protocol to date reflects a profound lack of transparency. Nonetheless, we understand this planned publication will fail to provide the following critical elements: (1) methods or scientific criteria by which sequencing analysis can discriminate between the positive control and wild-type virus; (2) information on how the method can be used to discriminate between infectious and non-infectious virus; (3) sufficient time for stakeholder review. Two weeks is not sufficient time, particularly when the assignment has been on hold for roughly two years.

It's also important to note that traditionally, BAM procedures do not require validation studies or "limits of the method" which establish it as "fit for purpose." It is unclear whether these studies were conducted, despite our previous requests for this information via letters to the agency and a Freedom of Information Act (FOIA) request, both of which have gone without response. A good example of a multi-country/lab validation published in peer-reviewed literature is Lowther et al., (2019), which provides method characteristics including limit of detection, limit of quantification, repeatability, and reproducibility in several food matrices.[32]

The agency should not resume the sampling assignment until a detailed protocol and information on validation is publicly released, with sufficient detail to allow replication of the test method externally for use in soft fruits. If the purpose of the sampling assignment is to better understand the prevalence and public health risk associated with the potential presence of enteric viruses in frozen berries, this goal cannot be achieved if FDA is the only body capable of using the relevant test method. Industry and independent laboratories need to have the ability to reproduce the test method so that additional testing can be done.

Again, we are aware of no other FDA sampling assignment where the agency has not published a replicable test method. A relevant example is recent agency work in developing *Cyclospora cayetanensis* detection methods, which were published in the BAM, revised Chapter 19B and linked publications. We are concerned about procedural fairness and the precedent that would be set if FDA does not provide sufficient detail to enable the method used to detect enteric viruses in frozen berries to be reliably reproduced.

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[32] Lowther J.A. et al. 2012. Two-year systematic study to assess norovirus contamination in Oysters from commercial harvesting areas in the United Kingdom. Applied and Environmental Microbiology 78(16):5812-5817.

4. FDA should convene an international panel of experts (including members from within FDA such as MOD 1, the Centers for Disease Control, USDA's Food Safety and Inspection Service, and academia) with expertise in virology and microbial risk assessments in foods, tasked with establishing risk-based interpretive criteria, including sample positivity and negativity criteria, for interpreting RT-qPCR tests of enteric viruses in soft fruit, as well as interpretative 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.

In mid-2020, AFFI constituted an international expert panel of virologists, food scientists, and researchers from academia, national government agencies, and industry to deliberate key scientific issues at the heart of enteric virus detection in berries. This included comparing the results from various surveillance studies of enteric viruses in foods outside the U.S. and identifying several important issues related to the application of RT-qPCR methods and interpretation of results that are pertinent to the agency's use of its current methods and interpretation of the data as related to potential public health impact. Some of the pertinent deliberations of the panel were previously shared with the agency both in writing and in virtual meetings with the expert researchers. We are awaiting the completion of the panel's white paper and encourage the agency to review and incorporate the panel's recommendations.

It is our understanding that FDA was also going to constitute an expert panel to consider the issues associated with enteric virus detection methods and interpretation of results. We would urge the 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. In light of the challenges posed by testing for non-cultivable pathogens and the impact product recalls have on the berry industry, it is critical that FDA establish credible sample positivity criteria and reliable confirmation methods. To the extent FDA has not already recruited such a panel, this work could be done by the panel AFFI convened or with assistance from the National Advisory Committee of Microbiological Criteria for Foods (NACMCF) or the Association of Official Analytical Collaboration (AOAC) International. Unless and until FDA has articulated a comprehensive scientific understanding of the implications of finding viral nucleic acid in foods and established sound criteria for interpreting the results from the sampling assignment, the sampling assignment should not proceed.

* * *

We appreciate the Commissioner's consideration of this petition. We look forward to working collaboratively with the agency to further our shared goals of advancing the safety of frozen berries while ensuring the integrity and validity of the scientific methods used.

Sincerely,

Alison Bodor
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On behalf of:

American Frozen Food Institute
Washington Red Raspberry Commission
Oregon Raspberry & Blackberry Commission

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