August 8, 2019

Donna M. Garren, Ph.D.
Executive Vice President, Science and Policy
American Frozen Food Institute
2345 Crystal Drive, Suite 801
Arlington, VA 22202

Dear Dr. Garren:

Thank you for your recent correspondence, on behalf of the American Frozen Food Institute (AFFI), regarding the U.S. Food and Drug Administration (FDA) microbiological sampling assignment for frozen berries. The FDA shares your concerns about the safety of the U.S. food supply and the importance of maintaining the public trust. By letter dated July 24, 2019,[1] we addressed the sampling assignment protocols, regulatory actions, and reporting issues raised in your letters of June 13, 2019, and July 2, 2019.

The purpose of this letter is to address your concerns about FDA's test methods for hepatitis A virus (HAV) and norovirus (NoV) in berries, raised in your earlier letters and a letter dated July 10, 2019, which asserts that FDA obtained a false positive test result for sample 1084927 due to laboratory contamination. The July 10 letter also expresses concern about information sharing. You request that FDA discontinue its frozen berries surveillance sampling program until and unless AFFI's concerns are addressed.

For the reasons explained below and in our earlier response, FDA is continuing with a modified frozen berries sampling assignment, consistent with our mission to help ensure the safety of the U.S. food supply.

FDA Sample 1084927 Matches a Clinical Specimen
No Laboratory Contamination

Your July 10 letter asserts that FDA's test result on sample 1084927 was a false positive. You indicate that an external expert conducted a comprehensive analysis of the HAV sequence FDA obtained, and the expert concluded that it is highly likely that FDA's result is a consequence of laboratory cross contamination.

[1] See Letter from Frank Yiannas, Deputy Commissioner, FDA, to Donna Garren, Executive Vice President, Science and Policy, AFFI, dated July 24, 2019, enclosed.

As discussed in FDA's letter of July 24, FDA's RT-qPCR detection assay for HAV and NoV method has undergone multi-laboratory validation, while viral extraction from soft fruit is a matrix extension of the FDA BAM method (Chapter 26B) that had undergone a multi-laboratory validation. 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.

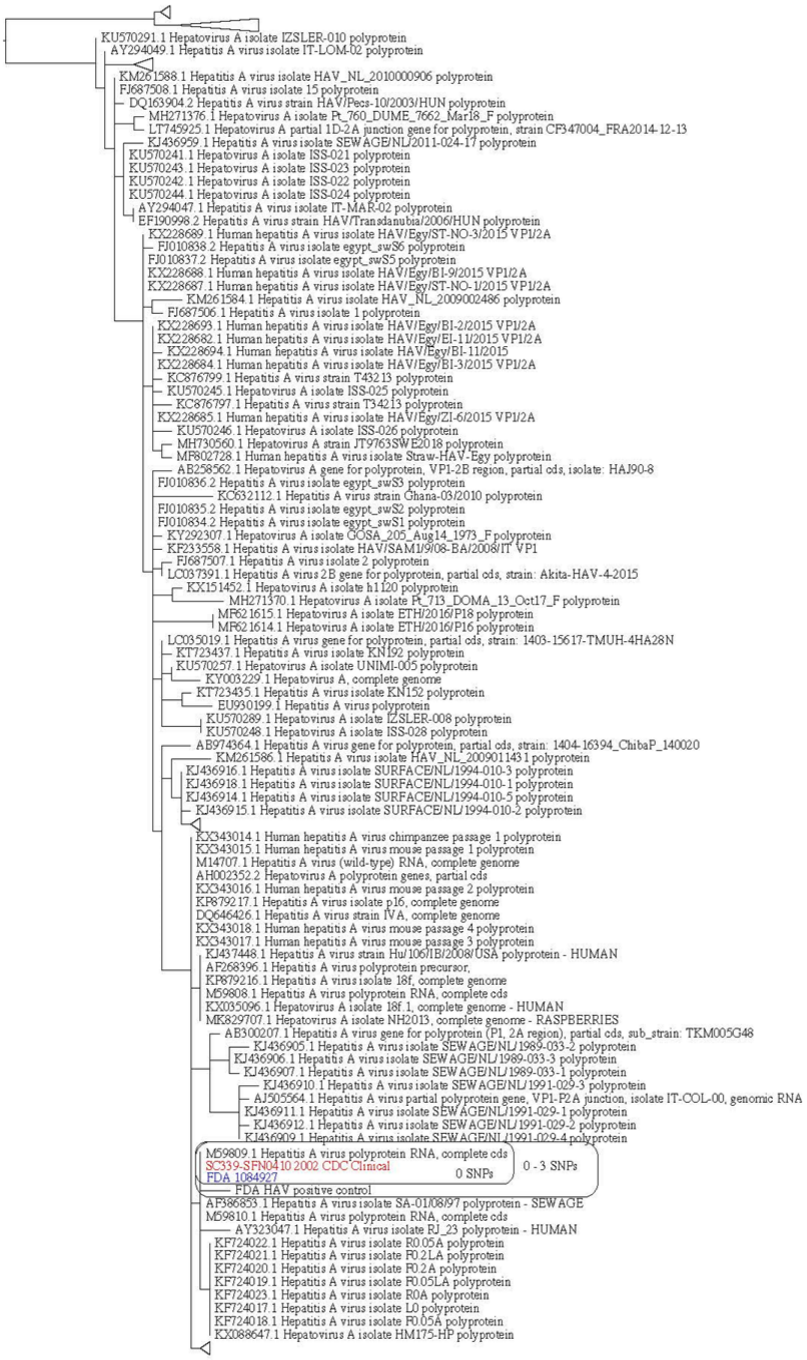
In analyzing the sequence of FDA sample 1084927, FDA found that the sequence obtained was consistent with other HAV sequences (M59809; most similar BLAST result) in the National Center for Biotechnology Information (NCBI) database.[2] This sequence comparison was not intended to determine HAV strain relatedness, for exclusionary purposes, nor to establish geographic origin.

FDA also submitted the HAV sequence from FDA sample 1084927 to the Centers for Disease Control and Prevention (CDC) for comparison to its non-public, internal HAV database of sequenced clinical specimens. After closely reviewing and comparing the sample sequence to its database, CDC experts concluded the HAV sequence from FDA sample 1084927 is a 100% match (315 of 315 base alignment) to an HAV positive specimen detected during CDC's clinical surveillance sampling in 2002.

FDA performed additional analysis using the CDC 2002 sequence, along with the best 250 matches, when BLAST-ing sample 1084927 against the nr/nt NCBI database to determine relatedness to other HAV strains. The agency then aligned sequences using muscle (Multiple Sequence Comparison by Log-Expectation) and sparsely populated flanking regions were trimmed. (NB: The one base difference between FDA1084927 and M59809 found by AFFI resided in the trimmed region.) Phylogenetic inference was performed using GARLI (Genetic Algorithm for Likelihood Inference) with the GTR (General Time Reversible) model of evolution and selecting the best of 10 non-bootstrapped replicates. DNA distances were computed using the Ape package in R with pairwise deletion set to true. Additionally, comparisons of FDA sample 1084927 to sequences in the public NCBI database showed other strains (from sewage and humans in addition to control strains) clustering closely with FDA sample 1084927. FDA is providing the results from the above analysis in the dendrogram below (Figure 1). This analysis confirmed that the HAV sequence from sample 1084927 is identical (315 of 315 bases align) to a CDC clinical HAV specimen from 2002. The laboratory positive control is 3 SNPs different from these matching sequences.

[2] NCBI provides public access to biomedical and genomic information, including genome sequences. BLAST is a program for sequence similarity searching developed at NCBI and is instrumental in identifying genes and genetic features.

Figure 1. Dendrogram demonstrating relatedness of the HAV sequence from sample 1084927, the laboratory positive control strain, a CDC clinical surveillance specimen (from CDC's internal database), and related strains from the NCBI public database.



Based on this analysis, FDA scientists have concluded that the HAV sequence FDA obtained from sample 1084927 is not the result of laboratory contamination.

Results with High Cycle Threshold Values Have Public Health Significance
Recommended Cut-off Value Unnecessary

Your letters recommend that FDA establish a cycle threshold (Ct) cut-off value above which RT-qPCR results should be considered false positives and not sequenced. The July 2 letter states: "In reviewing the scientific literature and discussing this issue with experts in the field, we understand that above Ct values of 37 or 38 the results are suspicious of being false positives due to the limits of the assay and potential for cross contamination." In support, you cite Gao *et al.* in which a sample with a Ct cut-off value of more than 38 was considered NoV negative.[3]

FDA disagrees with the assumption that all high Ct values should be considered false positives. We believe Gao *et al.* has been misinterpreted; it states:

> *"In this study, the CT cut-off value was used as a criterion for the determination of NoV contamination in berry samples. Although the method has been widely used (Jiang et al., 2018; Liu et al., 2016),* ***the use of the CT cut-off leads to underestimation of positive samples in practice****. Therefore, the positive rate of NoV contamination in berries collected in this work may be higher." (emphasis added)*

As discussed above, FDA successfully sequenced sample 1084927, in which HAV was detected through RT-qPCR with a Ct value of 42, and matched (315 of 315 bases) to a CDC clinical specimen. Based on that analysis, FDA scientists have concluded that the qPCR result on sample 1084927 is not a false positive, despite the high Ct value.

FDA achieves successful analytical results with:

1) An efficient extraction method to reduce the presence of potentially inhibitory material coextracted with viral RNA;
2) High amplification efficiency in detection and sequencing reactions; and
3) Sufficient quantity and quality of RNA for sequencing.[4]

Your July 2 letter also suggests that samples yielding Ct values of 42 or higher do not represent a demonstrated public health risk, and you recommend that FDA consider high-value Ct results to be negative for purposes of enforcement or other regulatory action. In the July 10 letter, you request that FDA issue a statement that any prior recalls or market withdrawals based on Ct values greater than 37 were unwarranted.

FDA has data that do not support this assumption. For the past decade, we have successfully sequenced samples of foods epidemiologically linked with HAV and NoV illnesses—with Ct values as high as 50. These data are reported in Table 1 (below).

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[3] Gao, *et al.*, 2019. Surveillance of norovirus contamination in commercial fresh/frozen berries from Heilongjiang province, China, using a TaqMan real-time RT-PCR assay. Food Microbiol. 82, 5812-5817.

[4] An inability to successfully sequence the targeted genome after an initial positive RT-qPCR should not necessarily be considered a false positive due to the expected low level of the viral RNA. Nonetheless, for this sampling assignment FDA will not request initiation of a voluntary recall unless further characterization (i.e., Sanger sequencing) is achieved.

Table 1. Analytical Results of Seafood and Berries Associated with Illness (2009-2018).

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 GII	42	NoV GII.2
Outbreak Related	Raspberries	2017	NoV GII	49	GII.17B
Outbreak Associated[a]	Crabmeat	2018	HAV	47	HAV IA

[a] Not meal remnant but associated lot

* 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 U.S. Food Microbiol. 59, 76-84.

Viray, M.A. *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.

FDA's experience and the published literature demonstrate that foods yielding high Ct values have been identified as the vectors for HAV and NoV outbreaks.

For the reasons explained above and in our earlier response, FDA intends to continue the frozen berries surveillance sampling program as modified and clarified.

FDA Procedures for Information Sharing

Your July 10 letter references blinded information that was shared in response to an inquiry from a state-chartered berry commission. FDA is committed to ensuring that sample results are shared in accordance with agency procedures, which we have reviewed and reinforced with agency staff.

In closing, we value the continuing collaboration with AFFI and its members and share the commitment to strengthening the safety of the U.S. food supply to protect American consumers.

Sincerely,

Frank Yiannas
Deputy Commissioner
Food Policy and Response

Enclosure
Letter from Deputy Commissioner Frank Yiannas
to the American Frozen Food Institute from July 24, 2019
https://www.fda.gov/media/129655/download