Development of a screening assay for hepatitis A virus which correlates to infectivity

Summary

Fresh and frozen berries have been associated with hepatitis A virus (HAV) outbreaks. HAV cannot be grown in the lab, hence reliance on molecular amplification (RT-qPCR) for detection. RT-qPCR detects the viral genetic material, which can persist after virus inactivation, so detection does not correlate with presence of infectious virus or public health risk. Nucleic acid intercalators (PMA/EMA/PMAxx) prevent detection of non-infectious virus by entering disrupted non-intact capsids, binding to viral RNA, and preventing amplification. They have been used for molecular-based infectivity discrimination assays, but with inconsistent performance. Similarly, platinum chloride ($PtCl_4$) or enzymatic pre-treatments can be used. This project seeks to optimize these methods to develop a rapid screening assay to detect infectious HAV to the exclusion of non-infectious virus, in berries.

Benefits to the Industry

Public concerns about the safety of the berry supply chain are on the rise, risking the competitiveness of a market valued over $1 billion. North American raspberry, blackberry, and strawberry growers and processors are increasingly required to provide certificates of analysis that their products are enteric virus-free, and are also struggling to ensure the safety of imported product. These testing requirements are burdensome, expensive, and time-consuming, and importantly, positive results do not reflect the presence of infectious virus and public health risk. Providing methodological additions that facilitate infectivity discrimination to standard detection protocols like ISO 15216 or FDA-BAM will provide greater assurance that test results are truly aligned with public health risk, keeping harmful product off the shelf and safe product from destruction or diversion.

Objectives

1. **Methods Optimization**: Improve performance of candidate molecular-based infectivity discrimination methods. This includes development of long-range RT-qPCR assay more amenable for use with RNase treatment; optimizing RNase treatment for increased performance in HAV genome regions with high degrees of secondary structure; and improving consistency of $PtCl_4$ pretreatments with high and variable organic loads typically found in berry samples.
2. **Screening**: Perform direct comparison between optimized methods, PMAxx, and infectivity assay as applied to fully infectious HAV, and that partially and fully inactivated by exposure to heat and sodium hypochlorite.
3. **Matrix Performance**: Evaluate the efficacy of the top-performing method(s) in a frozen berry matrix seeded with HAV (fully infectious, partially inactivated, and fully inactivated) processed for detection using the ISO 15216 protocol.

Methods

The long-range RT-qPCR method utilizes the existing forward primer and probe from the ISO 15216 method, while increasing the amplicon size using newly identified downstream reverse primers. Selection of reverse primer locations is being done by a combination of sequence alignment ('msa' package in R) and secondary structure modeling (The Vienna RNA Websuite, http://rna.tbi.univie.ac.at/).

For Objectives 2 and 3 (**Figure 1**, overview), HAV stocks with varying degrees of capsid damage (infectivity) will be generated using either heat or chlorine to represent different types of capsid damage and used to assess the performance of the optimized molecular infectivity discrimination methods in comparison to plaque assay. For Objective 3, the virus samples will be spiked into berries that have been processed through the ISO 15216 concentration protocol.

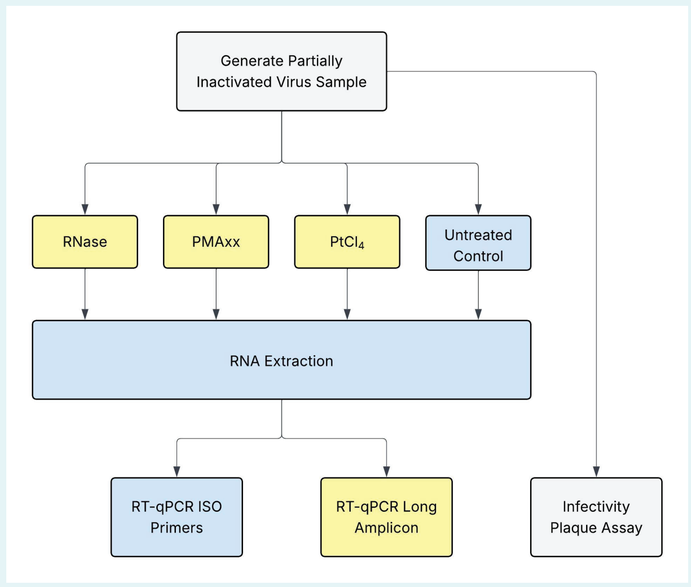

Figure 1: Experimental design for Objectives 2 and 3, with blue representing the RNA extraction and RT-qPCR detection of the current ISO 15216 method, and yellow representing the potential modifications to the method to account for viral infectivity.

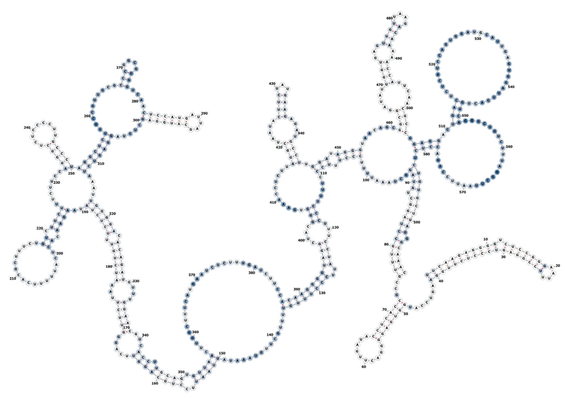

Figure 2: Centroid structure RNA model of the first 600 bases of HAV wild type strain HM-175 (M14707) at 37°C. Shading represents entropy, with darker colors being higher entropy (less stable).

Results to Date

RNA secondary structure modeling at 37°C (**Figure 2**) showed extensive regions of low entropy double-stranded helices in the RT-qPCR amplification region targeted in the ISO 15216 standard method (bases 68–240). Expanding the target region to include the relatively unstable region from bases 255–310 and the loop region from bases 355–385 could potentially improve efficacy of RNase treatment necessary to improve the assay performance. Two potential reverse primer locations (~340–360 and ~390–410) that are well-conserved amongst all HAV genotypes and have minimal secondary structure have been identified (**Figure 3**). We are in the process of validating these primers along with the reverse primer GAR1R used by the FDA BAM method (bases ~515–535) for use in a long-amplicon RT-qPCR assay.

Figure 3: Sequence alignment of consensus sequences for each human genotype of HAV.

Contact

Lee-Ann Jaykus, PhD (Co-PI)
Distinguished Professor Emeritus
North Carolina State University
Department of Food,
Bioprocessing and Nutrition Sciences
leeann_jaykus@ncsu.edu

Authors

Jeremy Faircloth, Lynette Johnston (PI),
Benjamin Chapman (Co-PI), Lee-Ann Jaykus (Co-PI)

Project funding dates

January 1, 2025 – December 31, 2025

Acknowledgements

We acknowledge Mr. Jeremy Faircloth for his efforts in planning and implementation of this research project. We also thank Dr. Sanjay Gummalla of American Frozen Foods Institute (AFFI) and Donna Browne (Naturipe Farms), Kinsey Clishe (North Bay Produce), and Rebecca Anderson (Global G.A.P) for their excellent stakeholder engagement efforts.