

# Hydrogel affinity particles to enable high throughput screening of soft fruits for intact HAV virions

## Summary

Polymerase chain reaction (PCR) is the gold standard for hepatitis A virus (HAV) testing. But PCR cannot discriminate between environmental genetic material, which poses no harm, and genetic material contained within intact virions, which are potentially infectious. This inability can result in large economic losses associated with product recalls due to harmless environmental genetic material. We propose the use of hydrogel nanoparticles (Nanotraps), designed to capture intact viruses, to enrich only intact virions during soft fruit PCR testing. If successful, Nanotraps would improve the accuracy of food safety testing by excluding environmental RNA during PCR-based analyses. The proposed method can be performed using liquid-handling equipment greatly increasing the number of fruit samples that could be tested while simultaneously decreasing the time and cost.

## Objectives

1. Adapt existing RT-qPCR HAV assays to multiplex RT-dPCR format and assess performance for quantification and detection of HAV RNA.
2. Assess the HAV RNA extraction efficiencies of two RNA extraction kits compatible with an automated workflow.
3. Compare Nanotrap concentration to azo-dye pretreatment for the detection and quantification of HAV RNA within intact virus capsids.
4. Assess the efficiency and performance characteristics of the complete workflow for the recovery and quantification of HAV RNA within intact virus capsids.

## Methods

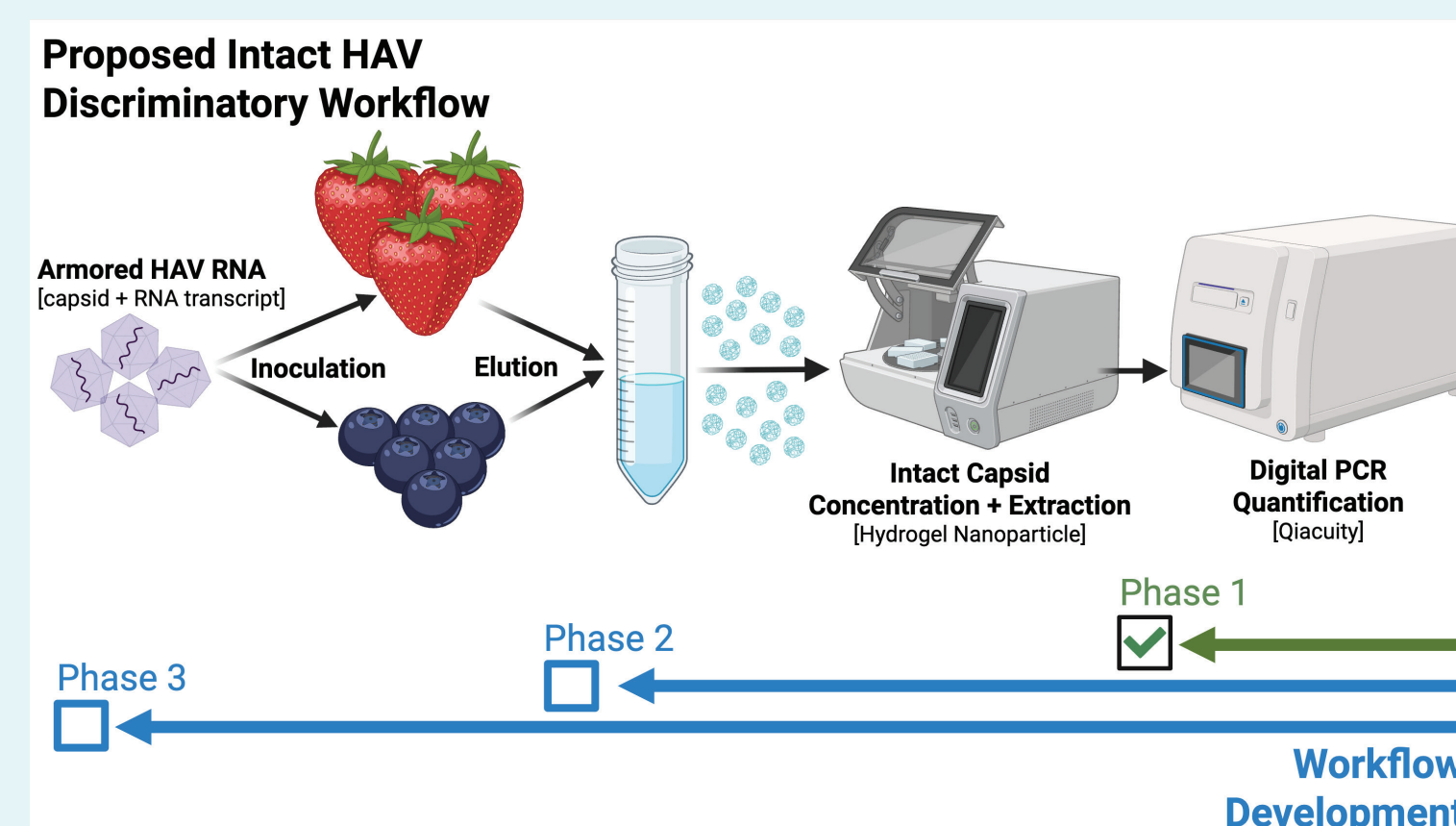
We propose a four-step method using Nanotrap particles and RT-dPCR to recover, enrich, and measure RNA from intact virions containing RNA from HAV (**Figure 1**). First, virions will be eluted from soft fruits using a buffer adopted from existing standard methods. Second, intact virions will be captured from the buffer using Nanotrap particles implemented onboard a KingFisher Apex. Third, the Nanotrap-captured virions will be lysed and extracted with a commercial RNA extraction kit, also performed on a KingFisher. Lastly, RNA targets will be measured using RT-dPCR. The method will be developed, optimized, and assessed using HAV Armored RNA control materials inoculated into the workflow in a step-by-step fashion proceeding upstream from the analytical endpoint to the soft fruit elution.

## Results to Date

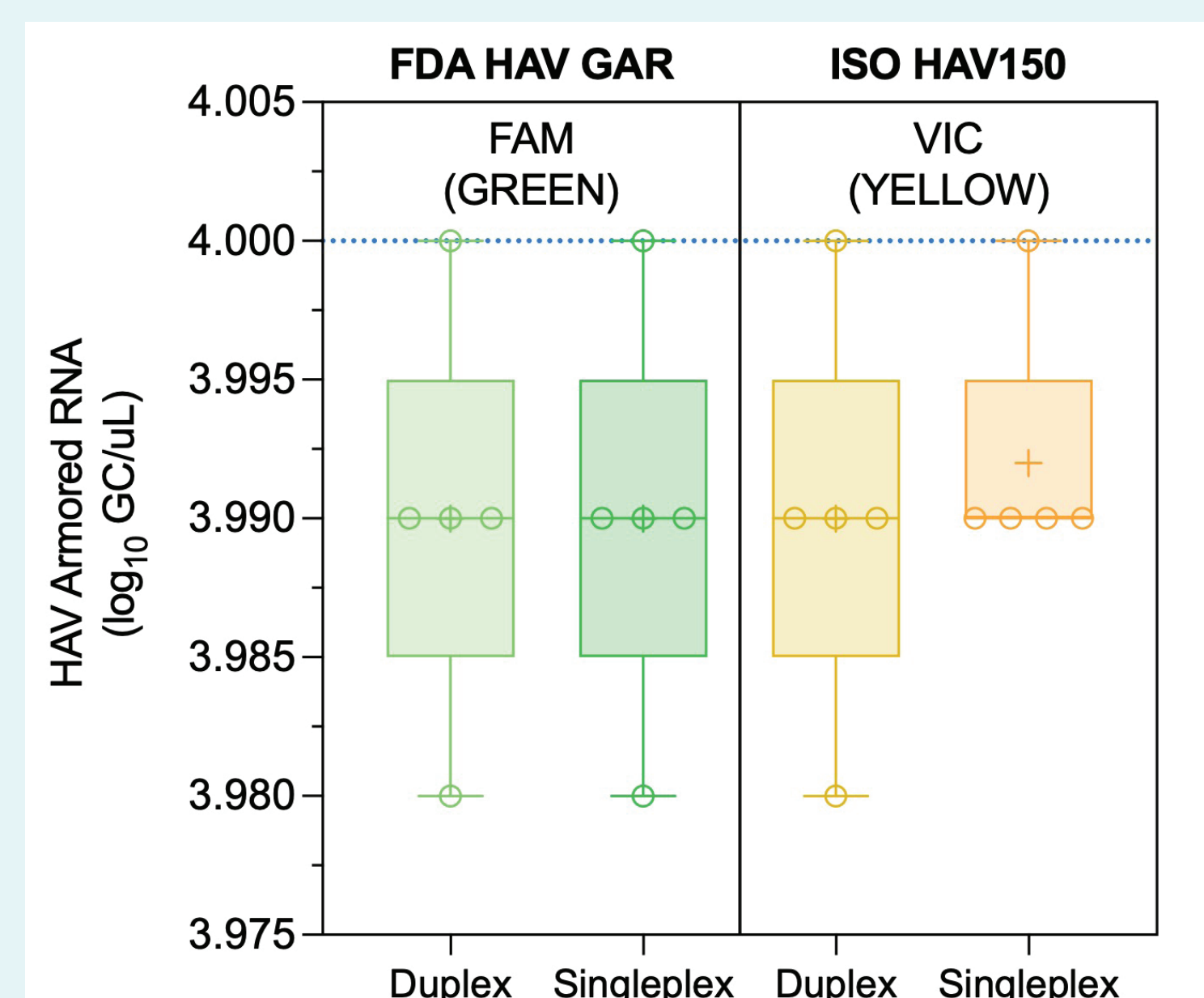
We have designed and procured an Armored RNA Quantitative control (BioTechne) that contains a 500-base single-stranded RNA transcript of the 5' UTR of the HAV genome, which contains both the ISO and FDA RT-qPCR assay targets. Using both cDNA control materials and heat-extracted HAV Armored RNA, we have verified the performance of the ISO and FDA RT-qPCR assays implemented in single-plex and duplex format on digital PCR (RT-dPCR, Qiacuity) format (**Figure 2**). We are now beginning head-to-head testing of RNA extraction performed onboard the KingFisher Apex (**Figure 3**) using two kits: the NucleoMag DNA/RNA Water Kit (Macherey-Nagel) and the MagMax Wastewater Ultra Nucleic Acid Isolation Kit (ThermoFisher).

## Benefits to the Industry

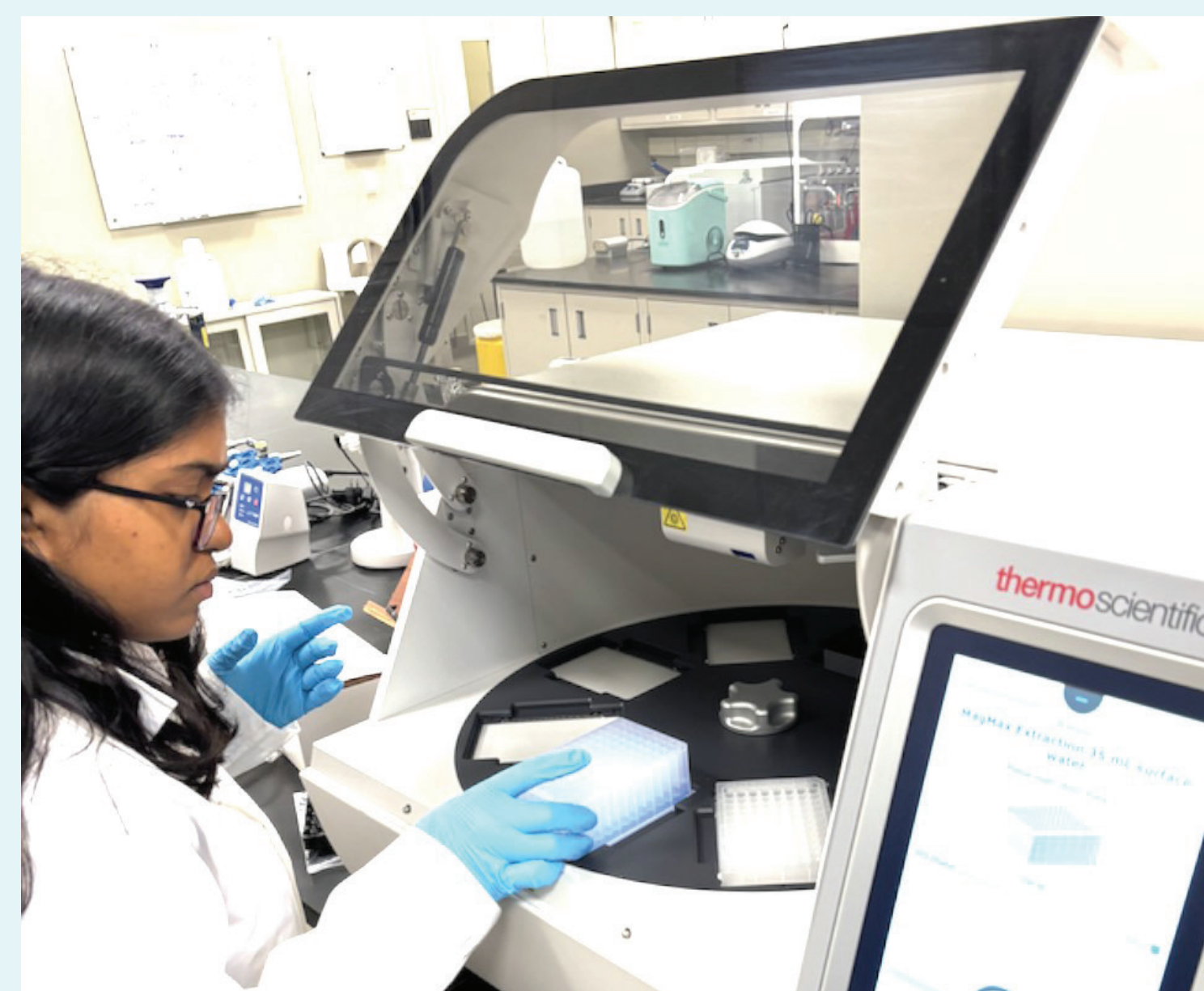
If the hydrogel-based method successfully discriminates between intact virions and environmental RNA the entire soft fruit supply chain would derive benefits. Soft fruit growers, buyers, and sellers could minimize the risk of economic losses associated with product recalls from non-discriminatory testing results that are not specific to potentially infectious intact virions. Regulators could benefit from a method that can be performed rapidly and at large scales via liquid handling robots. Finally, consumers would benefit from improved product safety owing to high-throughput screening and timely results. Consumers would also enjoy less inflationary pressure on the price of soft fruit products derived from further de-risking the supply chain.



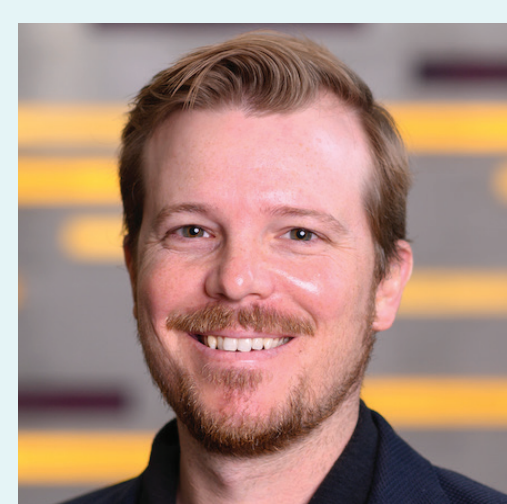
**Figure 1:** The proposed hydrogel-based workflow for concentrating intact HAV virions from soft fruit wash.



**Figure 2:** Preliminary RT-dPCR results for quantifying HAV Armored RNA using single-plex and duplex ISO and FDA HAV qPCR assays in single-plex and duplex formats.



**Figure 3:** Saborni Sen prepares an RNA extraction experiment on the KingFisher Apex.



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