

# Improving methods for the assessment of infectious human enteric virus survival in produce

## Summary

Viruses like human noroviruses (NoV) and hepatitis A virus (HAV) can cause gastroenteritis and hepatitis from consuming contaminated leafy greens and berries. Detecting these viruses is challenging due to the lack of straightforward automated methods. Moreover, new approaches are needed to confirm the infectivity of identified viral genomes. This project optimized an ultrafiltration-based concentration method for NoV and HAV analysis, comparing it to current reference protocols. The project has also refined capsid-integrity assays to estimate viral infectivity and explored the applicability of salivary-gland cell lines for detecting infectious NoV. These optimized procedures will help us understand how infectious viruses persist in leafy greens and berries during post-harvest storage and disinfection.

## Objectives

1. Optimize extraction and concentration procedures to detect infectious NoV and HAV in leafy greens and berries.
2. Optimize capsid-integrity molecular assays to better estimate NoV and HAV infectivity.
3. Investigate persistence of infectious viruses, capsid-protected genomes, and total genomes in lettuce and berries under different post-harvest conditions.

## Methods

**Optimization of virus concentration procedure:** Two alternative protocols were adapted: aluminum hydroxide adsorption-precipitation (AlCl<sub>3</sub>) and Concentrating Pipette Select (CP InnovaPrep, USA). Method effectiveness was compared to the current ISO and FDA reference protocols, using 6 matrices (romaine lettuce, spinach, baby leaves, strawberries, raspberries and blueberries) spiked with HAV, NoV genogroup I (GI), and NoV GII. Preservation of viral infectivity was confirmed for HAV (TCID<sub>50</sub> assay) and NoV (Human Intestinal Organoid (HIO) system). Infectivity of NoV was also assessed on the salivary gland (SG)-derived ductal (NS-SV-TT-DC) continuous cell line.

**Optimization of capsid-integrity assays:** Novel HAV and NoV PMAxx viability RTqPCR assays with x2-4-fold increase in amplicon lengths were designed and validated on leafy greens and berries using infectious and inactivated (5 min at 99°C) viruses.

## Results to Date

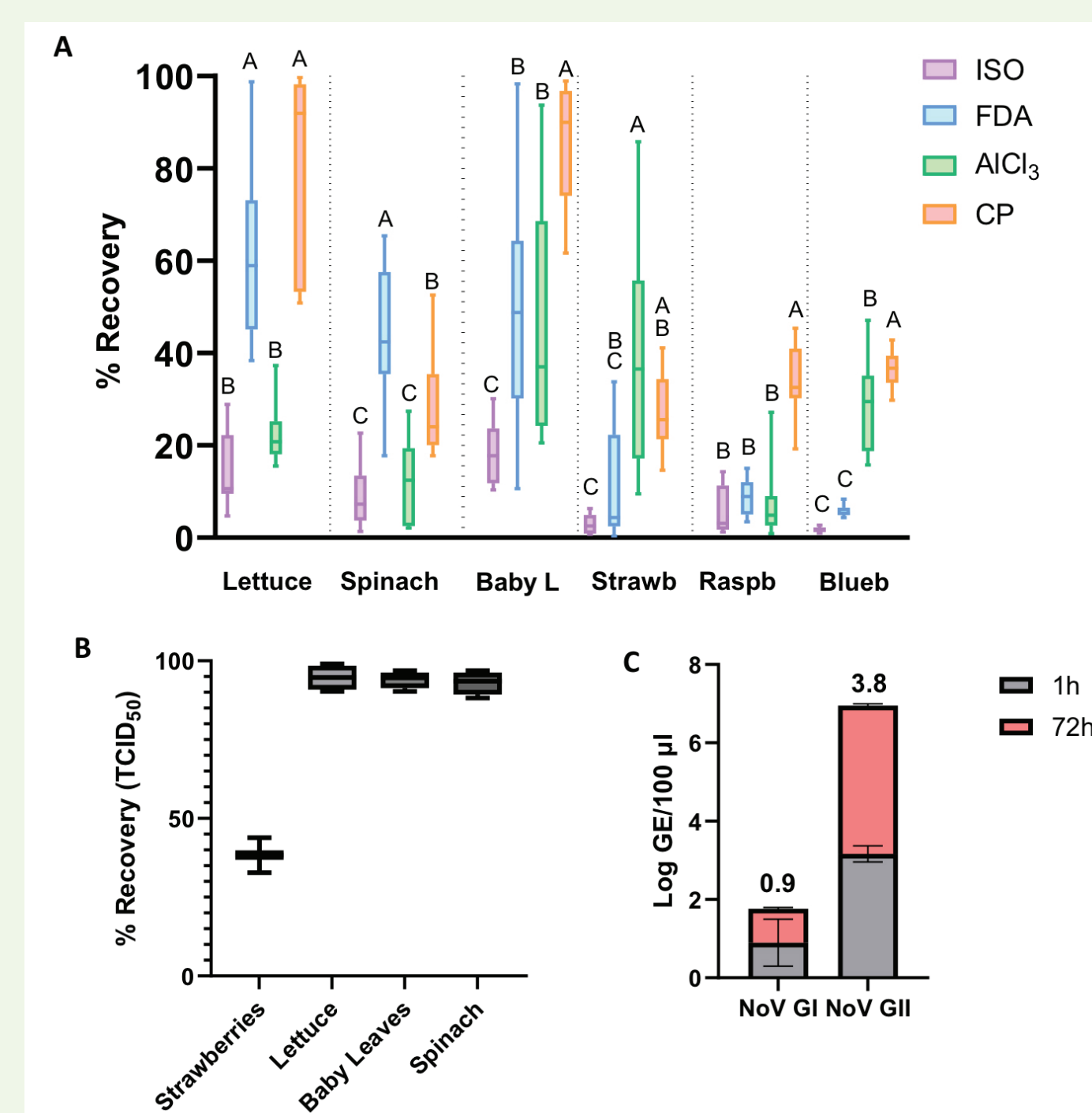
Mean recoveries of novel and reference protocols on leafy greens/berries were 26.5/24.7%, 64.1/32.5%, 14.0/3.5% and 50.9/8.4% for AlCl<sub>3</sub>, CP, ISO and FDA, respectively. CP shows better performance than AlCl<sub>3</sub> for 5 of 6 matrices, better than ISO for all 6 matrices, and better than or equal to FDA for 3 of 6 and 2 of 6 matrices (**Figure 1A**). Overall, CP enables a low turnaround time (<1 h), satisfactory recoveries for all matrices without requiring ultracentrifugation equipment, preservation of infectivity (**Figure 1B-1C**), and LoD<sub>95</sub> comparable to both reference methods (**Table 1**).

The use of the newly developed PMAxx RTqPCR assays based on long amplicons was validated on several food CP concentrates (**Figure 2**). Reduction in false positives or even complete loss of signal was obtained in samples containing heat-inactivated viruses for all 3 targets.

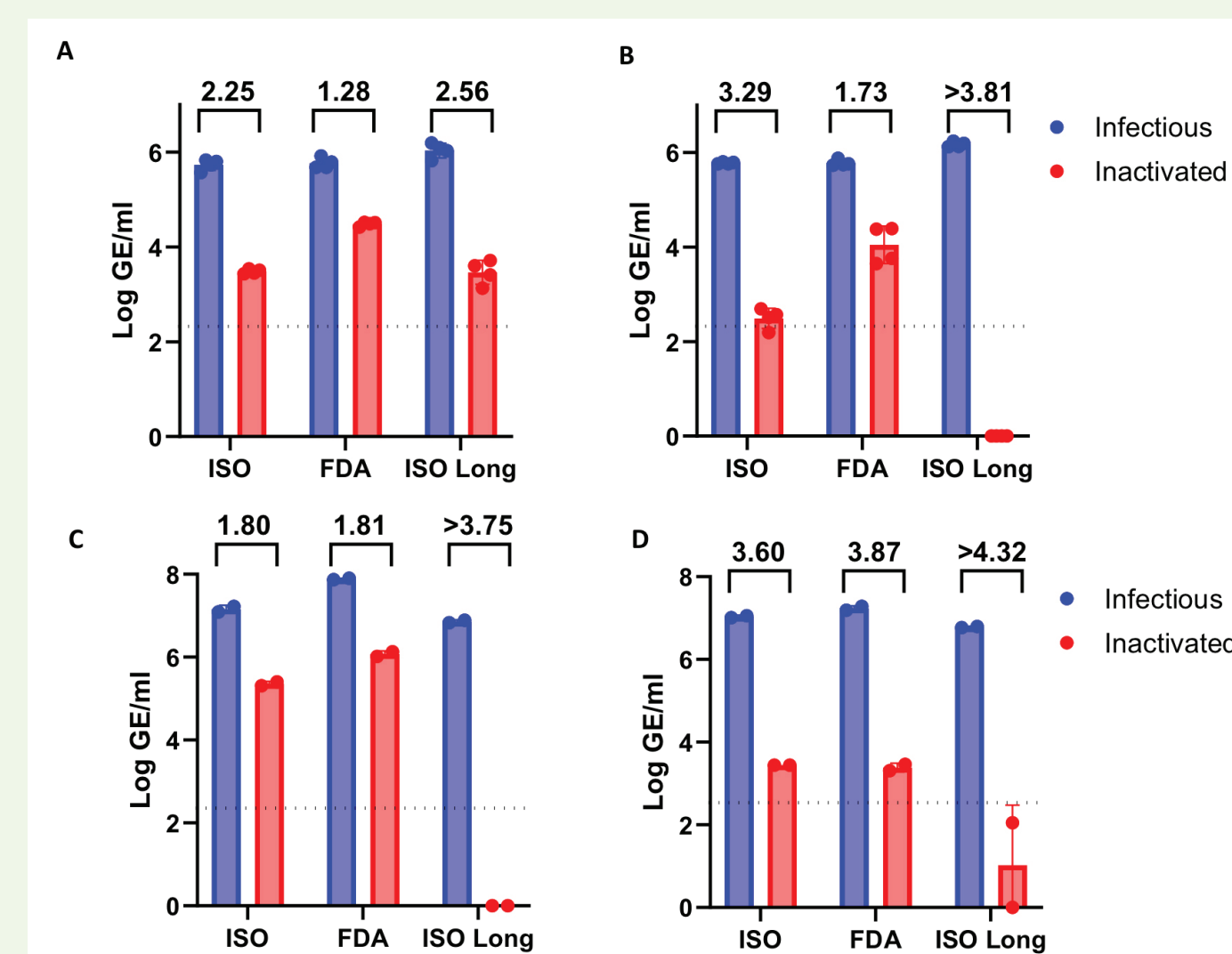
## Benefits to the Industry

The current project aims to provide the following benefits to the fresh produce processing industry:

- Optimized user-friendly Standard Operating Procedures for detection and quantification of NoV and HAV from leafy greens and berries, with reduced turnaround time and independent of ultracentrifugation equipment.
- Refined capsid-integrity assays to provide a better correlation with NoV and HAV infectivity.
- Knowledge of die-off rates of infectious NoV and HAV in leafy greens and berries in post-harvest scenarios to support design of effective practices.



**Figure 1 (A)** Overall recoveries (%) for all 3 target viruses for each type of food matrix. Values with different capital letters above the error bars are significantly different ( $p < 0.05$ ). Whiskers represent 10–90 percentile. **(B)** Recovery of infectious HAV from different food matrices using the CP method, as measured by cell-culture assay. **(C)** Confirmation of NoV infectivity extracted from strawberries using the CP method on HIOs. Grey shading indicates genome equivalents (GE) per well at 1 h post-infection (hpi), and red shading at 72 hpi. Values above the bars represent log<sub>10</sub> difference in viral growth (72h-1h).



**Figure 2:** Performance of PMAxx viability RTqPCR to discriminate between infectious and inactivated (5 min at 99°C) HAV on lettuce (**A**) and spinach (**B**), NoV GI on strawberries (**C**) and NoV GII on strawberries (**D**), using the ISO RTqPCR assay, the FDA RTqPCR assay and the modified ISO-Long RTqPCR assays. Number above each bar indicates the mean log<sub>10</sub>-reduction. Dotted line indicates the LoD of the ISO-Long RTqPCR assays.

Extraction/ Concentration	ISO RTqPCR						FDA RTqPCR					
	Romaine Lettuce			Fresh Spinach			Romaine Lettuce			Fresh Spinach		
	ISO	FDA	CP	ISO	FDA	CP	ISO	FDA	CP	ISO	FDA	CP
HAV	156.0	<8	29.9	121.0	110.0	293.0	227.4	21.1	51.8	287.6	146.4	848.2
GI	13.3	<8	<8	13.3	21.1	<8	<8	<8	<8	<8	<8	<8
GII	436.0	35.2	13.3	178.0	178.0	777.0	860.9	120.7	66.8	196.6	237.0	4150.9
	Strawberries			Blueberries			Strawberries			Blueberries		
	ISO	FDA	CP	ISO	FDA	CP	ISO	FDA	CP	ISO	FDA	CP
HAV	221.0	22.4	106.0	178.0	178.0	29.9	452.3	214.3	51.8	130.9	18.41	16.96
GI	18.4	<8	<8	13.5	<8	<8	<8	<8	<8	<8	<8	<8
GII	94.4	<8	63.7	197.0	178.0	<8	<8	<8	<8	13.3	<8	<8

**Table 1:** Limits of detection at 95% confidence (LoD<sub>95</sub>) for each extraction/concentration method expressed in genome equivalents (GE)/g, using ISO and FDA RTqPCR protocols for detection.



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