Optimizing methods for the detection and quantification of infectious human norovirus from fresh berries using human intestinal enteroids

Contact

Malak Esseili, PhD
University of Georgia
Center for Food Safety
malak.esseili@uga.edu

Authors

Akhila Pati (graduate student),
Issmat Kassem (Co-PI)

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Summary

Human norovirus (HuNoV) and hepatitis A virus (HAV) are responsible for the majority of foodborne outbreaks associated with berries. The current FDA standard method for detection of these viruses in berries relies on identification of viral RNA. A recent breakthrough in HuNoV cell culture allowed the detection of infectious HuNoV using human stem cells that are grown as 3D human intestinal enteroids (HIE). In this project, we will optimize the FDA standard method for recovery of infectious HuNoV and HAV from berries using HIE and FRhK-4 cells, respectively. The optimized method's percent recovery and detection limit, as well as the relationship between viral RNA and infectivity, will be detemined. The optimized method will be used to investigate the persistence of infectious viruses on berries.

Objectives

1. Optimize the FDA-based method for the detection of **infectious** HuNoV from various berries using HIE, establish the method's detection limit for infectious HuNoV from various berries, and determine the relationship between infectious HuNoV and viral RNA copies recovered from various berries. Tulane virus (a norovirus surrogate) and HAV will be initially used to optimize the method.
2. Determine the persistence of infectious HuNoV and HAV on various fresh berries under postharvest conditions.

Methods

The elution, clarification, and concentration steps of the FDA standard BAM method (**Figure 1**) for "Concentration and Extraction of Enteric Viruses from Soft Fruit" were examined for recovery of infectious Tulane virus (TV) spiked onto strawberries. For the elution step, several buffers were compared to the standard elution buffer, 6% Tris-Glycine Beef Extract (TGBE, pH 9.5). For the clarification step, several centrifugation speeds were compared to the standard speed of 10,000 ×g. For the concentration step, the speed and time of ultracentrifugation were varied to determine their effect on virus recovery. The limit of detection was determined by $TCID_{50}$ infectivity assay and RT-qPCR using 10-fold serially diluted TV spiked onto strawberries. The relationship between infectivity and Ct values was determined using linear regression analysis.

Results to Date

The FDA elution step using 6% TGBE recovered, on average, ~12% infectious TV from strawberries, while carbonated water recovered ~40% (**Figure 2**). The 6% TGBE inactivated ~90% of the infectious TV as compared to DMEM/FBS (**Figure 3A**). Of the remaining infectious viruses, the FDA clarification step by centrifugation recovered ~100% (**Figure 3B**). The FDA ultracentrifugation speed recovered ~1% of the infectious TV in DMEM while a lower speed/longer time was 3-fold better (**Figure 4**). The infectivity limit of detection for TV in media was 1–6 particles/ml (Ct 40-41) (**Figure 5A**), while it was ~60 infectious particles/50 g on strawberries (Ct ~37) (**Figure 5B**). Regression analysis showed a significant linear relationship between TV infectivity and Ct-values, making it possible to predict the initial infectious virus levels present on strawberries even at Ct >37(**Figure 5C**).

Benefits to the Industry

The current FDA standard method for virus detection in berries relies on detection of viral RNA by molecular assays which may or may not indicate the presence of infectious viruses. Having an optimized method for the detection of infectious viruses would provide the berry industry with a better understanding of the risks associated with the detection of viral RNA. In addition, the knowledge about the persistence of infectious viruses on berries under post-harvest conditions is important for devising better control strategies of these viruses on berries. Overall, the berry industry would benefit from reducing foodborne illnesses through consumption of safe food, reducing product recalls and the associated damage to brands.

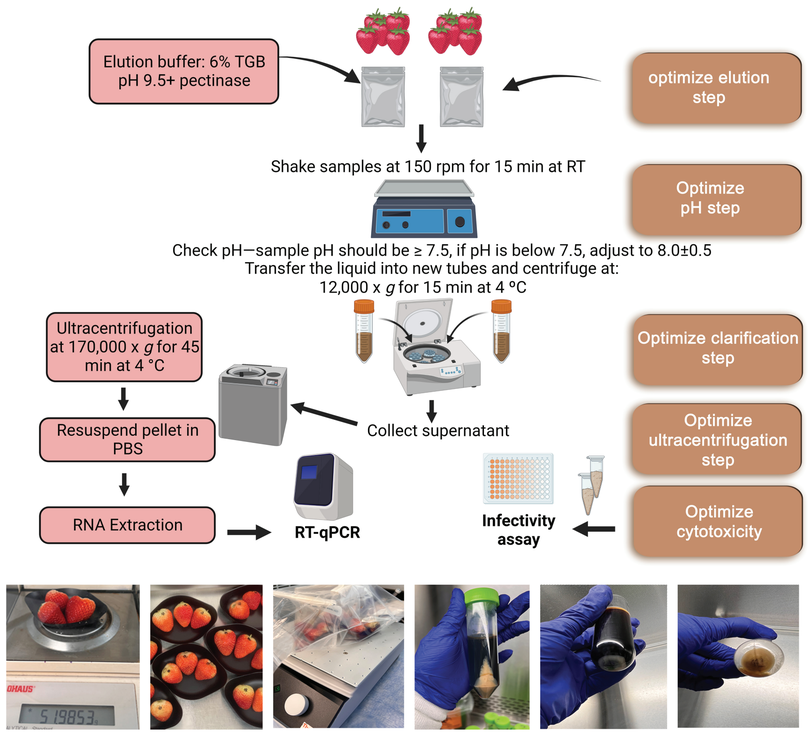

Figure 1: A flowchart showing the general steps of the FDA standard method for "Concentration, Extraction, and Detection of viruses in Soft Fruit" with RT-qPCR as an endpoint assay.

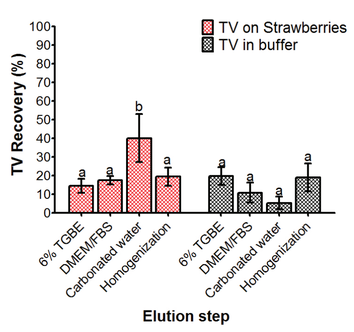

Figure 2: Percent recovery of infectious TV from media and strawberries.

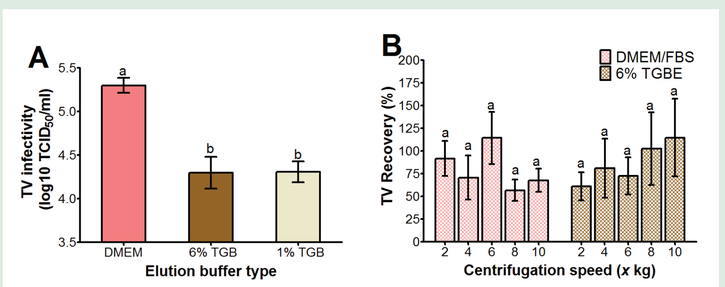

Figure 3: (**A**) Effect of elution buffer on infectious TV and (**B**) as affected by centrifugation speed.

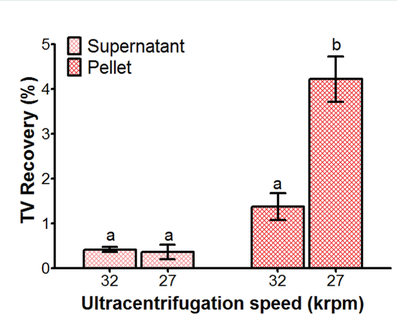

Figure 4: Recovery of infectious TV in media following ultracentrifugation.

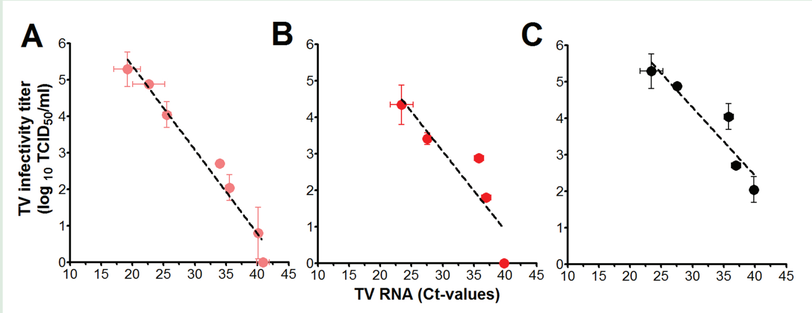

Figure 5: Limit of detection of TV in (**A**) media and (**B**) on strawberries. (**C**) Regression analysis showing relationship between the infectivity and Ct-values to predict initial infectious TV on strawberries.