Two validated first-level screening assays for infectious hepatitis A virus by detection of an intact capsid on frozen berries

Summary

In response to U.S. outbreaks linked to hepatitis A virus (HAV) in frozen berries, the FDA launched surveillance using RT-qPCR, which detects viral RNA but not infectivity. There is concern by industry because RT-qPCR may yield positive signals from non-infectious virus RNA. This project tests two methods to indicate infectious HAV. Both methods integrate RNase-pretreatment to assess capsid integrity. Method 1 adapts the ISO 15216-2:2019 standard; Method 2 replaces RT-qPCR with a rapid, cost-effective CRISPR-Cas13a-based assay. Methods will be validated in separate labs using infectious and inactivated HAV. Methods' sensitivity will be measured. The long-term project impact is to prevent unnecessary recalls, support consumer safety, and reduce food waste. Collaboration with the American Frozen Food Institute will identify adoption strategies in the frozen fruit industry.

Objectives

1. Develop and quantify the sensitivity of two first-level detection methods for infectious HAV on frozen strawberries and irrigation water by specific detection of viral capsid-protected RNA.
2. Replicate these two methods in separate laboratories and validate these two methods comparing their performance against infectious HAV, inactivated HAV and free HAV RNA.

Methods

Method 1 follows the standard ISO 15216-2:2019 protocol to detect HAV but adds an RNase pre-treatment step. RNase enzyme destroys free RNA, so only RNA protected inside an intact viral capsid is detected. An intact capsid suggests potential infectivity. Method 2 uses the same sample preparation as Method 1 but replaces RT-qPCR with a novel CRISPR-Cas13a system (**Figure 1A**). Specific regions of the HAV genome (capsid and RNA polymerase) were targeted using custom-designed primers and guide RNAs. The process involves amplification of viral RNA by an RPA isothermal amplification, transcription into RNA, and detection via Cas13a enzyme activation, which emits a fluorescent signal if the target RNA is present. Buffer conditions were tested to improve sensitivity of the detection reaction.

Results to Date

We developed two sets of RPA primers and Cas13a gRNAs targeting HAV capsid (VP1) and RNA polymerase genes (**Figure 1B**). Each forward primer includes a T7 promoter to enable RNA transcription after amplification. **Figure 2A** shows successful HAV RPA amplification by agarose gel electrophoresis. **Figure 2B** suggests that Genscript's Cas13a buffer produced the highest detection sensitivity. Transcribed RNA was detected using the CRISPR-Cas13a fluorescence assay, visualized under UV and fluorescence readers (**Figure 3**). These signals confirm that the novel CRISPR assay detects capsid and RNA polymerase gene targets. Work is ongoing to combine amplification and detection into a single step, with planned validation on frozen strawberries and irrigation water.

Benefits to the Industry

These methods address industry concerns by distinguishing infectious HAV from non-infectious RNA in frozen strawberries and irrigation water. By integrating RNase pretreatment, both approaches reduce false positives linked to degraded or environmental RNA, improving decision-making for recalls and market withdrawals. Method 2, using CRISPR-Cas, offers a faster, more cost-effective alternative to RT-qPCR and can be adopted in existing testing laboratories. Additionally, CRISPR-Cas detection is adaptable to portable formats, such as lateral flow assays or handheld fluorescence devices, enabling potential in-field or point-of-need testing. Together, these validated tools will help the frozen berry industry better protect consumers, reduce unnecessary product losses, and maintain confidence in the frozen produce supply chain.

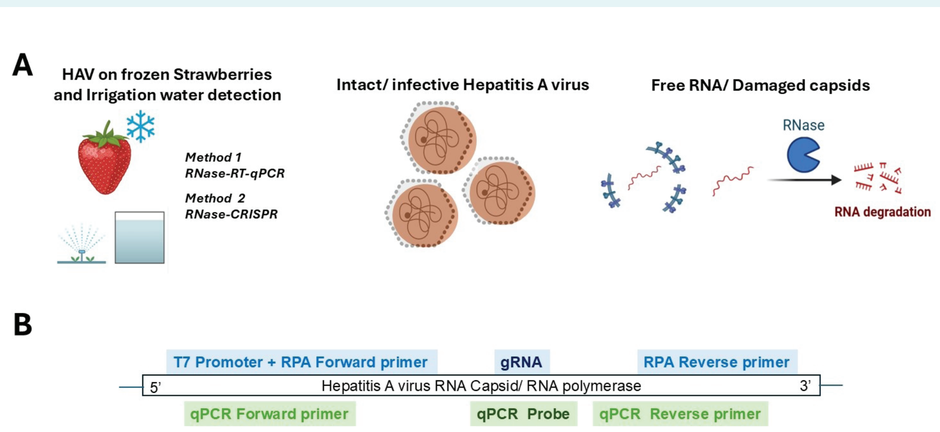

Figure 1. Schematic for the novel CRISPR-Cas 13a assay for infectious hepatitis A virus. **A**) Workflow. Hepatitis A virus is inoculated on frozen strawberries and irrigation water. Then it is tested by novel CRISPR based assay and compared to traditional RT-PCR assay. Addition of RNase (blue "pacman") eliminates non-infectious free RNA and RNA in damaged capsid. Assays then detect RNA in whole capsid and infectious hepatitis A virus. **B**) Design. Both the CRISPR (blue) and RT-PCR (green) assay detect a hepatitis A virus RNA target. Colored boxes represent the assay reagents."

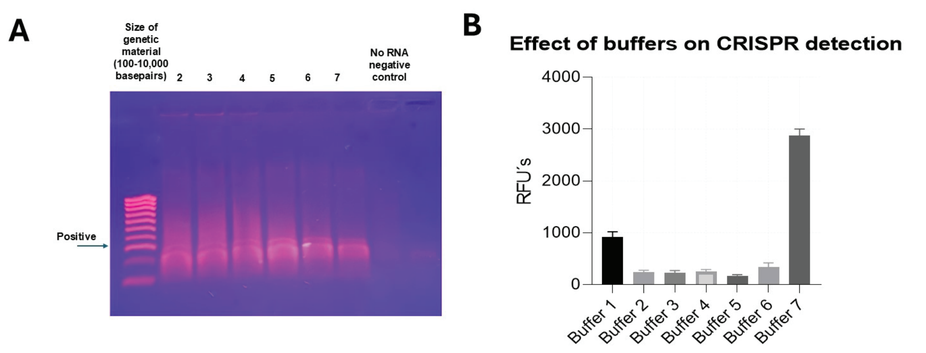

Figure 2. (A). RPA primers detect hepatitis A virus RNA. Agarose gel electrophoresis lanes 4 and 5 represent, positive HAV detection. Arrow shows the positive signal detection. (B). CRISPR detection highest with buffers 7 and 1. [Y axis] Assay fluorescence (RFU 470 nm). [X axis] Buffers include: NEB 3.1 (1), Zou1 (2), Sprangle (3), Zou2 (4), Kellner (5), NEB 2.1 (6) and GenScript Buffer (7).

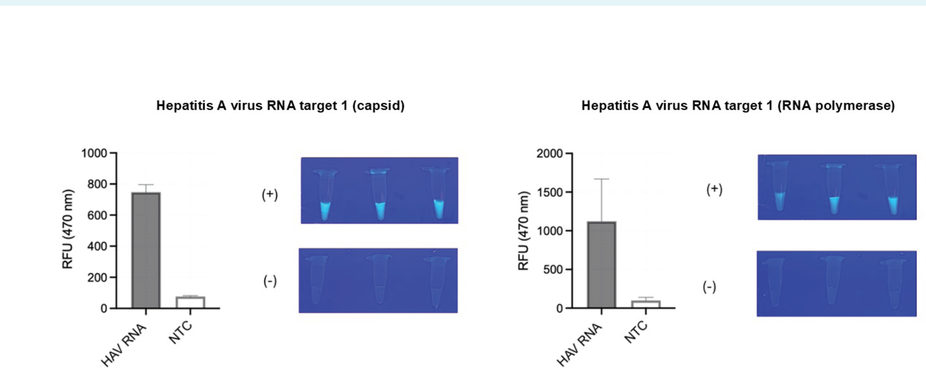

Figure 3. Novel CRISPR assay detects Hepatitis A virus RNA. Bar chart. Results in a fluorescence reader. [Y axis] Assay fluorescence (RFU 470 nm). [X axis] Hepatitis A virus RNA (HAV RNA), No RNA negative control (NTC). Tubes picture. Results under UV light . Each tube represents separate CRISPR reactions with Hepatitis A virus RNA (+), No RNA negative control (-). Light blue color in tube (top row) shows fluorescence (detection). Bottom clear color in tube (bottom row) shows no fluorescence (no detection). The results of these tubes are represented in the bar chart.

Contact

Jose Santos García Alvarado (Santos García), PhD
Facultad de Ciencias Biologicas, UANL
Jose.garcialv@uanl.edu.mx
santos@microbiosymas.com

Authors

Juan S. Leon, Kalmia E. Kniel, Norma L. Heredia (Co-PI), Santos García (PI)

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