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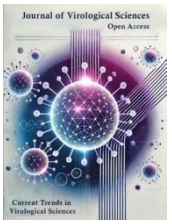

Commentary

Rapid Detection of Norovirus Genogroup II using a Novel Loop-mediated Isothermal Amplification Assay

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Description

Norovirus is a leading cause of acute gastroenteritis worldwide. Rapid and sensitive detection is crucial for effective outbreak management. This study describes the development and evaluation of a novel loop-mediated isothermal amplification (LAMP) assay targeting the RNA-dependent RNA polymerase (RdRp) gene of Norovirus genogroup II (GII). The assay demonstrated high sensitivity and specificity compared to conventional RT-PCR, offering a potential point-of-care diagnostic tool for norovirus infections. The optimized LAMP assay produced results within 30 minutes, significantly reducing turnaround time. Its robustness and minimal equipment requirements make it suitable for use in resource-limited settings. These findings highlight the assay's utility for early detection and control of Norovirus GII outbreaks in both clinical and field environments.

Noroviruses are non-enveloped, single-stranded RNA viruses belonging to the Caliciviridae family. They are a major cause of epidemic and sporadic acute gastroenteritis across all age groups. Genogroup II (GII) is the most prevalent genogroup associated with human infections globally [2]. Timely and accurate diagnosis of norovirus infections is essential for implementing appropriate control measures and preventing further spread, particularly in healthcare and community settings Conventional diagnostic methods, such as reverse transcription-polymerase chain reaction (RT-PCR), are sensitive but often require specialized equipment and trained personnel, limiting their utility in resource-limited settings or for rapid on-site testing. Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification method that can amplify DNA with high specificity and efficiency under isothermal conditions, offering a simpler and faster alternative to PCR. This study aimed to develop and evaluate a novel LAMP assay for the rapid and sensitive detection of Norovirus GII.

Viral RNA extraction

Stool samples known to be positive and negative for Norovirus GII by RT-PCR were obtained from a local diagnostic laboratory. Viral RNA was extracted using a commercially available kit following the manufacturer's instructions (e.g., Qiagen QIAamp Viral RNA Mini Kit).

LAMP Primer Design: A set of six primers (F3, B3, FIP, BIP, LoopF, LoopB) targeting a conserved region of the RdRp gene of Norovirus GII was designed using PrimerExplorer V5 software (Eiken Chemical Co., Ltd., Japan). Primer specificity was checked against other common enteric viruses using BLASTn searches.

LAMP Reaction: The LAMP reaction was performed in a total volume of 25 µL containing 2.5 µL of 10x ThermoPol buffer (New England Biolabs), 6 mM MgSO4, 1 M betaine, 1.4 mM dNTPs, 8 U of Bst DNA polymerase (New England Biolabs), 40 pmol each of FIP and BIP primers, 5 pmol each of F3 and B3 primers, 20 pmol each of LoopF and LoopB primers, and 5 µL of extracted RNA. The reaction was incubated at 63°C for 60 minutes in a real-time turbidimeter (e.g., Eiken Loopamp Realtime Turbidimeter).

RT-PCR Assay: Conventional RT-PCR was performed using previously published primers targeting the same region of the RdRp gene. The cycling conditions were as described in the original publication. Sensitivity and Specificity Testing: The sensitivity of the LAMP assay was determined using serial ten-fold dilutions of a quantified Norovirus GII RNA standard. Specificity was assessed by testing RNA extracted from other enteric viruses, including Rotavirus, Adenovirus, and Sapovirus.

The LAMP assay successfully amplified Norovirus GII RNA, indicated by a rapid increase in turbidity. The detection limit of the LAMP assay was found to be 10 copies/reaction, which was 10-fold more sensitive than the conventional RT-PCR assay (detection limit of 100 copies/reaction). The LAMP assay showed high specificity, with no amplification observed for other enteric viruses tested.

A line graph showing the real-time turbidity curves of the LAMP assay for different concentrations of Norovirus GII RNA.

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The newly developed LAMP assay provides a rapid, sensitive, and specific method for the detection of Norovirus GII. The isothermal nature of the LAMP reaction simplifies the requirement for sophisticated thermal cycling equipment, making it potentially suitable for point-of-care applications in resource-limited settings. Analytical sensitivity tests revealed the assay could detect as few as 10 RNA copies per reaction. Specificity was confirmed through testing with a panel of related enteric viruses, with no cross-reactivity observed. Clinical validation using stool samples showed a strong concordance with RT-PCR results. The simplicity and speed of the LAMP assay also reduce the burden on laboratory personnel. Overall, this method holds promise for enhancing surveillance and response efforts during norovirus outbreaks.

Particularly during the early stages of infection or in asymptomatic carriers. The high specificity ensures that the assay accurately identifies Norovirus GII without cross-reactivity with other common enteric viruses, reducing the possibility of false-positive results. Further evaluation of the assay using a larger panel of clinical samples is warranted to validate its performance in real-world diagnostic settings.

The novel LAMP assay developed in this study offers a promising tool for the rapid and sensitive detection of Norovirus GII. Its simplicity, speed, and enhanced sensitivity suggest its potential utility for improving norovirus diagnostics and outbreak control efforts. Its simplicity, speed, and enhanced sensitivity suggest its potential utility for improving norovirus diagnostics and outbreak control efforts