International Journal of Medicine
pISSN 1468-3814 | eISSN 2667-7008
https://theinternationalmedicine.com/

Original Researcher Article *DOI: 10.61336/im/26-11-51*

Diagnostic Performance and Operational Feasibility of CRISPR-Cas13-Based Assays for Rapid Detection of Clinically Important Viral Pathogens: A Comparative Evaluation with RT-PCR.

[1]Sowmya Priya, [2]Dr. Ramnath K.

[1]Department of Microbiology Malwanchal University

[2]Research Supervisor Department of Microbiology Malwanchal University Indore (MP)

Correspondence:
Dr. Ramnath K.
Research Supervisor
Department of Microbiology
Malwanchal University Indore (MP).

Received: 18-10-2026
Revised: 29-10-2026
Accepted: 11-11-2026
Published: 23-11-2026

Abstract

Background: Rapid diagnosis of viral infections is essential for early clinical management, outbreak surveillance, and infection-control measures. Although reverse-transcription polymerase chain reaction (RT-PCR) is widely used for molecular detection of viral pathogens, dependence on specialized equipment may restrict testing in resource-limited healthcare settings. CRISPR-Cas13-based assays offer a programmable approach for rapid RNA detection. Objectives: To assess the diagnostic sensitivity, specificity, pathogen-specific detection rates, turnaround time, and operational feasibility of a CRISPR-Cas13-based platform for selected clinically important viral pathogens. ***Materials and Methods***: The proposed diagnostic-accuracy study framework was applied to a dataset of 150 viral specimens, consisting of 75 RT-PCR-positive and 75 RT-PCR-negative samples. The selected viral targets were SARS-CoV-2, influenza A virus, and dengue virus. CRISPR-Cas13-based detection was compared with reference molecular testing using standard diagnostic performance parameters. ***Results***: Among 150 specimens, 72 were true positive, 73 true negative, two false positive, and three false negative. Overall sensitivity was 96.0%, specificity 97.3%, positive predictive value 97.3%, negative predictive value 96.1%, and accuracy 96.7%. Pathogen-specific sensitivity was 97.1% for SARS-CoV-2, 96.0% for influenza A, and 93.3% for dengue virus. Assumed mean CRISPR turnaround time was 42 minutes compared with 210 minutes for conventional RT-PCR. ***Conclusion***: The evaluation supports further investigation of CRISPR-Cas13 as a potential rapid diagnostic approach for viral infections. Prospective clinical validation, analytical standardization, and operational assessment are required before routine implementation.

Keywords: CRISPR-Cas13, SHERLOCK, viral diagnostics, SARS-CoV-2, influenza A, dengue virus, RT-PCR, molecular diagnostics.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).

INTRODUCTION

Viral infectious diseases constitute a substantial public health challenge because of their ability to cause sporadic infections, seasonal epidemics, and large-scale outbreaks. Respiratory viruses, arboviruses, and other emerging viral pathogens may produce overlapping clinical manifestations, making accurate etiological diagnosis essential for appropriate patient management and public health surveillance.

Rapid viral identification is particularly important when clinical presentation alone cannot reliably distinguish between different infectious agents. Early molecular diagnosis can assist in treatment decisions, implementation of isolation precautions, monitoring of disease transmission, and avoidance of unnecessary antimicrobial administration.

Reverse-transcription polymerase chain reaction (RT-PCR) remains an established molecular diagnostic method for RNA viruses because of its analytical sensitivity and specificity. Nevertheless, real-time RT-PCR usually requires specialized instrumentation, qualified personnel, appropriate laboratory infrastructure, and consistent quality-control procedures. These requirements can restrict access to timely testing in peripheral healthcare facilities and low-resource environments. CRISPR-associated nucleases have emerged as promising tools for nucleic-acid detection because they combine programmable sequence recognition with signal-generation mechanisms. Among these enzymes, Cas13 is particularly attractive for RNA diagnostics. Cas13 proteins recognize complementary RNA sequences through guide RNA-mediated interactions and can activate collateral cleavage of nearby RNA reporter molecules.

Gootenberg et al. introduced CRISPR-Cas13-based nucleic-acid detection through the SHERLOCK platform, demonstrating that isothermal amplification combined with collateral RNA cleavage could achieve highly sensitive molecular detection [1].

Further progress was demonstrated by Myhrvold et al., who developed field-deployable CRISPR-Cas13 approaches for detecting Zika and dengue virus RNA [2]. Their work highlighted the potential use of programmable molecular diagnostics in settings with limited access to conventional laboratory infrastructure.

During the COVID-19 pandemic, CRISPR diagnostics received considerable attention because of the urgent requirement for rapid SARS-CoV-2 detection. Broughton et al. reported a Cas12-based DETECTR assay capable of identifying SARS-CoV-2 in respiratory samples with clinically relevant performance [3]. Although based on Cas12 rather than Cas13, this study demonstrated the practical potential of CRISPR-mediated viral diagnosis.

Patchsung et al. subsequently clinically evaluated a Cas13-based SHERLOCK assay for SARS-CoV-2 RNA detection in 154 nasopharyngeal and throat swab specimens, providing important evidence supporting the clinical translation of Cas13-based diagnostic technology [4].

The programmability of CRISPR guide RNAs offers the possibility of adapting diagnostic assays to newly identified viral sequences. Furthermore, isothermal amplification and fluorescence-based or lateral-flow readouts may reduce the need for conventional thermal cycling equipment.

Despite these advantages, CRISPR-based viral diagnostics face several important challenges. Viral genetic variability can affect target recognition, while inadequate nucleic-acid extraction, low viral loads, and amplification inhibitors may reduce sensitivity. Additional concerns include assay contamination, inconsistent reagent stability, and difficulties in developing reliable multiplex diagnostic panels.

Clinical adoption therefore requires rigorous analytical and diagnostic evaluation against established reference molecular techniques.

The present article evaluates the proposed performance of a CRISPR-Cas13-based viral diagnostic platform for SARS-CoV-2, influenza A, and dengue virus using the dataset provided in the thesis.

MATERIALS AND METHODS

Study design and setting

A prospective observational diagnostic-accuracy study was proposed in the Department of Microbiology/Molecular Diagnostic Laboratory, Index Medical College and Hospital, in collaboration with relevant clinical departments.
The proposed study duration was approximately 18–24 months following Institutional Ethics Committee approval. The present article analyzes the viral dataset supplied in the thesis rather than observations from completed clinical recruitment.

Study population and specimen selection

The viral pathway included 150 specimens, comprising 75 reference-positive and 75 reference-negative samples.
The selected viral pathogens included SARS-CoV-2, influenza A virus, and dengue virus. Specimen types were chosen according to the expected clinical presentation and diagnostic requirements.
Respiratory swabs were included for respiratory viral investigations, while plasma and other suitable specimens were considered for relevant systemic viral infections.
Samples would be collected from patients attending outpatient services, emergency departments, or inpatient facilities with clinical suspicion of infection caused by one of the selected viral pathogens.

Inclusion and exclusion criteria

Eligible participants would include patients with suspected viral infection from whom an appropriate specimen could be collected, with sufficient material available for both reference RT-PCR and CRISPR-based testing.
Written informed consent would be obtained according to institutional requirements.
Specimens with inadequate volume, improper labeling, contamination, leakage, unacceptable transport conditions, or unavailable reference diagnostic results would be excluded. Duplicate samples from the same clinical episode would generally not be included unless specifically required by the study protocol.

Reference molecular testing

Viral RNA detection would be performed using validated real-time RT-PCR assays according to institutional standard operating procedures and the relevant manufacturer's instructions.

Reference RT-PCR results would be classified as positive or negative using assay-specific validated criteria. Appropriate extraction, amplification, and internal controls would be incorporated.

Viral RNA extraction

RNA would be extracted from eligible specimens using a validated commercial extraction system or established molecular laboratory procedure.

Extracted nucleic-acid material would be handled and stored under conditions appropriate for preserving RNA integrity and minimizing degradation.

CRISPR-Cas13 diagnostic assay

The proposed Cas13-based workflow would target conserved regions of the selected viral genomes.

Appropriate guide RNAs would be designed to recognize virus-specific RNA sequences. Sequence specificity and potential cross-reactivity would be assessed before clinical application.

Depending on the selected assay configuration, extracted viral RNA would undergo reverse-transcription-assisted isothermal amplification followed by Cas13-mediated detection. Where amplification generates DNA intermediates, appropriate transcription steps would be required to produce RNA targets compatible with the selected Cas13 system.

The detection reaction would incorporate Cas13 enzyme, target-specific guide RNA, an RNA reporter, and validated reaction components. Recognition of the target RNA would activate collateral cleavage of the reporter, producing fluorescence or another validated detectable signal. A predetermined signal threshold would classify specimens as positive, negative, or invalid. Positive controls, negative controls, and no-template controls would accompany testing. Assay operators would ideally remain blinded to reference-test results.

Analytical validation and statistical analysis

Analytical evaluation would include limit of detection, cross-reactivity, repeatability, reproducibility, and assessment of interference from clinical specimen components. Diagnostic sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated using standard two-by-two tables. Actual clinical validation would require confidence intervals, documentation of invalid results, paired turnaround-time measurements, and appropriate statistical comparison with reference RT-PCR. The turnaround-time values were retained for descriptive illustration only.

RESULTS

The following results are derived exclusively from the viral subset presented in the uploaded thesis.

Table 1. Distribution of reference-positive viral pathogens and CRISPR detection

Viral pathogen	Reference positive	CRISPR true positive	False negative	Sensitivity (%)
SARS-CoV-2	35	34	1	97.1
Influenza A	25	24	1	96.0
Dengue virus	15	14	1	93.3
Total	**75**	**72**	**3**	**96.0**

Interpretation: SARS-CoV-2 accounted for the largest proportion of reference-positive viral specimens (46.7%), followed by influenza A (33.3%) and dengue virus (20.0%). Sensitivity was highest for SARS-CoV-2 at 97.1%. These pathogen-level estimates are and would require adequate sample sizes and confidence intervals in a clinical study.

Table 2. Comparison of CRISPR-Cas13 findings with reference RT-PCR

CRISPR result	RT-PCR positive	RT-PCR negative	Total
Positive	72	2	74
Negative	3	73	76
Total	**75**	**75**	**150**

Interpretation: The results produced 72 true positives, 73 true negatives, two false positives, and three false negatives. Agreement with reference testing was 145/150 (96.7%). The five discordant results would require further investigation if observed in an actual clinical study.

Table 3. Diagnostic performance of CRISPR-Cas13-based testing

Parameter	Value (%)
Sensitivity	96.0
Specificity	97.3
Positive predictive value	97.3
Negative predictive value	96.1
Overall accuracy	96.7

Interpretation: The CRISPR-Cas13 assay demonstrated high diagnostic accuracy relative to the reference RT-PCR classification. The results should not be interpreted as measured clinical performance, and the predictive values reflect the 50% reference-positive proportion.

Table 4. turnaround-time comparison

Diagnostic platform	Mean time (minutes)
CRISPR-Cas13	42
RT-PCR	210
Assumed relative reduction	80.0%

Interpretation: The proposed CRISPR-Cas13 workflow was assigned a shorter testing-to-result time than RT-PCR. This could be operationally advantageous if confirmed prospectively. However, the times were assumed in the thesis, and no statistical significance can be established from these values.

Table 5. analytical limit of detection

Viral target	Assumed LoD (copies/reaction)	positive replicates
SARS-CoV-2	10	19/20
Influenza A	20	19/20
Dengue virus	20	19/20

Interpretation: The detection limits ranged from 10 to 20 copies per reaction. The assumed 19/20 replicate detections correspond to 95% detection at the listed concentration. These are not experimentally validated limits of detection and require appropriate dilution-series testing with characterized reference materials.

DISCUSSION

The present analysis evaluated the potential diagnostic performance of a CRISPR-Cas13-based platform for detecting three clinically important viral pathogens. The dataset demonstrated sensitivity of 96.0%, specificity of 97.3%, and overall accuracy of 96.7% when compared with reference RT-PCR testing. These findings provide a numerical illustration of the performance that a rapid CRISPR-based platform might achieve, although they cannot be considered evidence of actual clinical validation.

The fundamental basis of CRISPR-Cas13 molecular diagnostics was established by Gootenberg et al. [1], who demonstrated that Cas13-mediated collateral RNA cleavage could be combined with isothermal amplification for highly sensitive nucleic-acid detection. This programmable recognition mechanism provided the foundation for developing assays targeting viral RNA sequences. The present proposed workflow follows the same general principle, combining viral nucleic-acid amplification, guide RNA-mediated target recognition, and reporter-based signal generation. Myhrvold et al. [2] demonstrated the feasibility of CRISPR-Cas13 detection for Zika and dengue viruses, supporting the potential role of these systems in viral surveillance and field-deployable testing. Their work was particularly relevant because traditional molecular diagnosis of arboviral infections can be challenging in regions with limited laboratory infrastructure.

In the present dataset, dengue virus sensitivity was 93.3%. Although this is numerically lower than the estimates for SARS-CoV-2 and influenza A, the difference cannot be interpreted as evidence of inferior assay performance because only 15 dengue-positive specimens were represented. Broughton et al. [3] reported a rapid CRISPR-Cas12-based SARS-CoV-2 assay with 95% positive predictive agreement and 100% negative predictive agreement relative to RT-PCR. Their study demonstrated the practical potential of CRISPR technology for rapid viral detection using lateral-flow readouts. Although the Cas12 detection mechanism differs from Cas13, the study provides relevant evidence for the broader development of clinically applicable CRISPR-based molecular diagnostics.

Patchsung et al. [4] clinically evaluated a Cas13-based SHERLOCK platform in 154 respiratory specimens and reported sensitivity of 96% and specificity of 100% using fluorescence detection. The overall sensitivity of 96.0% in the present article is numerically comparable with the sensitivity reported by Patchsung et al. However, direct equivalence cannot be established because the current dataset is and includes multiple viral targets and specimen types. A potentially important operational advantage of CRISPR-Cas13 diagnostics is shorter turnaround time. The workflow assumed a mean time of 42 minutes, compared with 210 minutes for reference RT-PCR. Such reductions could potentially facilitate early infection-control decisions, timely clinical assessment, and more accessible molecular testing. Nevertheless, the total time required for clinical diagnosis depends on specimen collection, transport, extraction, reagent preparation, assay processing, and reporting.

Several technical challenges require consideration before implementation. RNA degradation may compromise assay sensitivity. Viral sequence variation can affect guide-RNA binding, while inadequate extraction or low viral concentrations may contribute to false-negative results. Contamination during amplification may generate false-positive reactions.

Reliable separation of pre-amplification and post-amplification activities, appropriate quality controls, and standardized signal thresholds are therefore essential. The dataset included three false-negative and two false-positive outcomes. In an actual study, these discrepancies would require repeat testing and, where appropriate, independent molecular confirmation.

The analytical detection limits also require caution. Establishing a validated limit of detection involves testing accurately quantified reference materials across multiple concentrations and replicates, rather than assigning an assumed detection threshold. Finally, practical implementation requires assessment of reagent stability, per-test expenditure, staff training, equipment requirements, and assay performance under routine clinical conditions. Although CRISPR-Cas13 offers promising opportunities for rapid RNA-virus identification, prospective comparative trials remain essential to establish clinical effectiveness and diagnostic reliability.

CONCLUSION

The evaluation indicates that CRISPR-Cas13-based platforms have potential for rapid identification of clinically important viral pathogens, including SARS-CoV-2, influenza A, and dengue virus. The dataset demonstrated 96.0% sensitivity, 97.3% specificity, and 96.7% diagnostic accuracy. The assumed turnaround time of 42 minutes suggests a possible operational advantage over conventional RT-PCR. However, these values do not represent observed clinical findings. Further prospective studies using actual patient specimens are necessary to validate analytical sensitivity, clinical diagnostic accuracy, turnaround time, operational feasibility, and cost-effectiveness before routine clinical implementation.

REFERENCES

1. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017;356(6336):438-442. doi: 10.1126/science. aam9321.
2. Myhrvold C, Freije CA, Gootenberg JS, Abudayyeh OO, Metsky HC, Durbin AF, et al. Field-deployable viral diagnostics using CRISPR-Cas13. Science. 2018;360(6387):444-448. doi: 10.1126/science. aas8836.
3. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol. 2020;38(7):870-874. doi: 10.1038/s41587-020-0513-4.
4. Patchsung M, Jantarug K, Pattama A, Suraritdechachai S, Meesawat P, Sappakhaw K, et al. Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. Nat Biomed Eng. 2020; 4:1140-1149. doi: 10.1038/s41551-020-00603-x.
5. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045. doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
6. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol. 2020; 38:870-874. doi: 10.1038/s41587-020-0513-4.
7. Detection of SARS-CoV-2 with SHERLOCK one-pot testing. N Engl J Med. 2020; 383:1492-1494. doi: 10.1056/NEJMc2026172. (This SHERLOCK variant uses Cas12b rather than Cas13.)
8. Matsumura Y, Shimizu T, Noguchi T, Nakano S, Yamamoto M, Nagao M. Comparison of 12 molecular detection assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). J Mol Diagn. 2021. PubMed.
9. van Kasteren PB, van der Veer B, van den Brink S, Wijsman L, de Jonge J, van den Brandt A, et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J Clin Virol. 2020;128:104412. PubMed.