RESEARCH ARTICLE

CRISPR-Cas13d Multiplexed Diagnostics for Simultaneous Detection of 12 Emerging Viral Pathogens in Under 30 Minutes

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Published: 2026-05-24 | GAST Vol. 1, No. 1 (2026)

Abstract: Rapid identification of emerging viral threats is critical for pandemic preparedness. Current diagnostic platforms are either fast but detect single targets (lateral flow assays) or comprehensive but slow (metagenomic sequencing). We present ViralSHIELD, a CRISPR-Cas13d-based multiplexed diagnostic system that simultaneously detects 12 high-priority viral pathogens — including SARS-CoV-2 variants, influenza A/B, RSV, MERS-CoV, Nipah, Ebola, Zika, and dengue serotypes — from a single nasopharyngeal swab in 28 minutes. The assay uses isothermal recombinase polymerase amplification (RPA) coupled with Cas13d collateral cleavage of orthogonal fluorescent reporters. Clinical validation on 2,450 patient samples across three hospital sites achieves 97.8% sensitivity and 99.4% specificity relative to RT-qPCR.

1. Introduction

The COVID-19 pandemic exposed critical gaps in global diagnostic infrastructure: rapid antigen tests sacrificed sensitivity for speed, while PCR-based methods required hours and centralized laboratories. For the next pandemic — which epidemiologists consider a matter of "when" rather than "if" — the world needs diagnostic platforms that are simultaneously fast, sensitive, multiplexed, and deployable at the point of care.

CRISPR-based diagnostics leverage the programmable nucleic acid recognition of Cas enzymes combined with their collateral cleavage activity to achieve PCR-like sensitivity without thermocycling. Cas13 family enzymes, which target RNA, are particularly suited for respiratory virus detection. However, existing CRISPR diagnostics detect only 1-2 targets per reaction, requiring multiple separate tests for differential diagnosis of febrile respiratory illness.

2. ViralSHIELD Platform

The 12-plex assay uses a single-pot isothermal amplification step (RPA, 37°C, 15 min) with 12 primer pairs, followed by Cas13d detection (37°C, 13 min) with 12 orthogonal crRNA-reporter pairs. Each reporter is labeled with a spectrally distinct fluorophore (6-FAM, HEX, Cy3, ROX, Cy5, Cy5.5 plus 6 quencher-shifted variants), enabling simultaneous

readout on a portable 12-channel fluorescence reader. The crRNA sequences are designed using a computational pipeline that ensures $<10^{-6}$ probability of cross-reactivity across all 12 targets.

3. Clinical Validation

Prospective validation across three hospital sites (Beijing, Boston, Nairobi) enrolled 2,450 patients presenting with acute febrile respiratory illness. ViralSHIELD correctly identified the causative pathogen in 97.8% of RT-qPCR-confirmed cases, including 23 co-infections that were detected as dual-positive signals.

Table 1. ViralSHIELD clinical performance by pathogen target (n = 2,450 patients)

Pathogen	True Positive	False Negative	Sensitivity (%)	Specificity (%)
SARS-CoV-2	312	5	98.4	99.6
Influenza A	285	8	97.3	99.5
Influenza B	142	4	97.3	99.7
RSV	198	5	97.5	99.4
Dengue (all)	89	3	96.7	99.2

4. Conclusions

ViralSHIELD demonstrates that CRISPR-Cas13d can enable rapid, multiplexed, and highly accurate point-of-care diagnosis of respiratory viral infections. The platform's modular crRNA design allows rapid reprogramming for novel pathogens within 48 hours of sequence availability, providing a critical tool for pandemic preparedness and routine syndromic testing of febrile illness.

References

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