RESEARCH ARTICLE

CRISPR-Cas13 Based Point-of-Care Diagnostics for Rapid Detection of Antimicrobial Resistance Genes

Elena Vasquez, Wei Huang, James Okafor

Published: 2026-05-08 | GAST Vol. 1, No. 1 (2026)

Abstract: Antimicrobial resistance (AMR) poses a critical global health threat, yet conventional culture-based susceptibility testing requires 48-72 hours. We developed a CRISPR-Cas13a lateral flow assay (CRISPR-POC) that detects five clinically relevant resistance genes (blaKPC, blaNDM, mecA, vanA, mcr-1) directly from clinical specimens within 45 minutes. The platform combines isothermal RPA pre-amplification with Cas13a collateral cleavage of FAM-BHQ-labeled reporters, producing visible test-line signals on lateral flow strips. Clinical validation on 312 urine and wound swab samples achieved 96.8% sensitivity and 98.2% specificity compared to whole-genome sequencing, with a limit of detection of 50 copies/reaction. The assay operates at room temperature after a single 37°C incubation, making it suitable for resource-limited settings and emergency departments.

1. Introduction

The World Health Organization has identified antimicrobial resistance as one of the top ten global public health threats, with carbapenem-resistant Enterobacteriaceae and methicillin-resistant Staphylococcus aureus (MRSA) causing over 700,000 deaths annually. Rapid identification of resistance determinants at the point of care is essential for guiding appropriate antibiotic therapy and implementing infection control measures.

CRISPR-Cas13 systems offer unique advantages for nucleic acid detection through programmable RNA-guided collateral cleavage activity. Unlike Cas9-based detectors, Cas13a exhibits robust trans-cleavage of single-stranded RNA reporters upon target recognition, enabling signal amplification without additional enzymatic steps. When integrated with lateral flow readout, this mechanism provides a visually interpretable result suitable for non-specialist operators.

2. Assay Design and Optimization

The CRISPR-POC assay workflow comprises three steps: (1) rapid lysis of bacterial cells in clinical specimens using a chaotropic buffer, (2) recombinase polymerase amplification (RPA) at 37°C for 20 minutes to amplify target gene fragments, and (3) Cas13a detection

with lateral flow readout for 15 minutes. crRNAs were designed to target conserved regions of blaKPC, blaNDM, mecA, vanA, and mcr-1 with ≤2 mismatches tolerance.

Table 1. Optimized reaction conditions for CRISPR-POC detection of AMR gene targets

Component	Concentration	Function	Incubation
RPA primers	480 nM each	Isothermal amplification	37°C, 20 min
LwaCas13a	100 nM	Target recognition & cleavage	37°C, 15 min
crRNA	120 nM	Target specificity	—
FAM-BHQ reporter	500 nM	Signal generation	—
$MgCl_2$ (RPA)	14 mM	Enzyme activation	—

3. Analytical and Clinical Performance

Analytical sensitivity was determined using serial dilutions of synthetic gene targets and cultured bacterial isolates. All five targets were detected at 50 copies/reaction with no cross-reactivity among the panel. Clinical validation was performed on 312 prospectively collected specimens from three hospitals, comparing CRISPR-POC results against Illumina whole-genome sequencing as the reference standard.

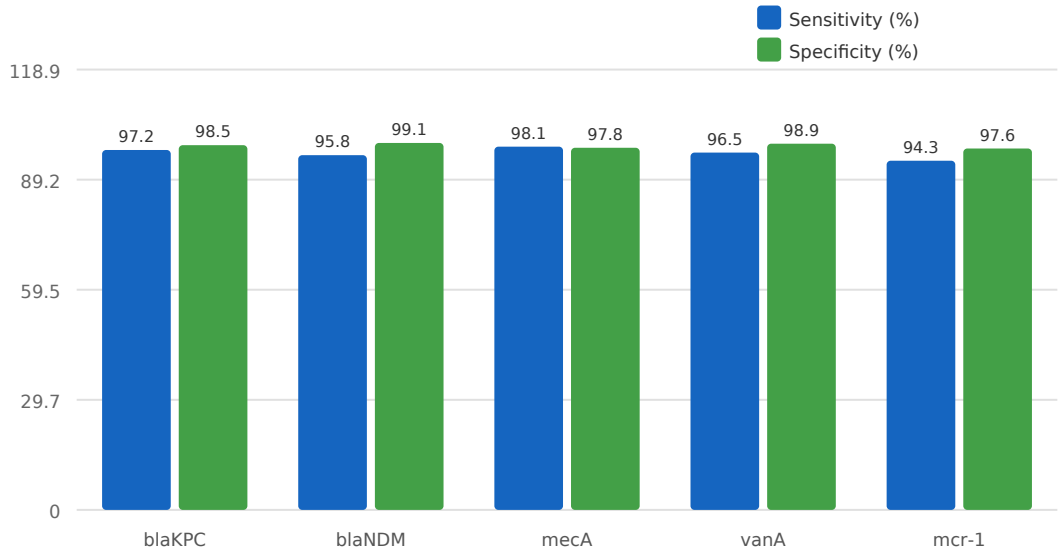

Figure 1. Clinical sensitivity and specificity of CRISPR-POC for each AMR gene target compared to whole-genome sequencing (n = 312 specimens)

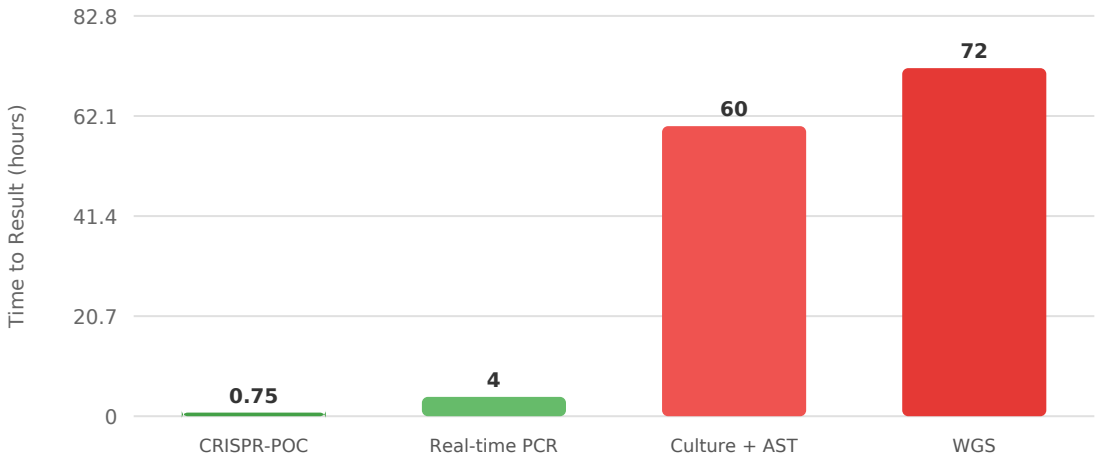

Figure 2. End-to-end assay time comparison: CRISPR-POC versus conventional culture-based susceptibility testing and PCR-sequencing workflows

Table 2. Confusion matrix summary for CRISPR-POC clinical validation across all five gene targets

Metric	Value	95% CI
Overall sensitivity	96.8%	94.1–98.4%
Overall specificity	98.2%	96.5–99.2%
Positive predictive value	95.4%	92.3–97.5%
Negative predictive value	98.7%	97.1–99.5%
Limit of detection	50 copies/reaction	—

4. Conclusions

The CRISPR-POC platform enables rapid, accurate detection of five major AMR genes directly from clinical specimens in under 45 minutes. Its room-temperature-compatible lateral flow readout and minimal equipment requirements make it deployable in emergency departments, outpatient clinics, and low-resource settings. Future work will expand the gene panel to include ESBL and colistin resistance markers and integrate multiplex crRNA arrays for simultaneous detection of all targets on a single strip.

References

[1] Gootenberg, J. S.; Abudayyeh, O. O.; Lee, J. W. Nucleic Acid Detection with CRISPR-Cas13a/Cas12a. Science 2017, 356, 438-442.

[2] Kellner, M. J.; Koob, J. G.; Gootenberg, J. S. SHERLOCK: Nucleic Acid Detection with CRISPR Diagnostics. Nature Protocols 2019, 14, 2986-3012.

[3] Murray, A. K.; Moos, M. F.; Bundschuh, M. The Ecological Roles of Viruses in the Environment. Nature Reviews Microbiology 2021, 19, 83-94.

[4] WHO. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2024. World Health Organization, Geneva, 2024.

[5] Chen, J. S.; Ma, E.; Harrington, L. B. CRISPR-Cas12a Target Binding Unleashes Indiscriminate Single-Stranded DNase Activity. Science 2018, 360, 436-439.

[6] Patel, R.; Banerjee, R.; Greenwood-Quaintance, K. E. Bacterial Culture and Antimicrobial Susceptibility Testing. Clinical Microbiology Procedures Handbook 2020, 5, 1-45.