Sensors & Actuators: B. Chemical 455 (2026) 139622

Contents lists available at ScienceDirect

Sensors and Actuators: B. Chemical

journal homepage: www.elsevier.com/locate/snb

A CRISPR/Cas12a–personal glucose meter biosensor for rapid and quantitative detection of *Burkholderia pseudomallei* DNA

Nini Luo [a,*], Yuping Ruan [a,1], Jian Luo [a,1], Shen Tian [a], Dai Kuang [a], Nan Zhang [a], Xiaoxia Xie [a], Zhangmeng Liu [a], Xiaobing Wang [a], Juan Yao [c,*], Huangxian Ju [b,*], Qianfeng Xia [a,*]

[a] *NHC Key Laboratory of Tropical Disease Control, School of Life Sciences and Medical Technology, Hainan Medical University, Haikou, Hainan 571199, China*
[b] *State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China*
[c] *Nanobiosensing and Microfluidic Point-of-Care Testing, Key Laboratory of Luzhou, Department of Clinical Laboratory, The Affiliated Traditional Chinese Medicine Hospital, Southwest Medical University, Luzhou, Sichuan 646000, China*

ARTICLE INFO

Keywords:
B. pseudomallei
CRISPR/Cas12a
DNA biosensor
Invertase
PGM

ABSTRACT

Melioidosis, caused by *Burkholderia pseudomallei (B. pseudomallei)*, is a fatal tropical infectious disease whose diagnosis is hindered by the lack of rapid and accessible detection methods. Here, we present a novel CRISPR/Cas12a-based biosensing platform integrated with a personal glucose meter (PGM) for rapid, quantitative, and amplification-free detection of *B. pseudomallei* DNA. In this system, target DNA activates Cas12a trans-cleavage, releasing invertase from an invertase-Linker-biotin probe. Subsequent magnetic separation removes the uncleaved complexes, and the invertase remaining in the solution catalyzes the conversion of sucrose to glucose, which is quantitatively measured by the PGM. The assay is completed within 50 min and achieves a detection limit of 0.1 pM with a linear dynamic range from 1 pM to 10 nM. The biosensor exhibits excellent specificity against single- and multi-base mismatched sequences, outstanding precision (CV < 10 %), and strong correlation with qPCR results in clinical samples (recovery: 98 % - 113 %). The reagents remain stable over four weeks of storage (CV $= 4.4$ %), confirming the robustness of the system. This amplification-free platform, independent of specialized laboratory instrumentation, represents an important step toward accessible molecular diagnostics, demonstrating the potential as a rapid, low-cost, and user-friendly solution for early melioidosis detection and potentially other infectious diseases. From a sensor-engineering standpoint, the CRISPR/Cas12a–PGM architecture constitutes a generalizable chemical/biosensing platform that can be readily reprogrammed for different nucleic-acid targets by simply switching the crRNA.

1. Introduction

Melioidosis is a severe infectious disease caused by *Burkholderia pseudomallei (B. pseudomallei)* [1], for which timely diagnosis remains a major clinical challenge. Current diagnostic strategies are largely incompatible with rapid decision-making and point-of-care testing. Bacterial culture [2], although considered the clinical gold standard, typically requires several days and well-equipped laboratories. Immunological assays [3] provide faster results but often suffer from insufficient sensitivity and cross-reactivity, particularly during early-stage infection. Molecular methods, including PCR [4] and isothermal amplification techniques [5] and Optical Sensor [6,7], offer improved analytical performance but depend on specialized instrumentation, controlled reaction conditions, and skilled operators. These requirements significantly limit their deployment in decentralized or resource-limited settings, where melioidosis is most prevalent. As a result, there is a critical unmet need for a diagnostic approach that combines molecular-level specificity with operational simplicity, portability, and rapid readout at the point of care.

To address these challenges, CRISPR-based nucleic acid detection has emerged as a promising strategy for point-of-care diagnostics [8]. Among various CRISPR effectors, Cas12a exhibits both specific cis-cleavage of target DNA and non-specific trans-cleavage of single-stranded DNA reporters once activated, enabling ultrasensitive and highly specific detection of nucleic acids [9]. This unique enzymatic property has spurred the development of numerous CRISPR-based

* Corresponding authors.
E-mail addresses: 396497795@qq.com (J. Yao), hxju@nju.edu.cn (H. Ju), xiaqianfeng@muhn.edu.cn (Q. Xia).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.snb.2026.139622

Available online 13 February 2026

biosensors, offering remarkable advantages in assay speed, programmability, and simplicity. However, most reported CRISPR/Cas12a systems depend on fluorescence or electrochemical signal readouts, which still require dedicated equipment, limiting their suitability for point-of-care (POC) or field applications [10,11]. Furthermore, given the complexity and genetic diversity of *B. pseudomallei*, detection platforms must achieve exceptional specificity to avoid false results caused by sequence variations [12].

Point-of-care testing (POCT) platforms that combine high analytical accuracy with portability and affordability are increasingly vital for infectious disease surveillance [13]. The personal glucose meter (PGM), one of the most ubiquitous diagnostic devices globally, presents a promising foundation for such applications. Originally designed for blood glucose monitoring, PGMs offer quantitative electrochemical detection, low cost, wide accessibility, and excellent operational robustness. By integrating molecular recognition elements with enzymatic signal transduction, PGMs can be repurposed to quantify diverse biomarkers beyond glucose—a strategy that has been successfully demonstrated in several recent biosensing innovations [14].

Building on these advances, we developed a novel CRISPR/Cas12a-PGM biosensing platform for rapid, quantitative, and amplification-free detection of *B. pseudomallei* DNA. In this system, the specific activation of Cas12a upon target recognition triggers the release of invertase, which catalyzes the hydrolysis of sucrose into glucose, subsequently quantified by a commercial PGM. This approach transforms complex nucleic acid recognition into a simple and quantitative biochemical output, bridging molecular diagnostics with consumer-grade technology. The proposed system aims to provide a reliable, low-cost, and deployable diagnostic solution for melioidosis, particularly suitable for use in low-resource or field settings where rapid decision-making is critical.

2. Experiment section

2.1. Reagents and apparatus

Tris-HCl, diethyl pyrocarbonate (DEPC) water, and all oligonucleotides listed in Table 1 were purchased from Sangon BioEngineering (Shanghai, China). 4-(N-Maleimidomethyl) cyclohexane-1-carboxylic acid succinimidyl ester (sulfo-SMCC) and invertase were acquired from Sigma-Aldrich (Shanghai, China), while sucrose was sourced from Dibo Biological (Shanghai, China). Cas12a enzyme, crRNA, and oligonucleotides were prepared as specified. Invertase and Linker were conjugated via a heterobifunctional crosslinker (Sulfo-SMCC) to generate the INV-linker-biotin probe. Streptavidin-modified magnetic beads were obtained from Thermobell Scientific (Shanghai, China), and EnGen® Lba Cas12a was procured from New England Biolabs (Shanghai, China). Phosphate-buffered saline (PBS, 0.1 M, pH 7.4) was supplied by Sangon Bioengineering (Shanghai, China). All chemical reagents were of analytical grade, and DEPC-treated water was used throughout the experiments. The specificity of the *B. pseudomallei* target DNA sequence was confirmed by BLAST analysis against the National Center for Biotechnology Information database (NCBI, USA, https://blast.ncbi.nlm.nih.gov/Blast.cgi).

For glucose detection, a YUWELL 590 PGM and its corresponding test strips (Jiangsu, China) were employed. Genomic DNA concentrations were quantified using a NanoDrop One spectrophotometer (Thermo Fisher, USA), and quantitative polymerase chain reaction (qPCR) assays were performed on a StepOnePlus Real-Time PCR System (Thermo Fisher, USA). Zeta potential measurements were conducted on a Zetasizer Nano ZS90 (Malvern, UK), and fluorescence spectra were recorded using an F-7000 fluorescence spectrometer (HITACHI, Japan).

2.2. Extraction of genomic DNA from B. pseudomallei clinical strains

A total of 10 clinical strains of *B. pseudomallei* were procured from clinical specimens of hospitalized patients at the Second Affiliated Hospital of Hainan Medical University. These strains were inoculated into Luria-Bertani (LB) medium and incubated at a constant temperature of 37°C for a duration ranging from 18 to 24 h. Subsequently, genomic DNA was extracted from *B. pseudomallei* strains using a commercially available bacterial genome extraction kit. The extraction procedure was carried out with strict adherence to the manufacturer's instructions to ensure the reproducibility and reliability of the results. Upon completion of the extraction process, the genomic DNA was stored at −20°C for subsequent experimental use. The concentration of the extracted genomic DNA was accurately determined using a Nanodrop One spectrophotometer.

2.3. Preparation of INV-linker

The catalytically active site of invertase predominantly comprises aspartate and glutamate residues. To maintain the enzymatic catalytic functionality, a non-active-site amine moiety on invertase was selected as the coupling site. In accordance with the standardized heterobifunctional conjugation protocol as reported by Xiang and Lu, the experimental procedure was executed as follows: Sulfo-SMCC was dissolved in DMSO to formulate a 10 mg/mL solution. Then, 200 μL of invertase solution (10 mg/mL) was mixed with 100 μL of the Sulfo-SMCC solution (10 mg/mL), followed by the addition of 300 μL of PBS (pH7.2, 10 mM) to obtain the reaction mixture. The mixture was vortexed for 5 min to ensure thorough mixing and homogeneity, then incubated at room temperature for 2 h. Subsequently, it was purified using an Amicon Ultra-10K centrifugal filter, with PBS employed for 8 consecutive exchange cycles. Next, the purified sulfo-SMCC-activated invertase was combined with 20 nmol of thiolated Linker (100 μM, 200 μl). The reaction was conducted in a thermomixer maintained at 4 ℃ with continuous shaking at 300 rpm overnight. The resultant solution was then subjected to further purification using an Amicon Ultra-100K centrifugal filter, with 8 consecutive exchange cycles employing PBS. The conjugated product (INV-Linker) was stored at 4 ℃ for utilization in subsequent experimental procedures.

2.4. Assembly and activity assessment of the CRISPR/Cas12a system

The CRISPR/Cas12a complex was assembled by combining 2 μL of crRNA (1 μM), 1 μL of LbCas12a enzyme (1 μM), 2 μL of Buffer 2.1, and 13 μL of nuclease-free water. To this mixture, 1 μL of target DNA at

Table 1
RNA and DNA Oligonucleotide Sequences Used in this experiment.

Name	Sequence (5'-3')
crRNA	UAAUUUCUACUAAGUGUAGAUACCUACAGCAAAGAUCCUGC
Target DNA	GCGGCACGACGAATTTCACCTACAGCAAAGATCCTGCG
Complementary DNA	CGCAGGATCTTTGCTGTAGGTGAAATTCGTCGTGCCGC
Linker	SH-TTT TTT TTT TTT -Biotin
Primer F	AACCGCGCGACGATCCTA
Primer R	TCGCCTTCCTGACCGGG
M-S	GCGGCACGACGAATTTCACCTATAGCAAAGATCCTGCGCCACGCGGC
M-M	GCGGCACGACCAATTTGACCTATAGCAAAGATCCTGCGCCACGCGGCA

varying concentrations and 1 μL of F-Q reporter probe (2 μM) was added. The resulting reaction solution was incubated at 37 °C for 40 min. Fluorescence measurements were carried out using a temperature-controlled HITACHI F-7000 fluorescence spectrometer to quantitatively monitor reaction kinetics and endpoint signals.

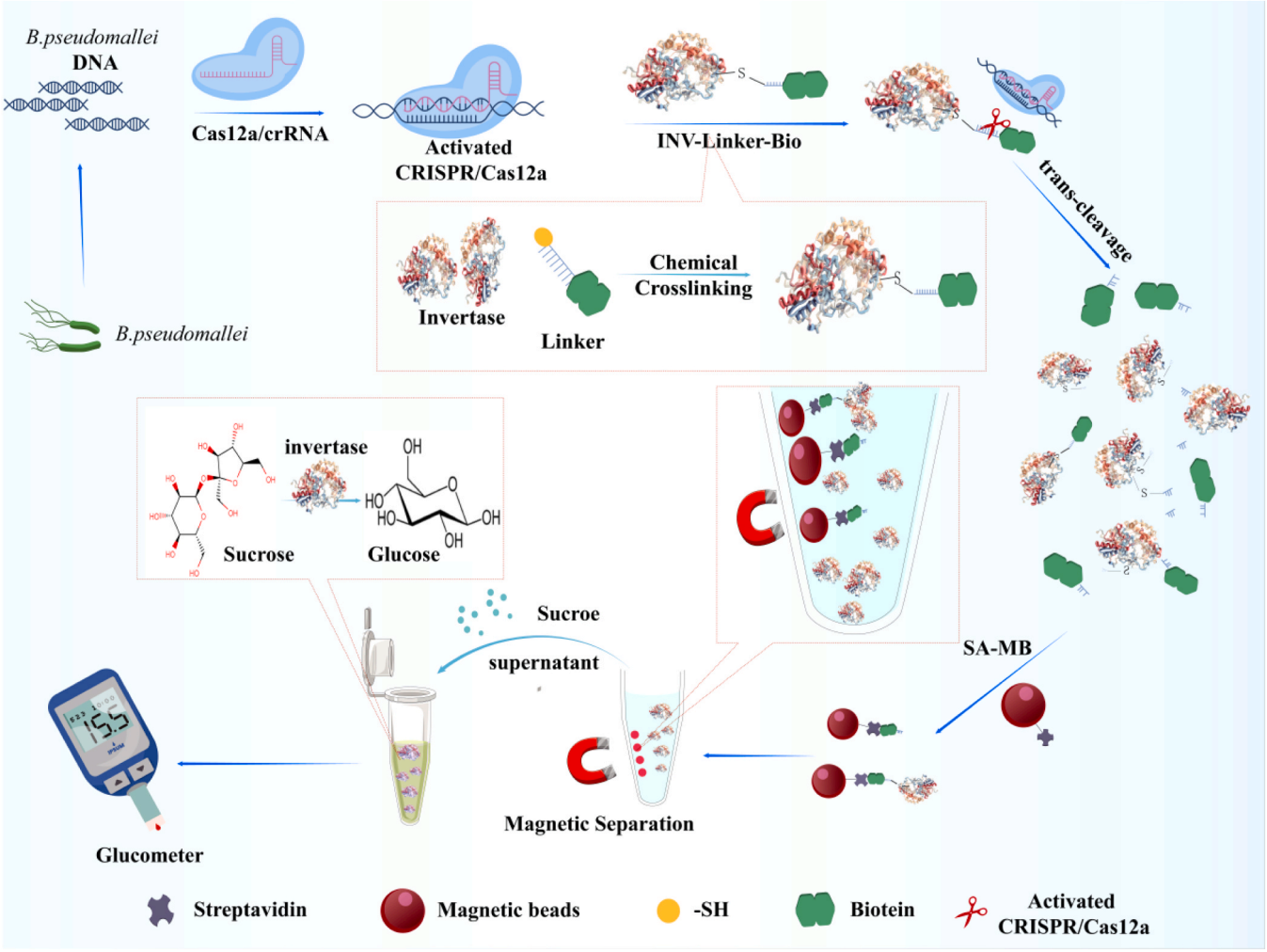
To assess the cleavage efficiency of Cas12a under the established conditions, the reaction products were analyzed by 12 % polyacrylamide gel electrophoresis (PAGE) in 1 × TBE buffer (pH 7.9). Each 6 μL electrophoresis sample contained 1 μL of 6 × loading buffer, 1 μL of GelGreen nucleic acid stain, and 4 μL of the Cas12a reaction mixture. After loading into the gel wells, electrophoresis was performed at a constant voltage of 110 V for 60 min. The gel was subsequently imaged using a ChemiDoc MP Universal Chemiluminescence Imaging System.

2.5. Detection of CRISPR/Cas12a trans-cleavage activity using PGM

The CRISPR/Cas12a system was assembled and reacted under the conditions described in Section 2.4, except that the F-Q probe was replaced with 1 μL of INV-linker. After the cleavage reaction, the resulting solution was introduced into 10 μL of streptavidin-modified magnetic beads (SA-MB, 10 mg/mL). The mixture was gently agitated at room temperature for 10 min to facilitate binding between the SA-MB and the cleaved products. Magnetic separation was then carried out, leaving unbound invertase in the supernatant. A 20 μL aliquot of the supernatant was carefully transferred and mixed with 50 μL of sucrose solution (0.5 M, pH 4.5). The mixture was incubated at 55 °C for 30 min to allow invertase-mediated hydrolysis of sucrose into glucose. Subsequently, 5 μL of the resulting solution was analyzed using a PGM to determine the glucose concentration. Based on the established correlation between glucose levels and the concentration of *B. pseudomallei* DNA, the target DNA concentration could be quantitatively inferred from the PGM readout.

2.6. Quantitative real-time PCR

Primers targeting the T3SS-1 ORF2 region of *B. pseudomallei* were designed using the NCBI Primer-BLAST tool, based on the reference sequence obtained from the NCBI database. The corresponding primer sequences are listed in Table 1. Each qPCR reaction was prepared in a total volume of 20 μL, consisting of 1 μL of *B. pseudomallei* DNA solution, 10 μL of 2 × SYBR qPCR Master Mix, 0.5 μL of each primer (10 μM), and 8 μL of DEPC-treated water. The amplification protocol began with an initial denaturation at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 30 s. Fluorescence signal was acquired at the end of each annealing/extension step. No-template controls (NTCs) were included in each run to monitor potential contamination and assess amplification specificity.



Scheme 1. Schematic of method using a CRISPR/Cas-PGM to detect *B. pseudomallei* DNA.

3. Results and discussion

3.1. Working principle

The detection mechanism, as illustrated in Scheme 1, follows a well-defined technical workflow for detecting the target gene of *B. pseudomallei* using the CRISPR/Cas- PGM system. The process begins with the design of a specific guide crRNA that recognizes the ORF2 fragment in the *B. pseudomallei* genomic DNA. When the target gene is present, the crRNA binds to it through complementary base pairing, forming a ternary complex with Cas12a and the DNA, which activates Cas12a's cleavage activity. Once activated, the Cas12a-crRNA-DNA complex cleaves the single-stranded DNA linker within the invertase-linker-biotin conjugate. This cleavage disrupts the structural integrity of the conjugate, releasing free invertase. To remove any uncleaved conjugates from the reaction system, streptavidin-functionalized magnetic beads (SA-MB) are used to capture and separate the invertase-linker-biotin conjugates through magnetic separation. After the magnetic beads are removed, the invertase remains in the supernatant. Upon adding sucrose to the system, the free invertase catalyzes the hydrolysis

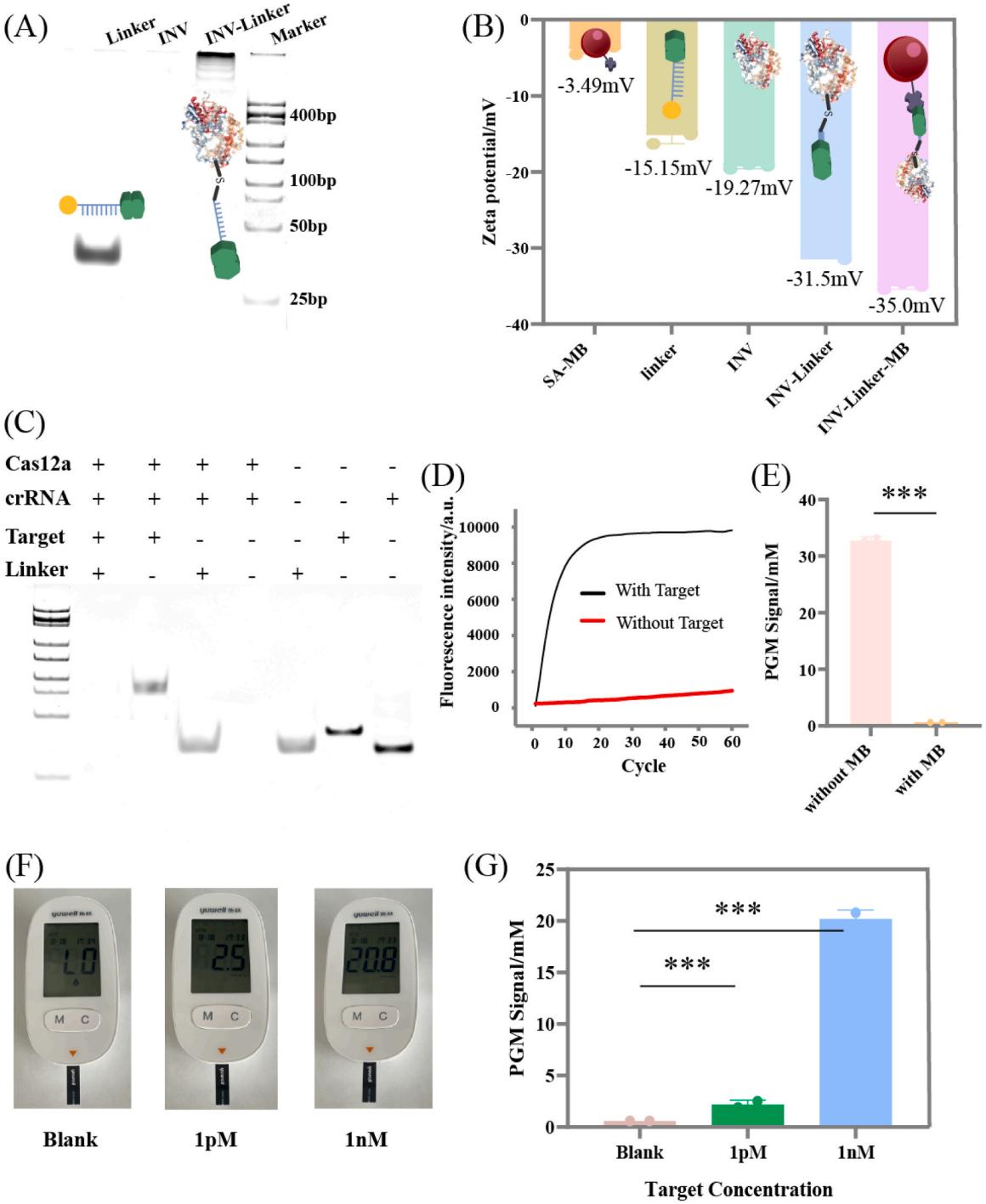


Fig. 1. Validation of INV-linker and INV-linker-MB fabrication by agarose gel electrophoresis (A) and Zeta potential analysis (B); Assessment of target recognition and cleavage by CRISPR/Cas12a using PAGE (C) and fluorescence spectroscopy (D); Evaluation of SA-MB effects on personal glucose meter detection (E) and feasibility analysis of PGM-based detection of *B. pseudomallei* DNA (F and G). ***: $P < 0.001$, Error bars represent SD ($n = 3$).

of sucrose into glucose. The released glucose is then quantitatively detected using a personal glucose meter (PGM). The concentration of glucose released is directly proportional to the amount of invertase present, which in turn is related to the concentration of the ORF2 gene in the sample. This allows for accurate quantification of *B. pseudomallei* DNA based on the glucose readings from the PGM, enabling rapid and sensitive detection of the pathogen.

3.2. Synthesis and characterization of INV - Linker and INV - Linker- MB

To validate the successful construction of INV-Linker and INV-Linker-MB, we performed agarose gel electrophoresis and surface potential analysis (Fig. 1A and Fig. 1B). The linker used was a polythymidine sequence functionalized with a thiol group at the 5'-end (5'-SH) and biotin at the 3'-end (3'-Bio). The thiol group allowed conjugation with Sulfo-SMCC-activated invertase via stable thioether bond formation, yielding the INV-linker complex. The 3'-Bio bound to streptavidin-modified magnetic beads, leading to uniform immobilization of the linker on the bead surface and generating INV-linker-MB. Gel electrophoresis revealed a markedly reduced migration rate for INV-linker compared to the unconjugated linker (Fig. 1A), confirming successful conjugation of invertase to the linker.

We further characterized the assembly of INV-linker-MB using Zeta potential measurements (Fig. 1B). The Zeta potentials of individual components were as follows: SA-MB (-3.49 mV $\pm$ 0.59 mV), linker (-15.15 mV $\pm$ 1.15 mV), and invertase (INV, -19.27 mV $\pm$ 0.31 mV). Upon covalent conjugation of INV, and Linker to form INV-Linker, the Zeta potential decreased to - 31.5 mV $\pm$ 0.2 mV. Subsequent immobilization onto streptavidin-functionalized magnetic beads further reduced the potential to -35.0 mV $\pm$ 0.4 mV. This negative shift can be attributed to the net negative charges carried by both nucleic acids and proteins, confirming the successful assembly of INV-linker on the magnetic bead surface. Moreover, the absolute Zeta potential value of INV-linker-MB exceeded 20 mV, indicating favorable colloidal stability of the complex in solution. In summary, both gel electrophoresis and Zeta potential analyses confirm the successful assembly of INV-Linker and INV-Linker -MB, with the resulting complexes exhibiting excellent stability in aqueous solution. These results confirm the successful construction of a stable sensing interface, which is crucial for reliable signal transduction in the CRISPR/Cas12a–PGM biosensor.

3.3. Characterization of target-activated CRISPR/Cas12a trans-cleavage activity

In the current study, the target DNA was meticulously selected from the T3SS1 gene of *B. pseudomallei* (GenBank accession number AF074878), which served as the analytical paradigm. The PAGE analysis (Fig. 1C) was firstly used to verify the dual CRISPR/Cas12a trans-cleavage. As shown in Fig. 1C, individual components including crRNA, target, and Linker exhibited fast electrophoretic mobility. When crRNA and Cas12a were present together, no distinct bands were observed. In contrast, when both crRNA and Target were included, a band with significantly reduced mobility appeared, indicating specific binding between crRNA and Target via complementary base pairing, and further confirming that the target sequence is essential for complex formation. After the addition of the Linker, no clear intact nucleic acid bands were detected in the corresponding lanes, suggesting that the formation of the crRNA/Cas12a/Target ternary complex activated the trans-cleavage activity of Cas12a, leading to fragmentation of the Linker. Concurrently, fluorescence-based verification demonstrated that the activation of Cas12a's bidirectional cleavage activity is strictly dependent on the presence of the target DNA (Fig. 1D). These results demonstrate the specific assembly of the Cas12a-crRNA complex upon target recognition and its ability to activate trans-cleavage of the linker.

To validate the binding capability of INV-Linker to streptavidin magnetic beads and its retained catalytic activity after binding, we compared the glucose concentrations in the supernatant of the reaction system in the presence or absence of magnetic beads. As shown in Fig. 1E, the glucose concentration in the supernatant was significantly lower when magnetic beads were present, indicating successful binding of INV-Linker to the beads and maintained sucrose hydrolysis function after binding. Furthermore, when the solution after Cas12a cleavage was incubated with streptavidin magnetic beads, the glucose conversion ability in the supernatant of the cleaved samples was significantly enhanced. This indicates that the Cas12a/crRNA-target DNA ternary complex can hydrolyze the Linker in INV-Linker, thereby releasing INV into the supernatant. As a result, the INV cannot be adsorbed by the magnetic beads, leading to increased hydrolysis of sucrose and a consequent rise in glucose content in the supernatant.

To evaluate the biosensor's practical detection capability, *B. pseudomallei* DNA was used as the target. As shown in Fig. 1F and Fig. 1G, only a weak PGM background signal was observed in the absence of the target (Blank), while a significant signal increase occurred in its presence. This indicates that without target DNA, the crRNA fails to activate the trans-cleavage activity of Cas12a, leaving INV-Linker intact and bound to magnetic beads. After magnetic separation, minimal invertase remains in the supernatant, resulting in a low PGM signal. In contrast, target presence activates Cas12a trans-cleavage, which cleaves INV-Linker, preventing its binding to beads. The released invertase in the supernatant catalyzes sucrose hydrolysis to glucose, thereby amplifying the PGM signal. These results demonstrate that the constructed sensor can effectively achieve visual detection of *B. pseudomallei* DNA.

3.4. Optimization of detection conditions

To enhance the overall performance of the biosensing strategy, we systematically optimized a range of experimental parameters. The signal-to-noise ratio (S/N) was used to accurately evaluate the detection performance by comparing the PGM signals obtained with 2.0 nM target DNA (Signal) to those of blank controls (Noise). As illustrated in Fig. 2A, increasing the Cas12a concentration from 20 nM to 40 nM resulted in a corresponding enhancement in both the target-induced PGM signal and the S/N ratio. However, further increasing the Cas12a concentration led to signal saturation and elevated background noise, causing a rapid decline in S/N. This may be attributed to cross-contamination of nucleic acids or unbound RNPs, which could lead to false-positive activation; higher Cas12a concentrations amplify these random signal fluctuations [15]. Therefore, 40 nM was identified as the optimal Cas12a concentration.

To ensure optimal formation of the CRISPR/Cas12a-crRNA (RNP) complex, improve target recognition efficiency, and avoid excessive non-specific activation, this study optimized the crRNA-to-Cas12a ratio. As shown in Fig. 2B, there was little change in the PGM signal as the ratio decreased. However, when the ratio was 1:5, the background signal significantly increased, indicating that an excessive Cas12a enzyme concentration might lead to excessive non-specific cleavage. This could be due to an imbalance between the free crRNA and Cas12a enzyme, resulting in incomplete RNP assembly and increasing the chances of non-specific binding and incidental cleavage. Based on a trade-off between cost and signal-to-noise ratio (S/N), the optimal crRNA-to-Cas12a ratio was determined to be 1:2.

To improve detection sensitivity while controlling background noise, ensuring the accuracy and reliability of the results, we investigated the Cas12a cleavage time (Fig. 2C). As the reaction time is extended, Cas12a's collateral activity is continuously activated, leading to the cleavage of a large number of reporter molecules, which causes the fluorescent signal to gradually increase and reach a steady state after approximately 15 min, maintaining a high plateau. However, the prolonged reaction time also inevitably exacerbates non-specific activation and the accumulation of background signals. In the negative control, the background signal gradually increases over time, especially after the

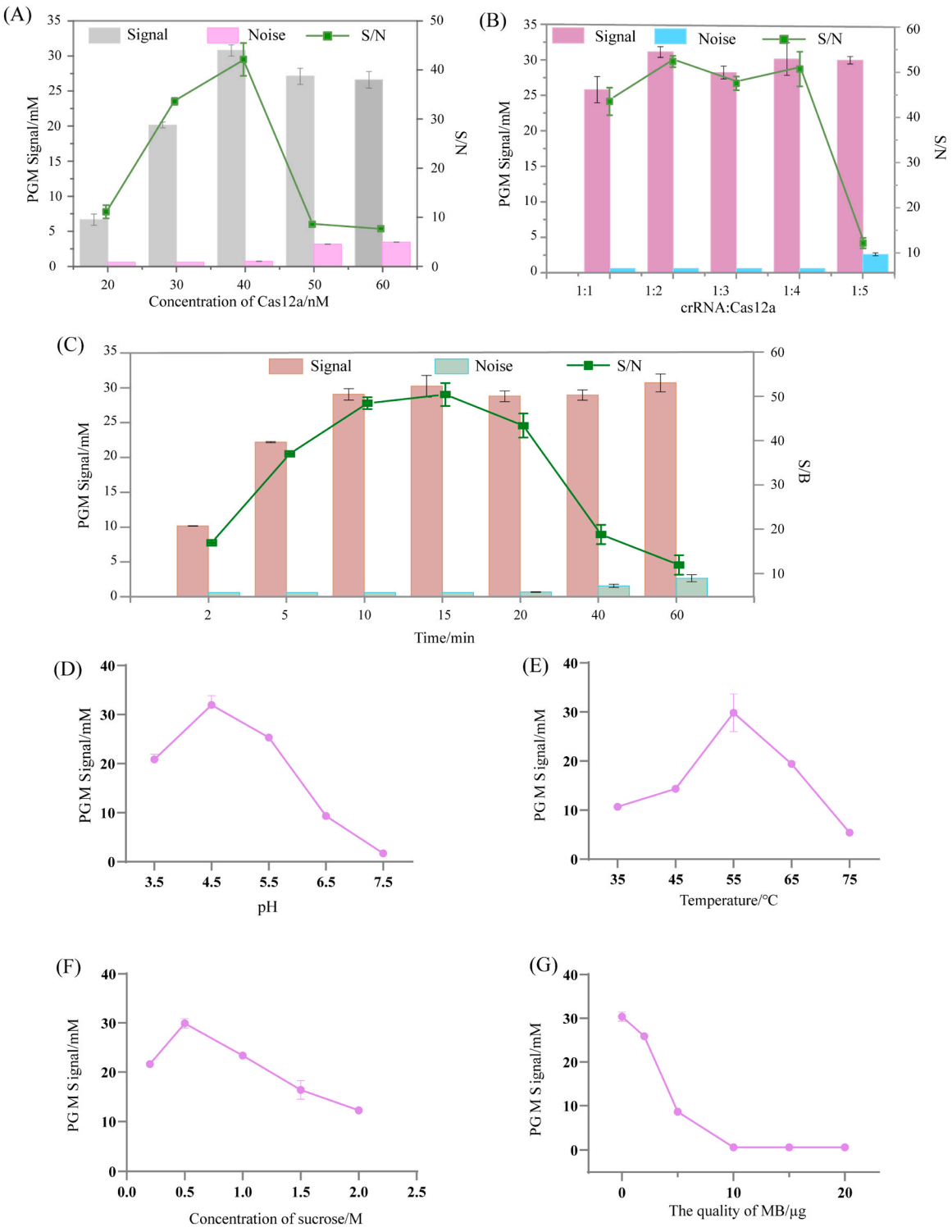


Fig. 2. Optimization of (A) Cas12a concentration, (B) concentration ratio of crRNA to Cas12a, and (C) Reaction time of CRISPR/Cas12a. Invertase reaction pH (D), and temperature (E). (F) Concentration of sucrose, and (G) the quality of SA-MB. Error bars represent SD (n = 3).

reaction exceeds 15 min, where the signal accumulation due to non-specific cleavage by Cas12a becomes more pronounced [16]. Although the signal intensity of the positive samples remains high at this point, the increase in the background of the control group ultimately leads to a decrease in the signal-to-noise ratio. Consequently, 15 min was selected as the optimal reaction time.

The effect of solution pH on invertase (INV) activity was examined, revealing maximum PGM response at pH 4.5 (Fig. 2D). Temperature optimization studies showed peak INV activity at 55 °C (Fig. 2E). Substrate concentration studies demonstrated that sucrose concentrations above 0.5 M led to reduced hydrolysis efficiency (Fig. 2F), establishing this as the optimal concentration. Finally, the amount of streptavidin magnetic beads (SA-MBs) was optimized to ensure complete removal of INV-Linker. The PGM signal decreased with increasing SA-MB amounts and stabilized at $\geq$ 10 μg (Fig. 2G), indicating quantitative capture of the INV-Linker complex and minimal false-positive risk.

3.5. Detection of *B. pseudomallei* DNA with PGM biosensing method

To comprehensively evaluate the analytical performance of the developed CRISPR/Cas12a-based personal glucose meter (PGM) biosensing platform, a series of systematic experiments were conducted. First, the response behavior toward varying concentrations of *B. pseudomallei* DNA was examined. As illustrated in Fig. 3A, the PGM readings showed a distinct concentration-dependent increase with rising target DNA levels. The dose-response curve demonstrated an excellent linear correlation between the PGM signal and the logarithm of target concentration (log *C*) across a wide dynamic range from 1 pM to 10 nM (Linear regression equation: $y = 7.27\ x + 2.6267$. y: PGM signals; x: log *C*) (Fig. 3B). This robust linearity ($R^2 > 0.99$) provides a reliable basis for quantitative analysis, indicating that within this range, the trans-cleavage activity of the CRISPR/Cas12a system is directly proportional to the *B. pseudomallei* DNA concentration, resulting in controlled cleavage of the INV-Linker and a linear glucose output.

The limit of detection (LOD) of the developed method was determined based on the lowest target concentration that produced a signal statistically distinguishable from the blank ($P < 0.05$). No significant difference was observed between the blank (PGM signal: L0) and 0.01 pM target concentrations (PGM signal: 0.7 ± 0.101), whereas a clear signal increase was detected at 0.1 pM (PGM signal: 1.34 ± 0.113). Therefore, the LOD of the assay was estimated to be 0.1 pM. When the target concentration exceeded 10 nM, the PGM signal reached a plateau, maintaining a consistently high level. Such saturation behavior is common in CRISPR-based biosensing systems and is mainly attributed to the exhaustion of critical components (e.g., Cas12a/crRNA complexes or INV-Linker substrates) or the reaction rate approaching its kinetic limit, preventing further signal enhancement at higher target concentrations.

The specificity of the developed method for *B. pseudomallei* DNA detection was evaluated. As shown in Fig. 3C and Fig. 3D, a strong PGM

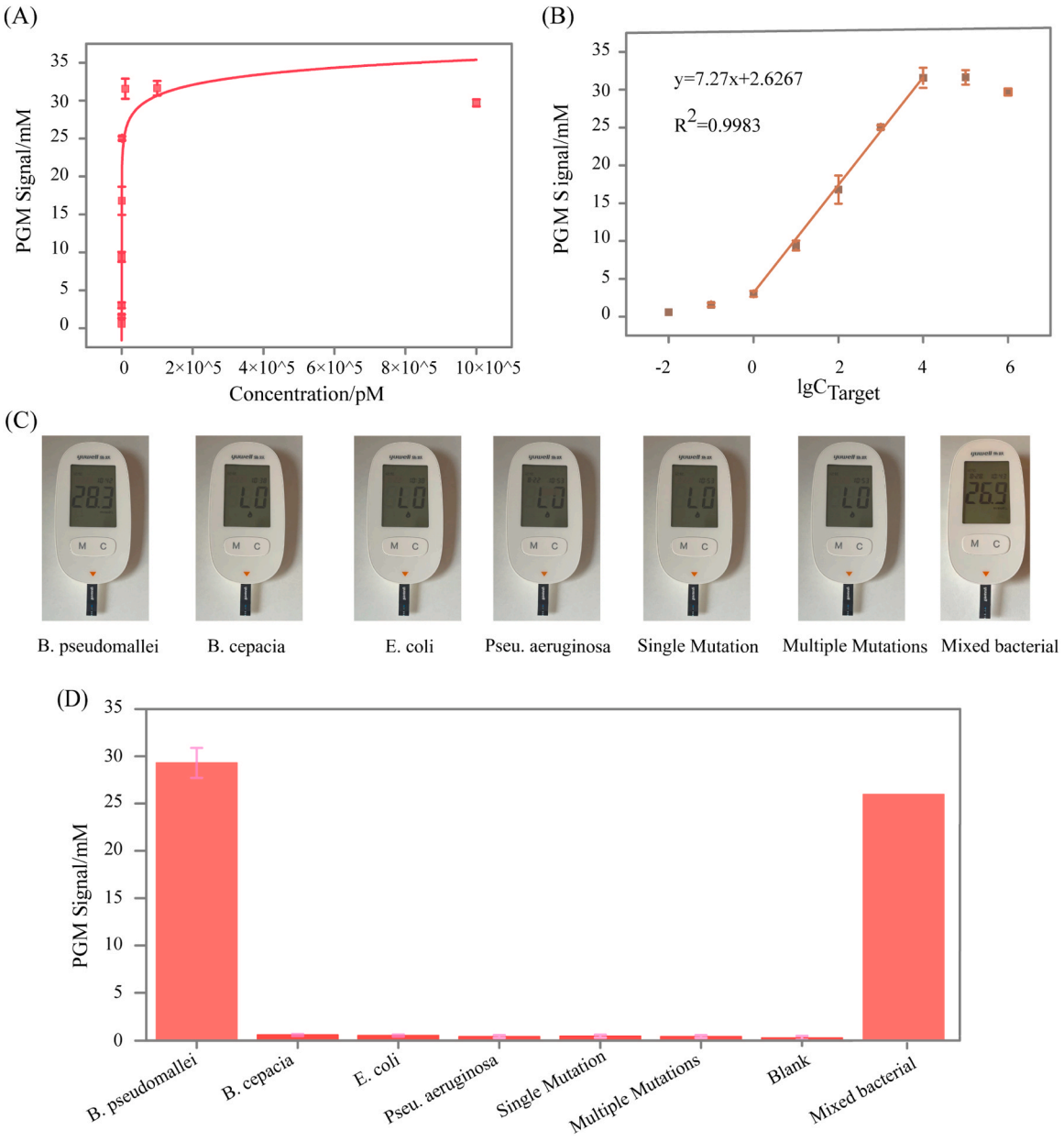


Fig. 3. Performance evaluation of the CRISPR/Cas12a-PGM biosensor for *B. pseudomallei* DNA detection. (A) PGM signals in response to different concentrations of *B. pseudomallei* DNA. (B) Calibration curve of the biosensor for *B. pseudomallei* DNA detection within the range of 1 pM to 10 nM. (C, D) Specificity evaluation of the biosensor. *B. cepacia*: *Burkholderia cepacia*; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; M-S: single-base mismatched target; M-M: multi-base mismatched target (All DNA concentrations were 10 nM). Error bars represent SD (n = 3).

signal was generated exclusively in the presence of *B. pseudomallei* DNA, whereas the response values for non-target bacterial DNA (e.g., *E. coli*, *B. cepacia*, and *P. aeruginosa* with a concentration of 10 nM) were indistinguishable from the blank control (L0, not detected). More importantly, the method exhibited excellent discrimination against genotypes containing single or multiple nucleotide substitutions within the target sequence, which failed to elicit any significant PGM signal. These results clearly demonstrate the high specificity of the proposed PGM-based detection assay. This specificity originates from the stringent recognition capability of the Cas12a/crRNA complex toward the PAM sequence and its adjacent protospacer regio[9]. Precise base pairing between the crRNA and target DNA is essential for activating Cas12a's trans-cleavage activity; mismatches or mutations at critical sites strongly inhibit this activation, thereby preventing non-specific signal generation. Consequently, the method provides a robust foundation for the accurate identification of *B. pseudomallei* in complex biological or environmental samples.

3.6. Stability and precision evaluation of the CRISPR/Cas12a-PGM biosensor

To ensure the practical applicability of the developed CRISPR/Cas12a-PGM biosensing system, its stability, repeatability, and reproducibility were comprehensively evaluated. In this study, the INV-linker-Bio probe and magnetic beads were stored at −20 °C and 4 °C, respectively. To assess its long-term stability, the PGM signal was monitored weekly over a four-week period. The PGM readings exhibited minimal fluctuation, with a coefficient of variation (CV) as low as 4.4 % (Fig. 4A and Fig. 4B). This value is well below the generally accepted threshold of 15 %, demonstrating the excellent stability and reproducibility of the developed detection system. This high stability can be attributed to the inherent robustness of the Cas12a enzyme and the catalytic resilience of invertase under optimized storage conditions. Together, these features ensure the reliability of the proposed method for potential use in real-world clinical sample testing.

The intra-assay precision was assessed by repeatedly detecting an ultra-low concentration of the *B. pseudomallei* DNA (0.1 pM) using the same operator, instrument, and day. The repeated PGM measurements exhibited minimal deviation, confirming the method's excellent repeatability (CV = 8.4 %, Fig. 4C). Even at concentrations approaching the limit of detection, the signal remained stable and consistent, suggesting that the biosensor performs reliably for trace-level quantification. This high precision reflects the strong control over Cas12a-mediated cleavage dynamics and the consistent enzymatic response of invertase, which collectively minimize random fluctuations during measurement. To evaluate inter-assay precision, independent experiments were performed by different operators at different time points using 1 pM and 1 nM target DNA samples. The results, presented in Fig. 4D and Fig. 4E, show strong consistency among repeated tests, with CV values remaining below 10 % (Fig. 4D: 7.5 %, Fig. 4E: 3.4 %), well within the acceptable range for bioanalytical methods. The absence of significant variation across operators and days demonstrates that the CRISPR/Cas12a-PGM system is highly reproducible and operator-independent, an essential feature for real-world applications and decentralized testing environments.

As summarized in Table 2, key analytical parameters including the detection limit and linear dynamic range were compared among different CRISPR/Cas–PGM-based biosensing strategies. It should be noted that while many reported methods focus primarily on achieving ultra-low detection limits, detailed quantitative characteristics such as linear range are not always systematically reported. In contrast, the proposed CRISPR/Cas12a–PGM biosensor not only achieves a low detection limit of 0.1 pM but also exhibits a well-defined linear range spanning from 1 pM to 10 nM. This wide linear dynamic range is particularly important for the quantitative analysis of clinical samples, where target DNA concentrations may vary significantly among patients.

Compared to some PGM sensing strategies that require the customization of functional DNA hydrogels [20] or complex solid-phase enzyme reporter systems for specific targets, the core advantage of our platform lies in the "sequence-independent" nature of its signal transduction module. The 'INV-linker-biotin/SA-MB' reporter system we developed is applicable to any ssDNA linker that can be released through Cas12a trans-cleavage. Therefore, when applying this platform to new nucleic acid targets, only the corresponding crRNA sequence needs to be replaced, without the need to redesign or optimize the downstream signal generation and readout modules. This feature greatly enhances the platform's versatility and reprogrammability, making it more suitable for constructing a modular and standardized CRISPR-PGM biosensing architecture. Although amplification-assisted CRISPR/PGM methods may offer higher absolute sensitivity, they often require longer assay times and more complex workflows. The amplification-free design presented here represents a balanced compromise between sensitivity, simplicity, and practical applicability, reinforcing its potential for point-of-care diagnostics.

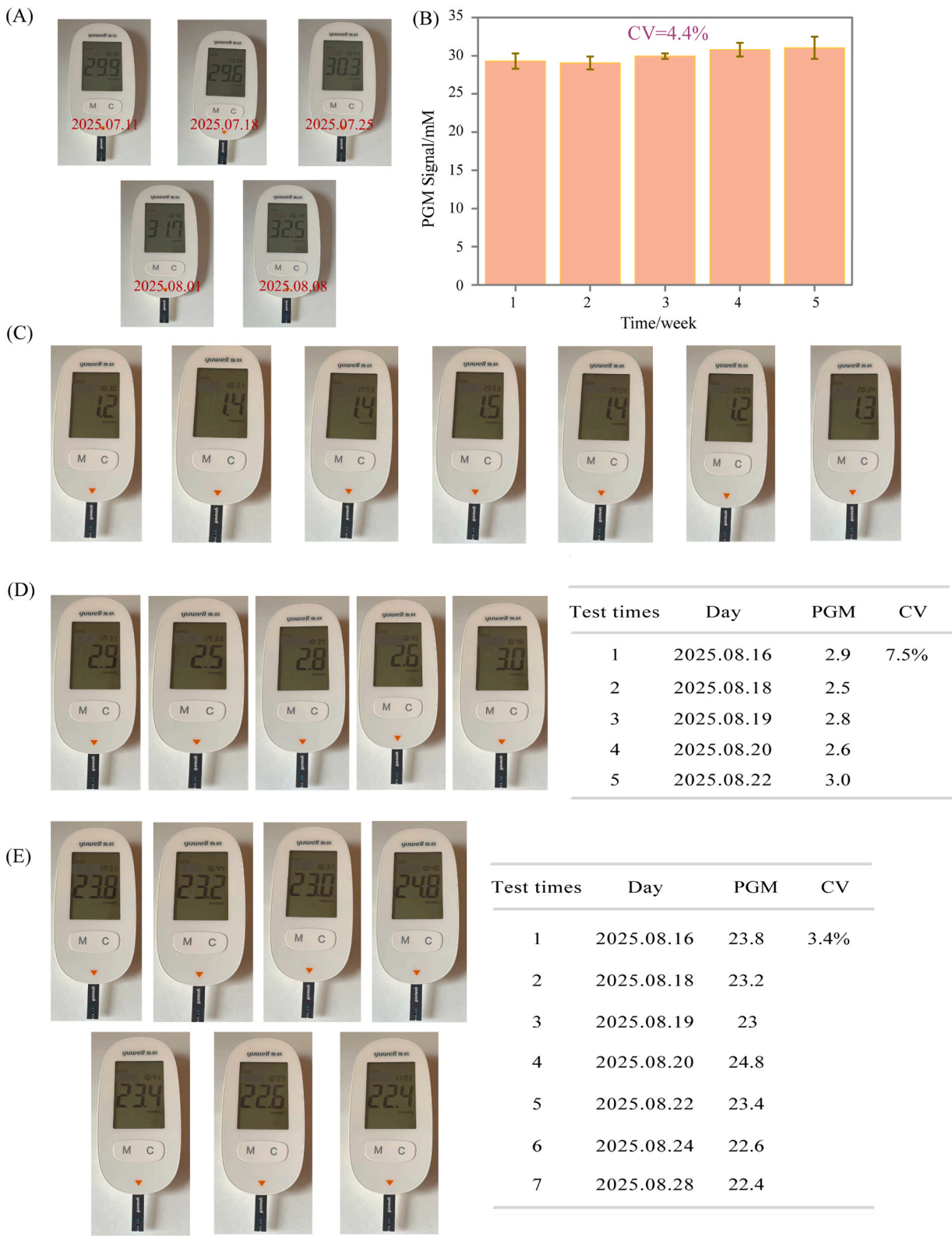
Compared to existing methods for detecting *B. pseudomallei* DNA [24,25], our CRISPR/Cas12a-PGM biosensor offers a unique combination of features: Amplification-free operation: Eliminates the need for target pre-amplification (e.g., PCR, RAA), reducing assay complexity, contamination risk, and reliance on precise temperature control. Rapid and instrument-light workflow: The complete assay takes ~50 min and uses a widely available, low-cost personal glucose meter as the readout device, bypassing the need for specialized laboratory equipment like fluorimeters, potentiostats, or thermocyclers. Excellent specificity and a wide linear range: The system discriminates against single- and multi-base mismatches and offers a broad quantitative range (1 pM – 10 nM), covering clinically relevant concentrations without sample dilution. Demonstrated practicality: The biosensor showed strong correlation with qPCR in clinical isolates, and stable performance over four weeks of storage, supporting its potential for real-world point-of-care use. Compared with the previously established detection methods in our research group, this approach simplifies the detection equipment by converting the detection signal of *B. pseudomallei* DNA into changes in glucose concentration, making it more suitable for point-of-care testing (POCT) [6,7,26,27].

To assess the anti-interference performance of the developed biosensing method, *B. pseudomallei* DNA was diluted to various concentrations in serum. As summarized in Table 3, the recovery rates ((Measured concentration in spiked sample) / (Theoretically added concentration)] × 100 %, with the background signal from unspiked serum subtracted prior to calculation) ranged from 98.0 % to 113.2 %, with standard deviations below 5 %, confirming the method's high accuracy and reliability for detecting *B. pseudomallei* DNA in complex biological matrices. Specifically, consistent recovery was achieved at 1 pM, 10 pM, and 100 pM, demonstrating stable quantitative performance across a broad dynamic range. These findings verify that the biosensor possesses excellent precision and robustness, highlighting its strong potential for clinical application as an efficient tool for *B. pseudomallei* DNA detection.

3.7. Testing of the real samples

To further validate the clinical applicability of the proposed strategy, DNA extracted from peripheral blood samples of ten patients diagnosed with melioidosis was analyzed using both the PGM-based biosensing method and qPCR as a reference technique. The concentrations of target DNA were quantified using the PGM standard curve established with standard DNA solutions (Fig. 3B) and the qPCR standard curve generated from serial dilutions of genomic DNA (Fig. 5B). The quantified DNA levels obtained by both methods and their comparison are summarized in Fig. 5C.

These results confirm that the PGM-based biosensing method



Test times	Day	PGM	CV
1	2025.08.16	2.9	7.5%
2	2025.08.18	2.5	
3	2025.08.19	2.8	
4	2025.08.20	2.6	
5	2025.08.22	3.0	

Test times	Day	PGM	CV
1	2025.08.16	23.8	3.4%
2	2025.08.18	23.2	
3	2025.08.19	23	
4	2025.08.20	24.8	
5	2025.08.22	23.4	
6	2025.08.24	22.6	
7	2025.08.28	22.4	

Fig. 4. Stability and precision analysis of the CRISPR/Cas12a-PGM biosensing system. (A) PGM readings obtained using reagents stored for different time intervals, showing minimal signal drift. (B) Corresponding bar chart of PGM responses, confirming stable performance over time (CV = 4.4 %). (C) Intra-assay precision analysis: repeated detections of 0.1 pM *B. pseudomallei* DNA under identical conditions (same operator, instrument, and day, CV = 8.4 %), demonstrating high repeatability. (D, E) Inter-assay reproducibility evaluation at 1 pM (CV = 7.5 %) and 1 nM (CV = 3.4 %) target concentrations tested by different operators on different days; results show strong agreement and consistent signal output across conditions. Error bars represent standard deviations (n = 3).

Table 2
Comparison of analytical performance of different CRISPR/Cas–PGM sensing strategies.

CRISPR/Cas system	signal amplification	LOD	linear dynamic range	detection time /min	Refer
CRISPR/Cas12a	free	57 pM HPV DNA	0.1 nM to 10 nM	120	[17]
CRISPR/Cas12a	SDA	55.1 fM HPV DNA	0.2–100 pM	45	[18]
CRISPR/Cas12a	RAA	5CFU/reaction	1–10^3 CFU/reaction	135	[19]
CRISPR/Cas12a	RCA,	2.6 fM	10 fM-1.0 nM.	75	[20]
CRISPR/Cas12a	RAA	0.011 pM	0.1 nM to 10 nM	87	[21]
CRISPR/Cas12a	RCA	30.3 fM	50.0 fM to 50.0 pM	180	[22]
CRISPR/Cas12a	RCA, RPA	10 pM	10.0 pM to 10.0 nM	80	[23]
CRISPR/Cas12a	free	0.1 pM	1 pM to 10 nM	50	This Work

Table 3
The recovery experiment of PGM using synthetic *B. pseudomallei* DNA strands piked serum sample. (n = 3).

Samples	Added(pM)	Found(pM)	Recovery (%)	RSD(%)[a]
1	1	1.132	113.2	1.6
2	10	9.805	98.0	2.1
3	100	105.723	105.7	2.2

[a] Precision expressed as a percentage of the relative standard deviation

exhibits high concordance with the gold standard qPCR for detecting clinical samples, underscoring its accuracy and reliability. This comparison represents a crucial step in advancing the sensor from proof-of-concept to practical clinical application. The strong agreement observed, coupled with the PGM's inherent portability, low cost, and ease of use, suggests that this strategy holds significant potential as a next-generation molecular diagnostic tool for primary care settings or point-of-care testing (POCT). It offers an accurate, efficient, and highly accessible solution for the rapid diagnosis of melioidosis and other infectious diseases.

4. Discussion

This work presents an amplification-free CRISPR/Cas12a–PGM biosensing strategy for quantitative detection of *B. pseudomallei* DNA. By coupling target-activated Cas12a collateral cleavage with an invertase-DNA-magnetic bead reporter and readout on a commercial personal glucose meter (PGM), the system achieves a limit of detection of 0.1 pM with a linear range from 1 pM to 10 nM within 50 min, while using only widely available instrumentation. These analytical figures of merit, together with good recovery in spiked serum and strong agreement with qPCR for clinical isolates, indicate that the platform strikes a practical balance between sensitivity, assay time, and operational simplicity suitable for point-of-care testing (POCT) scenarios.

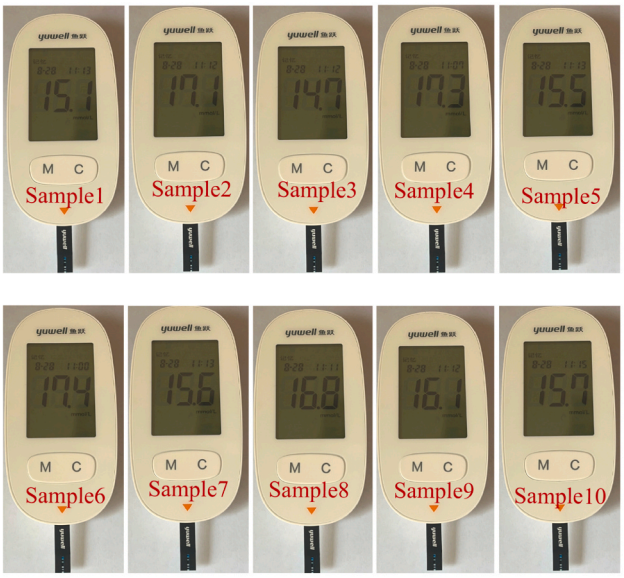
CRISPR/Cas biosensors have rapidly evolved from fluorescence-based assays (e.g. DETECTR and HOLMES) into a diverse family of point-of-care biosensors that exploit the collateral nuclease activity of Cas12 and Cas13 for nucleic-acid detection [9,10,28]. However, most CRISPR assays still rely on benchtop fluorescence readers, plate readers, or electrochemical workstations, which limit their deployment outside centralized laboratories. In parallel, PGMs have been repurposed as generic chemical sensors by using functional DNA or enzymatic circuits to convert non-glucose targets into glucose signals, enabling portable, quantitative detection with minimal hardware [29,30]. The present study integrates these two lines of development by installing a CRISPR/Cas12a sensor into a "sweet" readout format, in which the amount of invertase released by Cas12a trans-cleavage is translated into a glucose concentration measurable by any standard PGM.

A growing number of studies have demonstrated that CRISPR–PGM integration is a powerful strategy for POCT. For example, Gong *et al.* used a duplex-specific nuclease-assisted CRISPR/Cas12a system combined with a PGM to detect microRNAs with picomolar-level sensitivity, but the method required an additional enzymatic pre-amplification step and multistep reagent handling [29]. Tanifuji *et al.* further embedded a Cas12a-PGM assay into a paper-based analytical device, simplifying the workflow and demonstrating quantitative nucleic acid detection in a PAD format [20]. Zhou *et al.* reported a CRISPR Cas12a-based "sweet" biosensor coupled with PGM readout for *Salmonella* testing in food, using recombinase-aided amplification (RAA) to achieve very low detection limits but at the cost of extra amplification reagents and temperature control [17]. More recently, several groups have developed versatile CRISPR–PGM platforms for protein biomarkers or viral nucleic acids, such as AFP or human papillomavirus, often employing solid-phase invertase reporters and isothermal amplification to compensate for the moderate sensitivity of PGMs [29,31–33]. A recent review systematically summarized these CRISPR/Cas-based PGM strategies, highlighting their promise for home-based and decentralized diagnostics while also pointing out current limitations in assay integration, multiplexing and robustness [34]. Within this context, the present work advances the field by demonstrating that an amplification-free Cas12a-PGM configuration can still reach 0.1 pM LOD with a relatively short assay time, while maintaining a simple reagent architecture and relying only on a standard glucometer as readout.

It should be noted that the "amplification-free" strategy used in this study, while simplifying the workflow, also demands stricter control over non-specific signals. As shown in Fig. 2C, the trans-cleavage activity of Cas12a is accompanied by the accumulation of background signals in the later stages of the reaction. To address this, we systematically optimized the reaction conditions, precisely controlling the reaction time to 15 min and setting the ratio of Cas12a to crRNA at 1:2. These conditions effectively suppressed the background increase caused by free Cas12a or non-specific binding, while maximizing the target activation signal. This achieves an optimal balance in practical terms between "detection sensitivity," "ease of operation," and "result reliability." This highlights the importance of engineering the CRISPR/Cas12a system when adapting it to the PGM readout platform.

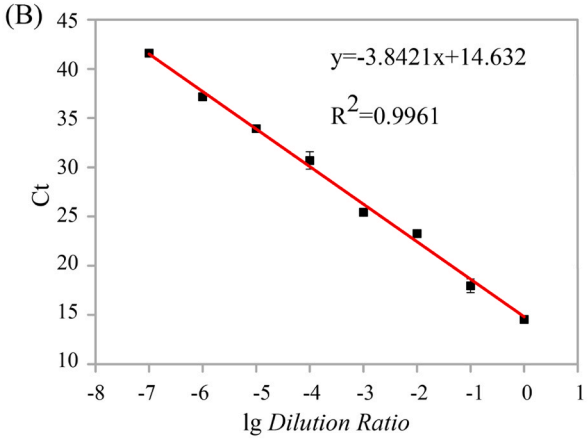
When compared with other rapid detection methods for *B. pseudomallei* and melioidosis, the proposed biosensor represents a distinct design trade-off. Conventional bacterial culture remains the gold standard but typically requires several days and BSL-3 facilities, which is incompatible with urgent clinical decision-making [1,2]. qPCR and other nucleic-acid amplification tests significantly shorten the

(A)



Samples	PGM-1	PGM-2	Concentration ng/μL	
1	15.1	15.3	93.47	102.51
2	17.1	17.1	219.18	224.51
3	14.7	14.6	78.44	75.06
4	17.3	17.1	237.12	224.51
5	15.5	15.2	111.62	97.73
6	17.2	17.3	234.67	237.12
7	15.6	15.5	116.47	111.62
8	16.8	16.6	196.86	180.46
9	16.1	15.9	144.80	132.62
10	15.7	15.6	121.83	116.47

(B)



Samples	ct-1	ct-2	Concentration ng/μL	
1	16.15	16.97	108.12	90.64
2	15.31	14.84	178.86	170.75
3	16.21	18.66	103.57	111.62
4	15.29	14.26	182.10	172.21
5	16.08	17.28	112.66	100.04
6	14.82	13.60	240.98	221.19
7	16.04	17.23	115.91	105.28
8	15.33	12.61	177.54	134.69
9	15.52	14.84	157.99	159.46
10	15.87	14.84	128.24	133.06

(C)

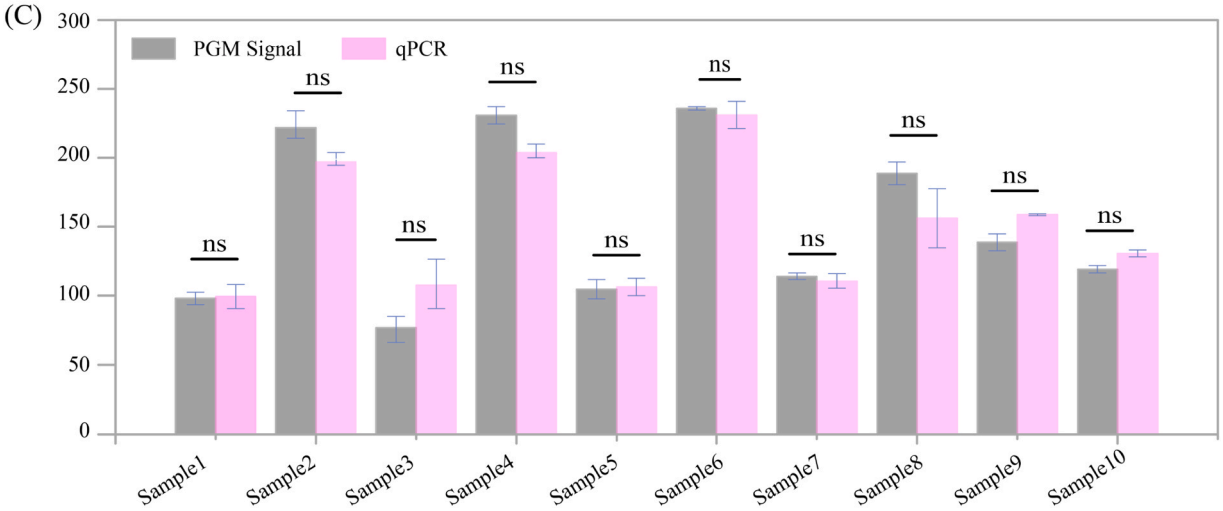


Fig. 5. (A) Representative detection results of ten clinical isolates obtained using the PGM-CRISPR biosensor. (B) qPCR standard curve showing the linear correlation between Ct values and log-transformed DNA concentration. (C) Comparative bar chart of *B. pseudomallei* DNA concentrations in clinical isolates measured by qPCR and the PGM-CRISPR biosensor. ns: No statistical significance, Error bars represent SD (n = 3).

turnaround time, yet they rely on thermocyclers or specialized isothermal amplification devices and trained personnel [13]. Recently, several high-performance electrochemiluminescent and electrochemical biosensors targeting *B. pseudomallei* have achieved limits of detection in the 11 fM to 57 pM range, often within 120–180 min, by integrating CRISPR, nucleic-acid amplification and nanomaterial-modified electrodes [18,22,35]. While these approaches offer excellent analytical sensitivity, they require customized electrodes, potentiostats or ECL

analyzers and multi-step surface modification procedures. By contrast, the CRISPR/Cas12a-PGM biosensor reported here delivers slightly higher LOD but relies on no bespoke hardware or electrode fabrication; instead, it leverages mass-produced PGMs already available in primary and secondary healthcare settings. For applications where ease of deployment, cost and user familiarity outweigh marginal gains in sensitivity, such as screening, triage or surveillance in resource-limited settings, this trade-off is highly attractive.

From the broader POCT perspective, this work aligns with the recognized need for diagnostic tools that are affordable, sensitive, specific, user-friendly, rapid and robust (ASSURED) [13,30]. The use of a commercial PGM as the signal transducer is particularly advantageous, because these devices are low-cost, battery-powered, widely distributed and well accepted by both healthcare workers and patients. Moreover, translating nucleic-acid detection into a glucose readout allows direct use of the PGMs' built-in calibration, display and data-logging capabilities [30,34]. Nevertheless, the current CRISPR-PGM landscape still faces several unresolved challenges. Many reported systems depend on nucleic-acid amplification (LAMP, RAA, RPA) to push the LOD into the fM or aM regime, which increases assay complexity and the risk of contamination [20,29,31–33]. Sample preparation (e.g. nucleic-acid extraction from whole blood, sputum or environmental matrices) is often performed off-chip and manually. In addition, most assays are single-plex and optimized for one specific pathogen, with limited demonstrations of multiplexing or broad panel testing. Addressing these issues will be essential for translating CRISPR-PGM biosensors into truly routine, field-deployable diagnostics [34].

The present platform also has several intrinsic limitations that should be acknowledged. First, although the assay is amplification-free at the nucleic-acid level, it still involves multiple manual steps, including genomic DNA extraction, Cas12a reaction, magnetic separation, sucrose incubation and PGM readout. This workflow is simpler than many electrode-based CRISPR sensors, but it is not yet a fully integrated "sample-to-answer" system. Second, the current study only evaluated a modest number of clinical isolates and spiked serum samples; larger-scale clinical validation, as well as testing in more complex matrices such as whole blood, sputum or environmental water and soil, will be necessary to fully establish diagnostic performance and robustness. Third, while the analytical sensitivity is sufficient for many clinically relevant *B. pseudomallei* DNA levels, it remains lower than that of the best amplification-assisted CRISPR or electrochemiluminescent sensors [5,20,29,32,35], and further optimization of enzyme loading, probe design and reaction kinetics may narrow this gap. Finally, the current design is single-plex and tailored to the ORF2 target of *B. pseudomallei*; adapting the platform to detect multiple pathogens or resistance genes in parallel will require additional engineering of crRNA pools and signal decoding strategies.

Despite these limitations, the CRISPR/Cas12a-PGM biosensor described here offers a realistic path toward deployment in several application scenarios. In endemic regions with limited laboratory infrastructure, such as rural hospitals and community clinics in Southeast Asia and northern Australia, the platform could serve as a frontline screening or triage tool, complementing culture and qPCR by providing rapid, on-site risk assessment. In peripheral or field laboratories, the assay may be used for environmental surveillance of *B. pseudomallei* in soil and water, helping to map environmental reservoirs and guide public-health interventions. With further integration into microfluidic cartridges, pre-loaded lyophilized reagents and simplified sample preparation, the same sensing principle could be extended to home-based or self-testing formats for high-risk occupational groups. More generally, because the sensing architecture is determined by the crRNA sequence rather than the PGM itself, the platform is readily reconfigurable for other bacterial, viral or even non-infectious nucleic-acid targets, positioning it as a versatile CRISPR–PGM framework for next-generation point-of-care biosensing.

5. Conclusion

In conclusion, we have developed a CRISPR/Cas12a-PGM biosensing platform capable of detecting *B. pseudomallei* DNA rapidly, accurately, and cost-effectively. The assay can be completed within one hour without sophisticated instrumentation and shows excellent agreement with the gold-standard qPCR in clinical validation. Its portability, stability, and simplicity suggest its strong potential for POCT in both hospital and field settings. These analytical and practical features highlight a balanced compromise between sensitivity, simplicity and operational robustness, which is critical for real-world point-of-care deployment. Beyond providing an efficient tool for early melioidosis diagnosis, this work establishes a generalizable chemical/biosensing architecture for CRISPR-based signal transduction on consumer electronics. Because target recognition is encoded by the crRNA sequence rather than the hardware itself, the CRISPR/Cas12a-PGM platform can, in principle, be reprogrammed for a wide range of nucleic-acid targets by straightforward crRNA redesign. With further integration of sample preparation, reagent lyophilization and microfluidic automation toward true sample-to-answer operation, the proposed system has strong potential to evolve into a versatile, scalable sensing platform for decentralized molecular diagnostics in both clinical and field settings.

CRediT authorship contribution statement

Xiaoxia Xie: Methodology. **Nan Zhang:** Data curation. **Zhangmeng Liu:** Methodology. **Jian Luo:** Methodology, Data curation. **Yuping Ruan:** Writing – original draft, Methodology, Investigation, Data curation. **Dai Kuang:** Methodology. **Shen Tian:** Methodology. **Juan Yao:** Methodology, Investigation. **Xiaobing Wang:** Methodology. **Qianfeng Xia:** Writing – review & editing, Methodology, Funding acquisition. **Nini Luo:** Writing – original draft, Methodology. **Huangxian Ju:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No. 82370018), the National Natural Science Foundation of China's Regional Innovation and Development Joint Fund Project (U24A20747), and State Key Research Development Program of China (2022YFC2305000, 2022YFC2305003).

Data availability

Data will be made available on request.

References

[1] W.J. Wiersinga, H.S. Virk, A.G. Torres, B.J. Currie, S.J. Peacock, D.A.B. Dance, D. Limmathurotsakul, Melioidosis, Nat. Rev. Dis. Prim. 4 (1) (2018), https://doi.org/10.1038/nrdp.2017.107.

[2] B. Currie, Melioidosis: evolving concepts in epidemiology, pathogenesis, and treatment, Semin. Respir. Crit. Care Med. 36 (01) (2015) 111–125, https://doi.org/10.1055/s-0034-1398389.

[3] M. Picardeau, R.L. Houghton, D.E. Reed, M.A. Hubbard, M.J. Dillon, H. Chen, B. J. Currie, M. Mayo, D.S. Sarovich, V. Theobald, D. Limmathurotsakul, G. Wongsuvan, N. Chantratita, S.J. Peacock, A.R. Hoffmaster, B. Duval, P.J. Brett, M.N. Burtnick, D.P. AuCoin, Development of a Prototype Lateral Flow Immunoassay (LFI) for the Rapid Diagnosis of Melioidosis, PLoS Negl. Trop. Dis. 8 (3) (2014), https://doi.org/10.1371/journal.pntd.0002727.

[4] N. Somprasong, J.P. Hagen, J.W. Sahl, J.R. Webb, C.M. Hall, B.J. Currie, D. M. Wagner, P. Keim, H.P. Schweizer, A conserved active site PenA β-lactamase Ambler motif specific for Burkholderia pseudomallei/B. mallei is likely responsible

for intrinsic amoxicillin-clavulanic acid sensitivity and facilitates a simple diagnostic PCR assay for melioidosis, Int. J. Antimicrob. Agents 61 (3) (2023), https://doi.org/10.1016/j.ijantimicag.2023.106714.
[5] J.-X. Zhang, J.-H. Xu, B. Yuan, X.-D. Wang, X.-h Mao, J.-L. Wang, X.-L.-L. Zhang, Y. Yuan, Detection of Burkholderia pseudomallei with CRISPR-Cas12a based on specific sequence tags, Front. Public Health 11 (2023), https://doi.org/10.3389/fpubh.2023.1153352.
[6] C. Li, C. Liu, R. Liu, Y. Wang, A. Li, S. Tian, W. Cheng, S. Ding, W. Li, M. Zhao, Q. Xia, A novel CRISPR/Cas14a-based electrochemical biosensor for ultrasensitive detection of Burkholderia pseudomallei with PtPd@PCN-224 nanoenzymes for signal amplification, Biosens. Bioelectron. 225 (2023), https://doi.org/10.1016/j.bios.2023.115098.
[7] Y. Wang, R. Chen, B. Shen, C. Li, J. Chen, Y. Wang, S. Tian, X. Li, N. Luo, R. Liu, S. Ding, C. Zhu, Q. Xia, Electrochemiluminescent (ECL) biosensor for Burkholderia pseudomallei based on cobalt-doped MOF decorated with gold nanoparticles and N-(4-aminobutyl)-N-(ethylisoluminol), Microchim. Acta 189 (9) (2022), https://doi.org/10.1007/s00604-022-05402-6.
[8] J.S. Gootenberg, O.O. Abudayyeh, J.W. Lee, P. Essletzbichler, A.J. Dy, J. Joung, V. Verdine, N. Donghia, N.M. Daringer, C.A. Freije, C. Myhrvold, R. P. Bhattacharyya, J. Livny, A. Regev, E.V. Koonin, D.T. Hung, P.C. Sabeti, J. J. Collins, F. Zhang, Nucleic acid detection with CRISPR-Cas13a/C2c2, Science 356 (6336) (2017) 438–442, https://doi.org/10.1126/science.aam9321.
[9] E.M.Janice S. Chen, Lucas B. Harrington, Maria Da Costa, Xinran Tian, Joel M. Palefsky, Jennifer A. Doudna, CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity, Science 360 (6387) (2018) 436–439, https://doi.org/10.1126/science.aar6245.
[10] S.-Y. Li, Q.-X. Cheng, J.-M. Wang, X.-Y. Li, Z.-L. Zhang, S. Gao, R.-B. Cao, G.-P. Zhao, J. Wang, CRISPR-Cas12a-assisted nucleic acid detection, Cell Discov. 4 (1) (2018), https://doi.org/10.1038/s41421-018-0028-z.
[11] B. Wang, R. Wang, D. Wang, J. Wu, J. Li, J. Wang, H. Liu, Y. Wang, Cas12aVDet: a CRISPR/Cas12a-based platform for rapid and visual nucleic acid detection, Anal. Chem. 91 (19) (2019) 12156–12161, https://doi.org/10.1021/acs.analchem.9b01526.
[12] L.A. Morici, E.P. Price, D.S. Sarovich, J.R. Webb, J.L. Ginther, M. Mayo, J.M. Cook, M.L. Seymour, M. Kaestli, V. Theobald, C.M. Hall, J.D. Busch, J.T. Foster, P. Keim, D.M. Wagner, A. Tuanyok, T. Pearson, B.J. Currie, Accurate and rapid identification of the Burkholderia pseudomallei near-neighbour, Burkholderia ubonensis, using real-time PCR, PLoS ONE 8 (8) (2013), https://doi.org/10.1371/journal.pone.0071647.
[13] A.B.-M. ThomasR.Kozel, Point-of-care testing for infectious diseases past, present, and future, J. Clin. Microbiol. 55 (8) (2017) 2313–2320, https://doi.org/10.1128/JCM.00476-17.
[14] J. Zhang, Y. Lu, Biocomputing for portable, resettable, and quantitative point-of-care diagnostics: making the glucose meter a logic-gate responsive device for measuring many clinically relevant targets, Angew. Chem. Int. Ed. 57 (31) (2018) 9702–9706, https://doi.org/10.1002/anie.201804292.
[15] I. Strohkendl, F.A. Saifuddin, J.R. Rybarski, I.J. Finkelstein, R. Russell, Kinetic Basis for DNA Target Specificity of CRISPR-Cas12a, Mol. Cell 71 (5) (2018) 816–824.e3, https://doi.org/10.1016/j.molcel.2018.06.043.
[16] M. Rossetti, R. Merlo, N. Bagheri, D. Moscone, A. Valenti, A. Saha, Pablo R. Arantes, R. Ippodrino, F. Ricci, I. Treglia, E. Delibato, J. van der Oost, G. Palermo, G. Perugino, A. Porchetta, Enhancement of CRISPR/Cas12a trans-cleavage activity using hairpin DNA reporters, Nucleic Acids Res. 50 (14) (2022) 8377–8391, https://doi.org/10.1093/nar/gkac578.
[17] Y. Tanifuji, G. Tong, Y. Hiruta, D. Citterio, Paper-based analytical device for point-of-care nucleic acid quantification combining CRISPR/Cas12a and a personal glucose meter, Analyst 149 (19) (2024) 4932–4939, https://doi.org/10.1039/d4an00905c.
[18] T. Li, J. Wang, J. Fang, F. Chen, X. Wu, L. Wang, M. Gao, L. Zhang, S. Li, A universal nucleic acid detection platform combing CRISPR/Cas12a and strand displacement amplification with multiple signal readout, Talanta 273 (2024), https://doi.org/10.1016/j.talanta.2024.125922.
[19] C. Zhou, D. Huang, Z. Wang, P. Shen, P. Wang, Z. Xu, CRISPR Cas12a-based "sweet" biosensor coupled with personal glucose meter readout for the point-of-care testing of Salmonella, J. Food Sci. 87 (9) (2022) 4137–4147, https://doi.org/10.1111/1750-3841.16287.
[20] W. Ma, M. Liu, S. Xie, B. Liu, L. Jiang, X. Zhang, X. Yuan, CRISPR/Cas12a system responsive DNA hydrogel for label-free detection of non-glucose targets with a portable personal glucose meter, Anal. Chim. Acta 1231 (2022), https://doi.org/10.1016/j.aca.2022.340439.
[21] B. Fang, Z. Jia, C. Liu, K. Tu, M. Zhang, L. Zhang, A versatile CRISPR Cas12a-based point-of-care biosensor enabling convenient glucometer readout for ultrasensitive detection of pathogen nucleic acids, Talanta 249 (2022), https://doi.org/10.1016/j.talanta.2022.123657.
[22] R. Liu, Y. Hu, Y. He, T. Lan, J. Zhang, Translating daily COVID-19 screening into a simple glucose test: a proof of concept study, Chem. Sci. 12 (26) (2021) 9022–9030, https://doi.org/10.1039/d1sc00512j.
[23] H. You, J. Shan, Y. Bai, R. Yang, S. Tan, Y. Gu, B. Li, H. Wu, Y. Liu, CRISPR/Cas-powered "sweet" DNA nano-sponge for highly sensitive detection of pathogen nucleic acid with portable personal glucometer, Chem. Eng. J. 507 (2025), https://doi.org/10.1016/j.cej.2025.160618.
[24] M. Nakase, J. Thapa, V. Batbaatar, O. Khurtsbaatar, B. Enkhtuul, J. Unenbat, B. Lkham, S. Fujita, A. Koshikawa, A. Tuanyok, V. Saechan, H. Higashi, K. Hayashida, Y. Suzuki, C. Nakajima, T. Kimura, A novel ready-to-use loop-mediated isothermal amplification (LAMP) method for detection of Burkholderia mallei and B. pseudomallei, BMC Microbiol. 25 (1) (2025), https://doi.org/10.1186/s12866-024-03737-z.
[25] A.G. Torres, S.P. Wongpalee, H. Thananchai, C. Chewapreecha, H.B. Roslund, C. Chomkatekaew, W. Tananupak, P. Boonklang, S. Pakdeerat, R. Seng, N. Chantratita, P. Takarn, P. Khamnoi, Highly specific and sensitive detection of Burkholderia pseudomallei genomic DNA by CRISPR-Cas12a, PLOS Negl. Trop. Dis. 16 (8) (2022), https://doi.org/10.1371/journal.pntd.0010659.
[26] Shoujia Lin, J.W.Yingzi Lin, Guangming Li, N.L.Xiaotian Wu, Wenting Li, Chuanlong Zhu, Rui Liu, Qiqi Xu, Qianfeng Xia, Huangxian Ju, A ratiometric fluorescent biosensor for rapid detection of Burkholderia pseudomallei by dual CRISPR/Cas12a trans-cleavage assisted signal enhancement, Sens. Actuators B Chem. 379 (2023) 133204, https://doi.org/10.1016/j.snb.2022.133204.
[27] X. Wang, L. Wang, H. Zhu, C. Wang, X. Zhu, Reliable detection of Burkholderia pseudomallei using multiple cross displacement amplification label-based biosensor, BMC Microbiol. 22 (1) (2022), https://doi.org/10.1186/s12866-022-02485-2.
[28] J.S. Gootenberg, O.O. Abudayyeh, M.J. Kellner, J. Joung, J.J. Collins, F. Zhang, Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6, Science 360 (6387) (2018) 439–444, https://doi.org/10.1126/science.aaq0179.
[29] X. Zhu, S. Wang, Y. Xue, X. Wang, S. Hu, T. Liang, W. Liu, Coupling CRISPR-Cas and a personal glucose meter with an enzymatic reporter for portable detection of human papillomavirus in biological samples, Theranostics 15 (7) (2025) 2870–2882, https://doi.org/10.7150/thno.106490.
[30] H. Chen, K. Liu, Z. Li, P. Wang, Point of care testing for infectious diseases, Clin. Chim. Acta 493 (2019) 138–147, https://doi.org/10.1016/j.cca.2019.03.008.
[31] C.-H. Lau, Q.-L. Liang, X. Chen, J. Li, Y. Shi, Y. Huang, Q. Xia, H. Zhu, G. Chen, CRISPR/Dx-based colorimetric and electrochemical detection systems for POCT applications, Biosens. Bioelectron. 288 (2025), https://doi.org/10.1016/j.bios.2025.117778.
[32] Z. Jia, Z. Li, C. Liu, CRISPR-powered biosensing platform for quantitative detection of alpha-fetoprotein by a personal glucose meter, Sens. Actuators B Chem. 390 (2023), https://doi.org/10.1016/j.snb.2023.133994.
[33] M. Liu, W. Ma, Y. Zhou, B. Liu, X. Zhang, S. Zhang, A label-free photoelectrochemical biosensor based on CRISPR/Cas12a system responsive deoxyribonucleic acid hydrogel and "Click" chemistry, ACS Sens. 7 (10) (2022) 3153–3160, https://doi.org/10.1021/acssensors.2c01636.
[34] J. Li, C.-H. Lau, H. Zhu, CRISPR/Cas-based personal glucose meters for nucleic acid detection, Trends Biotechnol. (2025), https://doi.org/10.1016/j.tibtech.2025.07.006.
[35] M.J. Kellner, J.G. Koob, J.S. Gootenberg, O.O. Abudayyeh, F. Zhang, SHERLOCK: nucleic acid detection with CRISPR nucleases, Nat. Protoc. 14 (10) (2019) 2986–3012, https://doi.org/10.1038/s41596-019-0210-2.

Nini Luo is currently working at the School of Life Sciences and Medical Technology, Hainan Medical University. Her main research focuses on the screening of diagnostic markers for intracellular bacteria and the development of detection methods.

Yuping Ruan graduated from the School of Tropical Medicine and Laboratory Medicine, Hainan Medical University with a master's degree in 2025. Her research direction is clinical diagnostic technology and biosensors.

Jian Luo is currently a lecturer at the School of Life Sciences and Medical Technology, Hainan Medical University. Her research mainly focuses on pathogen–host interactions.

Shen Tian is currently working at the School of Life Sciences and Medical Technology, Hainan Medical University. His main research interest is the pathogenicity of melioidosis and phage therapy.

Dai Kuang is currently a faculty member at the School of Life Sciences and Medical Technology, Hainan Medical University. His main research interests include: (1) bacterial resistance and pathogenic mechanisms; (2) rapid detection of pathogenic bacteria and phage control; (3) discovery of emerging infectious diseases and molecular epidemiological surveillance.

Nan Zhang is currently working at the School of Life Sciences and Medical Technology, Hainan Medical University. Her major research focuses on the drug resistance mechanism of *Burkholderia pseudomallei*.

Xiaoxia Xie is currently working at the School of Life Sciences and Medical Technology, Hainan Medical University. Her research fields involve the design and application of biosensors.

Zhangmeng Liu is currently working at the School of Life Sciences and Medical Technology, Hainan Medical University. His research fields mainly involve functional and environmentally friendly green energy materials.

Xiaobing Wang is currently working at the School of Life Sciences and Medical Technology, Hainan Medical University. His main research interests the structure and interface design of weak-electricity-driven nanozymes.

Juan Yao is currently working in the Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University. Her research interest is the development of biosensing strategy for clinical diagnosis.

Qianfeng Xia received his BS，MS and PhD degrees from College of Laboratory Medicine, Chongqing Medical University, and then became associate professor and professor in School of Life Sciences and Medical Technology, Hainan Medical University. His research interests focus on the novel methods and key technologies for clinical molecular diagnostic testing,tropical disease prevention and control.He has published more than 50 papers as the first or coresponding author.

Huangxian Ju received his BS, MS and PD degrees from Nanjing University during1982–1992.He was a postdoc in Montreal Usiversity (Canada) in 1996–1997.He became an associate and full professor of Nanjing University in 1993 and 1999.He is currently the director of State Key Laboratory of Analytical Chemistry for Life Science, and Fellows of the International Society of Electrodhemistrand the Royal Society of Chemistry. He won the National Funds for National Distinguished Young Scholars in2003 and was selected as a Changjiang Professor by Education Minisry of China in 2007.His research interests focus on analytical biochemistry,biosensingand molecular diagnosis.He has published 13 books and 836 papers in different journals with SClE h-index of 102(Google Scholar h- index 111 with more than 47000 citations)