REVIEW ARTICLES

Trends in the development of diagnostic methods for detecting *Burkholderia pseudomallei* (2010-2025): A global and Vietnamese perspective

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ABSTRACT

Objectives: To describe the global and national trends in the development of diagnostic methods for detecting *Burkholderia pseudomallei (B. pseudomallei)* and to analyze the diagnostic performance of these detection methods during the period 2010-2025.

Subjects: Published studies and reports on diagnostic methods for *B. pseudomallei* conducted worldwide and in Vietnam from 2010 to 2025.

Methods: A systematic literature review was performed using PubMed and Google Scholar databases. A total of 34 eligible studies were included. Data was synthesized and analyzed using Microsoft Excel and Google Sheets and reference management was conducted with Zotero 7.0.

Results: Between 2010 and 2025, diagnostic approaches for *B. pseudomallei* were classified into four main categories: Culture-based methods, molecular biology techniques, Matrix-Assisted Laser Desorption/Ionization - Time of Flight (MALDI-TOF) mass spectrometry, and immunological assays. Immunoassays were the most widely applied due to their speed and low cost, though sensitivity and specificity varied. Culture-based methods remained indispensable as the diagnostic gold standard, despite moderate sensitivity and longer turnaround time. Molecular biology techniques, particularly Polymerase Chain Reaction (PCR) and Loop-Mediated Isothermal Amplification (LAMP) achieved high sensitivity and specificity, capable of detecting *B. pseudomallei* at very low DNA concentrations. MALDI-TOF mass spectrometry demonstrated nearly perfect accuracy in species identification from cultured colonies.

Conclusions: Diagnostic methods for *B. pseudomallei* have progressively evolved toward more rapid and precise technologies. Immunoassays are appropriate for preliminary screening, while culture remains essential for confirmation. Molecular biology- and MALDI-TOF-based techniques provide high accuracy and reliability, representing the future direction for diagnostic standardization and clinical application.

Keywords: *B. pseudomallei*, diagnostic methods, melioidosis, global, Vietnamese.

INTRODUCTION

Melioidosis is a life-threatening infectious disease caused by the Gram-negative bacterium *Burkholderia pseudomallei (B. pseudomallei)*. The organism is naturally found in soil and surface water, particularly in Southeast Asia and Northern Australia, where it represents a major cause of community-acquired pneumonia and sepsis. Despite appropriate antimicrobial therapy, the case fatality rate remains high-up to 40% in some regions (1,2). Clinical manifestations are highly diverse and often mimic tuberculosis

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or other bacterial infections, making early and accurate diagnosis essential (3).

Isolation and identification of *B. pseudomallei* from clinical specimens remain the gold standard for definitive diagnosis. However, culture-based methods show limited sensitivity (approximately 60%) and require 2–7 days to produce results, a delay that can be fatal in severe cases (4,5). Furthermore, bacterial culture and identification demand biosafety level 2 (BSL-2) facilities, trained personnel, and specialized equipment resources that are often scarce in low- and middle-income countries, including Vietnam (3).

To address these limitations, several diagnostic approaches have been developed or optimized since 2010. These can be broadly classified into four major categories: Culture-based methods, molecular biology techniques, Matrix-Assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF) mass spectrometry, and immunological assays. Culture-based methods, notably MALDI-TOF mass spectrometry, which enables rapid and accurate bacterial identification within minutes (6). Molecular biology techniques, such as Real-time Polymerase Chain Reaction (PCR) and loop-mediated isothermal amplification (LAMP), which allow rapid detection of bacterial DNA with high sensitivity and specificity (7,8). Immunological assays, including lateral flow immunoassays (LFI) targeting capsular polysaccharide (CPS) or hemolysin co-regulated protein 1 (Hcp1) antigens, providing results within 15-30 minutes, as well as serological methods such as Enzyme-Linked Immunosorbent Assay (ELISA) and indirect hemagglutination assays (IHA), which continue to be refined (9-11).

In Vietnam, reported cases of melioidosis have been increasing, particularly in central provinces, suggesting that the disease burden is likely underestimated. Nevertheless, diagnostic capacity at primary healthcare levels remains limited (12). Although, several domestic studies have investigated PCR-, LAMP-, and immunoassay-based methods; large-scale implementation remains challenging due to infrastructure and resource constraints.

To provide a comprehensive and updated overview of current diagnostic practices, we conducted this systematic review to describe the global and national trends in the development of diagnostic methods for detecting *B. pseudomallei* and to analyze the diagnostic performance of these detection methods during the period 2010-2025.

RESEARCH METHODS

Subjects

The review included peer-reviewed articles, conference reports, and professional proceedings evaluating diagnostic assays for *B. pseudomallei* detection. Eligible studies, both international and Vietnamese, were selected based on the predefined inclusion criteria.

Search Strategy

Databases

Relevant studies were identified through two major online medical databases: PubMed and Google Scholar covering all publications up to August 31, 2025.

Keywords for English-language literature, searches were performed in PubMed using the following keywords in the title or abstract fields: *"Burkholderia pseudomallei"*, *"assay"*, *"melioidosis"*, *"diagnosis"*, *"testing"*, *"method"*, and *"value"*.

Advanced search filters and Boolean operators were applied to construct the combined search strategy as follows:

(*Burkholderia pseudomallei*[Title/Abstract] AND testing[Title/Abstract]) OR (*Burkholderia pseudomallei*[Title/Abstract] AND diagno*[Title/Abstract])

OR (*Burkholderia pseudomallei*[Title/Abstract] AND method[Title/Abstract]) OR (melioidosis[Title/Abstract] AND diagno*[Title/Abstract]) OR (melioidosis[Title/Abstract] AND testing[Title/Abstract]) OR (melioidosis[Title/Abstract] AND diagno* value[Title/Abstract]) OR (melioidosis[Title/Abstract] AND assay[Title/Abstract])

For Vietnamese-language literature, the Google Scholar database was searched using the following keywords: ("Xét nghiệm" OR "Phát hiện" OR "Chẩn đoán") AND ("*Burkholderia pseudomallei*" OR "Melioidosis" OR "Whitmore")

Document Selection

All titles and abstracts retrieved from the database search were independently screened by three reviewers from Nghe An Friendship General Hospital, Nghe An Mental Hospital and Quang Tri General Hospital to determine eligibility based on the predefined inclusion and exclusion criteria. Any discrepancies in study selection were resolved through discussion and consensus.

The reference management and citation tracking were conducted using Zotero version 7.0.

Inclusion and exclusion criteria

Inclusion Criteria

Studies, articles, or reports that presented diagnostic methods for detecting *B. pseudomallei* published between January 1, 2010, and August 31, 2025 were included. Eligible publications were required to be available in English or Vietnamese and to involve human specimens or clinically relevant samples.

Exclusion Criteria

Studies were excluded if they lacked full-text access, were not published as original research articles, or had unverifiable sources and unclear research subjects (non-human studies). Publications in languages other than English or Vietnamese were also excluded.

Document selection and management method

All identified records underwent a three-stage screening process. **Round 1:** Three independent reviewers performed research using the same predefined keywords. Title and abstract were assessed, and studies not meeting the inclusion criteria were excluded; **Round 2:** Only studies selected by at least two reviewers proceeded to the next round; **Round 3:** Full-text versions of the remaining studies were retrieved and reviewed in detail. Each article was discussed collectively by all three reviewers, and inclusion was confirmed when at least two reviewers agreed on eligibility. To ensure completeness, the reference lists of all included studies were also screened to identify any additional relevant publications (Figure 1).

Document screening results

A total of 1,091 records were initially identified from the databases with 993 from PubMed and 98 from Google Scholar. After three rounds of screening, 34 studies met the inclusion criteria and were included in the synthesis (31 in English and 3 in Vietnamese). Among the excluded articles, 229 lacked full-text access, 5 were in languages other than English or Vietnamese, and 28 were excluded after full-text review due to irrelevance, absence of diagnostic data, broken links, or non-human sample types.

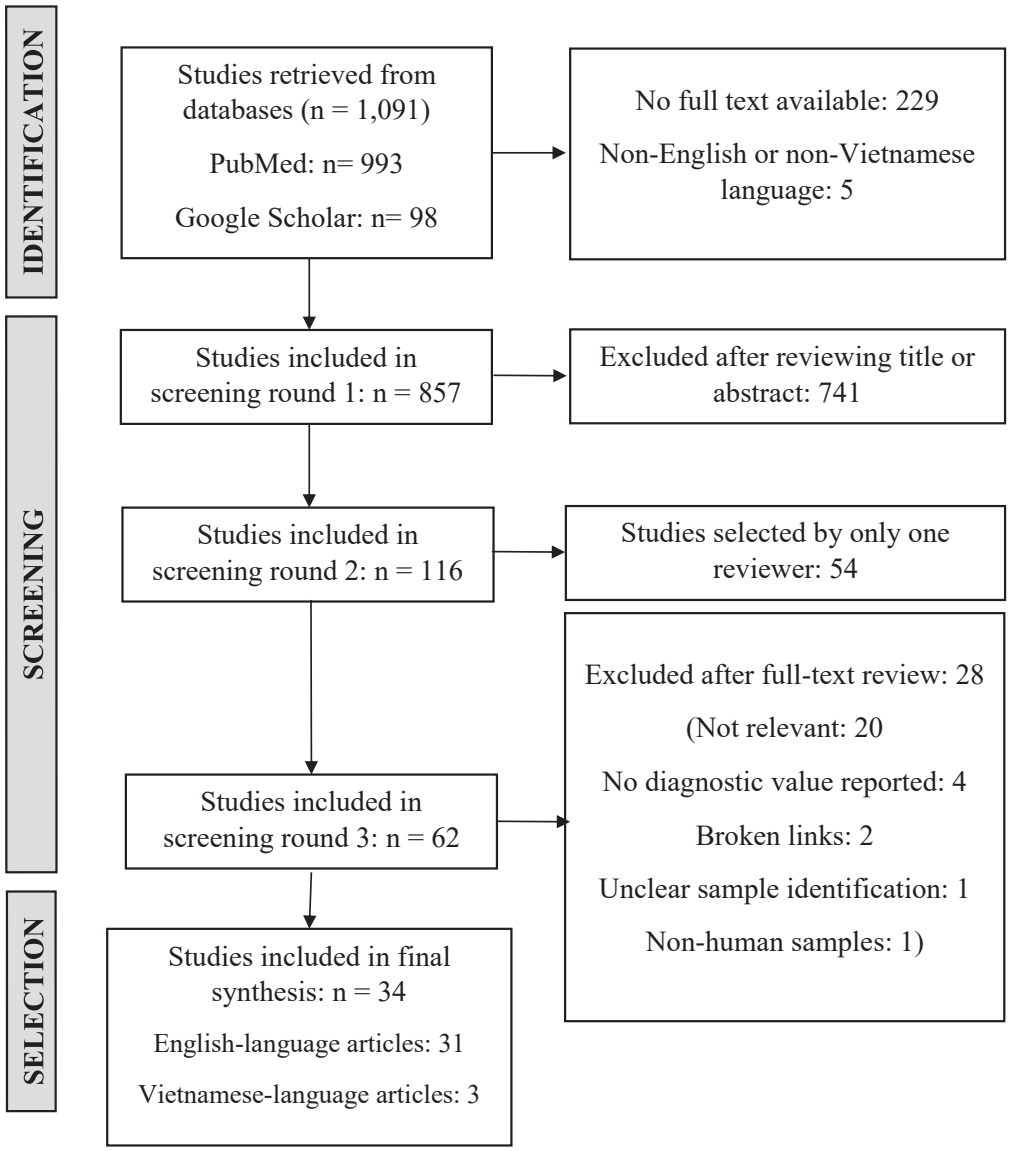

Figure 1. Flow diagram of the search and selection process for studies included in the systematic review.

Characteristics of the selected documents

Among the 34 articles selected for the synthesis of research findings on *B. pseudomallei* detection methods during the period 2010-2025, 31 were published in English and three in Vietnamese (Figure 1).

Data Analysis

All extracted data were descriptively analyzed using Microsoft Excel and Google Sheets. Comprehensive summary tables were compiled to outline the key study features, diagnostic methodologies, and performance parameters including sensitivity, specificity, and limit of detection.

RESULTS

Trends in diagnostic methods for detecting *B. pseudomallei* (2010-2025)

Table 1. Diagnostic methods for the detection of *Burkholderia pseudomallei* (*B. pseudomallei*) worldwide (2010-2025)

Method	Location	Year	Reference
Rapid test	Thailand	2014	Houghton, et al. (9)
	India	2018	Shaw T, et al. (13)
	Cambodia	2018	Peeters M, et al. (14)
	Thailand	2018	Wongsuvan G, et al. (15)
	Thailand	2018	Phokrai P, et al. (10)
	Laos	2018	Woods KL, et al. (16)
	Laos	2019	Rizzi MC, et al. (17)
	Thailand	2020	Wagner GE, et al. (11)
	Thailand	2022	Amornchai P, et al. (18)
ELISA	Malaysia	2013	Hara Y, et al. (19)
	Thailand	2016	Suttisunhakul V, et al. (20)
	Malaysia	2017	Hii SYF, et al. (21)
	Thailand	2021	Yatsomboon A, et al. (22)
	Thailand	2022	Wajanarogana S, et al. (23)
Immunofluorescence	Thailand	2013	Tandhavanant S, et al. (24)
	Thailand	2013	Chantratita N, et al. (25)
	Thailand	2016	Dulsuk A, et al. (26)
	Australia	2023	Settles EW, et al. (27)
Latex agglutination	Thailand	2014	Duval BD, et al. (28)
	Thailand	2015	Suttisunhakul V, et al. (20)
	Thailand	2021	Muangsombut V, et al. (29)
PCR	Thailand	2012	Price EP, et al. (8)
	Malaysia	2019	Ali MRM, et al. (30)
	India	2024	Yadav PK, et al. (31)
MALDI-TOF	Australia	2019	Gassiep I, et al. (6)
MALDI-TOF, Culture, Rapid test	Australia	2024	Campbell S, et al. (32)
LAMP, Rapid test	China	2022	Wang X, et al. (33)
PCR, LAMP	Malaysia	2020	Chua KH, et al. (34)
Rapid test, ELISA	Thailand	2021	Amornchai P, et al. (35)
Rapid test, ELISA, PCR	Thailand	2023	Noparatvarakorn C, et al. (36)

Table 1 summarizes diagnostic methods for detecting *B. pseudomallei* globally between 2010 and 2025. Analysis of 30 international studies revealed that diagnostic research during this period primarily focused on four major methodological categories. Immunological assays received the greatest attention, appearing in 25 of 30 studies, most of which centered on developing rapid diagnostic tools. The LFI was the most frequently investigated

technique, reported in 13 studies conducted mainly in Southeast Asia between 2014 and 2020. The ELISA method was utilized in seven studies from Thailand and Malaysia (2016–2023), while immunofluorescence assays (IFA) and latex agglutination tests were less common, reported in four and three studies, respectively, primarily from Thailand. Especially between 2020 and 2023, molecular biology techniques expanded markedly, with seven studies employing PCR and real-time PCR as the principal tools, and three studies evaluating isothermal amplification methods such as LAMP and multiple cross displacement amplification. Despite ongoing innovation, three studies (2021–2024) reaffirmed that culture-based methods remain the diagnostic gold standard. Additionally, MALDI-TOF mass spectrometry, examined in two studies (2019 and 2024), demonstrated high accuracy for direct species identification from cultured colonies.

Table 2. Diagnostic methods for the detection of *Burkholderia pseudomallei* (*B. pseudomallei*) in Vietnam (2010-2025).

Method	Location	Year	Reference
ELISA	Bac Giang, Nghe An, Ha Tinh, Binh Dinh	2020	Tran Thi Le Quyen, et al. (37)
PCR, LAMP	Nghe An	2020	Bui Thi Lan Anh, et al. (38)
Culture	Nghe An, Ha Tinh	2018	Trinh TT, et al. (12)
	Ha Tinh	2020	Hoang Viet Ha, et al. (39)

In Vietnam, research on *B. pseudomallei* diagnostics was primarily concentrated during 2018–2020, with four studies meeting the inclusion criteria (Table 2). These comprised two culture-based studies, one molecular study, and one ELISA-based study. Notably, all studies were conducted in the central region of Vietnam, an area recognized as endemic for *B. pseudomallei*. The findings reflect the limited but regionally focused diagnostic research efforts in the country, with a predominant reliance on conventional culture methods and emerging applications of molecular and immunological techniques for confirmatory and surveillance purposes.

In summary, between 2014 and 2020, rapid diagnostic tests represented the most extensively investigated approaches in Southeast Asian countries, followed by ELISA-based methods, which were widely applied from 2016 to 2023. After 2020, there was a marked expansion in the development and application of molecular biology techniques, particularly PCR and LAMP, reflecting the growing emphasis on rapid and sensitive molecular diagnostics. Nevertheless, despite notable technological advancements, culture-based methods continued to be explored and validated by several researchers, reaffirming their fundamental and irreplaceable role as the gold standard for *B. pseudomallei* detection.

Diagnostic Performance of Methods for Detecting *B. pseudomallei* (2010-2025)

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Table 3. The value of methods for detecting *B. pseudomallei*

Method	Sample size	Sensitivity (%)	Specificity (%)	Reference
Rapid test	113	98.7	97.2	Houghton RL, et al. (9)
	63	85.7	93.6	Shaw T, et al. (13)
	295	96.5	100	Peeters M, et al. (14)
	758	31.3	98.8	Wongsuvan G, et al. (15)
	986	88.3	86.1-100	Phokrai P, et al. (10)
	893	47-100	100	Woods KL, et al. (16)
	250	65.4	87.2	Rizzi MC, et al. (17)
	235	92	97	Wagner GE, et al. (11)
	614	27	97	Amornchai P, et al. (18)
ELISA	68	65	99	Hara Y, et al. (19)
	419	71.6	95.7	Suttisunhakul V, et al. (20)
	258	76.1-84.7	90.2-93.6	Hii SYF, et al. (21)
	94	93.7	84.4	Tran Thi Le Quyen, et al. (37)
	20	44.5	~100	Yatsomboon A, et al. (22)
	220	94.7	95.1	Wajanarogana S, et al. (23)
Immunofluorescence	88	48.4	99.8	Tandhavanant S, et al. (24)
	541	97.4	100	Chantratita N, et al. (25)
	545	100	99.6	Dulsuk A, et al. (26)
	188	90	93	Settles EW, et al. (27)
Latex agglutination	146	98.7	97.2	Duval BD, et al. (28)
	342	69.5-84.4	56.9-63.8	Suttisunhakul V, et al. (20)
	300	98	83	Muangsombut V, et al. (29)
PCR	2332	99.9-100	99.7-100	Price EP, et al. (8)
	20	86.3	100	Ali MRM, et al. (30)
	65	100	100	Yadav PK, et al. (31)
PCR, LAMP	121	97.5	PCR: 100 LAMP: 95	Bui Thi Lan Anh, et al. (38)
Culture	94	Accuracy: 100		Trinh TT, et al. (12)
	169	Accuracy: 100		Hoang Viet Ha, et al. (39)
MALDI-TOF	40	100	98	Gassiep I, et al. (6)
MALDI-TOF, PCR, Culture, Rapid test	43	MALDI-TOF: 100 Culture: 83 qPCR(*): 100 Rapid test: 100	MALDI-TOF: 90 Culture: 88 qPCR: 100 Rapid test: 100	Campbell S, et al. (32)
LAMP, Rapid test	38	100 fg DNA	100	Wang X, et al. (33)
PCR, LAMP	122	100	95-100	Chua KH, et al. (34)
Rapid test, ELISA	694	67.7	95	Amornchai P, et al. (35)
Rapid test, ELISA, PCR	173	Rapid test: 74.5 TTS1-PCR: 78.2 Combined: 98.2	Rapid test: 89.8 TTS1-PCR: 100 Combined: 89.8	Noparatvarakorn C, et al. (36)

**qPCR: quantitative real-time polymerase chain reaction*

Between 2010 and 2025, based on the analysis of 34 studies presented in Table 3, various diagnostic techniques for *B. pseudomallei* were evaluated across different methodological groups. Three studies assessed the diagnostic value of the culture method combined with bacterial identification using biochemical characteristics on the Vitek 2 automated system, while two others focused on antibiotic susceptibility testing for bacterial identification. The biochemical identification approach achieved 83% sensitivity and 88% specificity, whereas the antibiotic susceptibility-based technique demonstrated 100% accuracy in detecting *B. pseudomallei*.

As demonstrated in Table 3, the immunological assay (rapid tests, ELISA, IFA, and latex agglutination) was the most extensively studied, representing 26 out of 34 studies. Thirteen studies focused on rapid diagnostic tests, targeting antigenic proteins such as Hcp1, CPS, and OPS in sample. Five studies employed LFI on culture-enriched blood samples, reporting sensitivities ranging from 96.5% to 100% and specificities from 97.2% to 100% (9,14,16,32,33). Three studies evaluated LFI for detecting Hcp1 antibodies in serum, with sensitivities of 74.5%-92% and specificities of 89.8%-97% (10,11,36). In contrast, the remaining five studies applied LFI directly to clinical specimens for antigen detection and observed considerably lower performance, with sensitivities of only 27%-31% for blood samples. Sensitivity for urine specimens ranged from 65.4% to 85.71%; however, the interpretability of these findings is limited by the small sample sizes in these studies (<100 specimens). One study demonstrated that combining a rapid test with ELISA improved sensitivity from 31.3% to 67.7%, with only a minor reduction in specificity (98%-95%).

Among the eight studies using ELISA-based methods, seven studies evaluated serological assays for the diagnosis of *B. pseudomallei* infection, focusing on the detection of antibodies against Hcp1, rGroEL-FLAG300, and O-polysaccharide (OPS). The sensitivity of assays detecting anti-Hcp1 antibodies ranged from 70.9% to 93.7%, while specificity ranged from 84.4% to 99% (19,36,37). For ELISAs targeting anti-OPS antibodies, reported sensitivities were 69.1%-71.6% and specificities were 89.8%-95.7% (20,35,36). Additionally, one study using an ELISA to detect anti-rGroEL-FLAG300 antibodies demonstrated high diagnostic performance, with a sensitivity of 94.74% and specificity of 95.05% (23). Another study employed an ELISA to detect exopolysaccharide antigen in blood samples; however, this assay showed low sensitivity (44.5%) despite achieving 100% specificity, based on a sample size of 20 cases (22).

Furthermore, IFA were evaluated in four studies. Three of them used monoclonal antibody-based IFA (Mab-IFA) and demonstrated outstanding performance, with sensitivities of 97.4%-100% and specificities of 99.6%-100% when applied to cultured blood samples. In contrast, one study that directly tested clinical specimens (respiratory fluids, urine, pus, and body fluids) reported a lower sensitivity of 48%, though specificity remained high (99.8%). Another study utilizing a multiplex bead-based immunofluorescence system (MAGPIX, Luminex) for IgG and IgM detection achieved 90% sensitivity and 93% specificity. Especially, latex agglutination assays were investigated in three studies. Two studies testing *B. pseudomallei* from cultured colonies achieved sensitivities of 98% and 98.7%, and specificities of 83% and 97.2%, respectively. Another study detecting antibodies in patient serum reported 69.5% sensitivity for CPS-latex and 84.4% for OPS-latex, with corresponding specificities of 63.8% and 56.9%.

Molecular diagnostic methods including PCR, real-time PCR, and LAMP consistently demonstrated high specificity (95%-100%) and excellent sensitivity. Detection limits ranged from 0.2 to 0.688 pg/µL for PCR assays, while LAMP achieved a lower limit of 68.8 fg/µL, approximately ten times more sensitive than conventional PCR (Table 3).

Finally, the MALDI-TOF mass spectrometry technique was evaluated in two studies for bacterial identification from cultured colonies. Both reported nearly absolute sensitivity (100%) and specificity between 90% and 100%, depending on the database configuration used for spectral matching (Table 3).

DISCUSSION

Trends in diagnostic methods for detecting *B. pseudomallei* (2010-2025)

Melioidosis, caused by *B. pseudomallei*, remains a major public health concern in endemic regions, particularly in Southeast Asia and northern Australia. Early and accurate diagnosis is essential for reducing mortality, and consequently, the period from 2010 to 2025 has witnessed rapid advancement and diversification in diagnostic technologies worldwide. Analysis of 30 international studies revealed a clear shift from traditional, time-consuming diagnostic approaches toward faster, more sensitive, and field-deployable technologies. The most notable progress was observed in immunological methods, which accounted for 25 of the 30 studies published during this period, underscoring a global emphasis on developing point-of-care diagnostic tools. Among them, LFI were the most extensively investigated, particularly between 2014 and 2020. From 2016 to 2022, research interest expanded to ELISA-based assays, while IFA and latex agglutination techniques were evaluated only sporadically between 2013 and 2023. During 2020–2023, molecular diagnostic techniques gained significant traction, with increasing applications on PCR, real-time PCR, and LAMP, demonstrating the transition toward modern molecular tools suitable for clinical practice. Despite these advancements, culture-based methods continued to be utilized in studies from 2021 to 2024, reaffirming their irreplaceable role as the diagnostic reference standard. Additionally, the introduction of MALDI-TOF mass spectrometry marked a new phase in integrating high-throughput analytical technologies into microbiological identification.

In Vietnam, diagnostic research was primarily concentrated between 2018 and 2020, encompassing diverse methodological approaches including culture, ELISA, and molecular assays. All studies were conducted in the central region, where *B. pseudomallei* is endemic, reflecting a research effort closely aligned with field needs for melioidosis surveillance and diagnosis.

Diagnostic Performance of Methods for Detecting *B. pseudomallei* (2010-2025)

Traditionally, *B. pseudomallei* infection is diagnosed through bacterial culture, the recognized gold standard due to its ability to provide definitive identification and enable antimicrobial susceptibility testing. However, culture is limited by its long turnaround time (2-7 days) and requirement for biosafety level 2 laboratories with trained personnel (4,5). Molecular techniques, real-time PCR, have been the most extensively studied and applied molecular diagnostic method for *B. pseudomallei*. Assays targeting specific genes such as TTS1 have demonstrated near-perfect sensitivity and specificity (99%-100%) with extremely low detection limits, capable of identifying only a few DNA copies

per reaction (8,31,32). The high specificity of real-time PCR has been validated in clinical settings, and its utility has expanded through multiplex PCR assays that enable simultaneous detection of *B. pseudomallei* alongside other pathogens, such as *Leptospira spp.* (30,36). Despite its advantages, real-time PCR remained limited by its high cost and dependence on advanced laboratory infrastructure, which restrict its use in low-resource or field settings. To overcome these limitations, LAMP has been developed. These techniques eliminate the need for thermal cyclers, produce rapid results, and allow visual interpretation, making them highly suitable for point-of-care and field application (33,34).

In parallel, immunological assays targeting *B. pseudomallei* antigens or host antibodies have been optimized for rapid, on-site diagnosis. Among the earliest, latex agglutination offers a simple and fast preliminary identification method. While its accuracy is nearly perfect when applied to cultured colonies, sensitivity and specificity decrease substantially when used for serum antibody detection (20,28,29). ELISA has been widely adopted for detecting both antibodies (IgM, IgG) and antigens. ELISA assays detecting anti-Hcp1 and anti-OPS antibodies demonstrated moderate sensitivity and high specificity, suggesting their potential for implementation at primary healthcare levels (19,20,36,37). Notably, the anti–rGroEL-FLAG300 antibody exhibited superior diagnostic performance, indicating promise as a rapid screening tool; however, the current evidence is based on a single study, and further validation in larger populations is required (23). In contrast, direct detection of B. pseudomallei antigen in whole blood samples remains a major challenge. The study by Yatsomboon, et al reported a sensitivity of only 44.5%, highlighting considerable limitations for clinical application (22).

To further enhance accessibility and reduce turnaround time, LFI has been extensively evaluated (9,13,16). LFI provides simplicity and speed, though sensitivity remains limited, particularly in direct clinical specimens like blood or serum (15,17,18). Rapid tests used to detect *B. pseudomallei* in enriched culture samples may serve as a good option for rapid diagnosis while awaiting biochemical identification, as they demonstrate relatively high sensitivity (96.5%–100%) and specificity (97.2%-100%)(9,14,16,32,33). The LFI technique for antibody detection also shows reasonably good diagnostic performance, with sensitivity ranging from 74% to 92% and specificity from 89.8% to 97%, and can be applied directly to clinical specimens without the need for an additional 1–3 days of enrichment culture (10,11,36). Newer multiplex LFI formats incorporating multiple antibody targets have shown promise for improved diagnostic sensitivity (11). In contrast, rapid tests that directly detect antigens from clinical specimens generally demonstrate low sensitivity and considerable variability, largely depending on the bacterial concentration in the sample, and therefore are unlikely to serve as reliable screening tools (13,15,17,18,35).

In addition, other immunological techniques, including IFA, offer rapid results within a few hours and high specificity using monoclonal antibodies. Nevertheless, their sensitivity decreases with low bacterial loads, and reliance on fluorescence microscopy limits their application in resource-limited environments (24–27). In modern diagnostic laboratories, MALDI-TOF mass spectrometry has emerged as a reliable and rapid method for bacterial identification. Several studies confirm its ability to identify *B. pseudomallei* from cultured isolates with nearly 100% accuracy within minutes. Although, it requires prior culture and

a high initial investment, MALDI-TOF substantially reduces consumable costs and turnaround time compared with conventional biochemical identification (6,32).

Overall, no single diagnostic method provides complete reliability. Combining complementary techniques enhances diagnostic accuracy. For example, pairing rapid tests with confirmatory PCR or ELISA can increase overall sensitivity to over 98% (35,36). A tiered diagnostic approach is therefore recommended using rapid assay for preliminary screening at community and primary healthcare levels, followed by confirmatory culture, immunological, and molecular assays in reference laboratories equipped with appropriate biosafety and expertise. In Vietnam, the continued evaluation and adoption of emerging molecular and rapid diagnostic technologies such as LAMP and next-generation lateral flow assays, along with laboratory network strengthening and biosafety standardization, will be vital to improving melioidosis diagnosis and control.

The limitation of this review was primarily descriptive and did not include a formal assessment of methodological quality or risk of bias using standardized tools such as QUADAS-2, which may affect the reliability of the reported diagnostic performance estimates. In addition, the analysis was limited to sensitivity and specificity due to incomplete reporting in the primary studies, precluding evaluation of other clinically relevant metrics such as predictive values and likelihood ratios. Restriction to English-language publications introduces potential language bias, and substantial heterogeneity across studies prevented quantitative synthesis. Consequently, the findings should be interpreted as descriptive trends rather than definitive pooled estimates for clinical decision-making

CONCLUSION

Between 2010 and 2025, diagnostic methods for *B. pseudomallei* have progressed toward faster and more accurate detection. Immunological assays remain useful for rapid screening, while molecular techniques and MALDI-TOF mass spectrometry provide high diagnostic accuracy. Despite technological advances, culture continues to serve as the irreplaceable gold standard. Strengthening laboratory capacity and applying advanced tools in endemic regions like Vietnam are crucial for improving early diagnosis and disease control.

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