SCIENCE LETTERS

ISSN: 1675-7785
eISSN: 2682-8626

Available online at
http://myjms.mohe.gov.my/index.php/sl

Science Letters 20(2) 2026, 1-15

Science Letters

# Evaluation of lateral flow immunoassay for melioidosis detection targeted capsular polysaccharide of *Burkholderia pseudomallei* among orangutans in Bukit Merah Orang Utan Island, Perak, Malaysia

Nurul Iman Mohamad[1,2*], Farida Zuraina Mohd Yusof[1], Nurul Aili Zakaria[1], Zakuan Zainy Deris[2], Sabapathy Dharmalingam[3]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA*
[2]*Department of Medical Microbiology & Parasitology at Universiti Sains Malaysia*
[3]*Bukit Merah Orang Utan Island Foundation, Malaysia*

ARTICLE INFO

*Article history:*
Received 13 May 2025
Revised 19 May 2026
Accepted 19 May 2026
Published 30 June 2026

*Keywords:*
*melioidosis*
*Burkholderia pseudomallei*
*wildlife*
*orangutan*
*lateral flow immunoassay*
*BMOUI*

DOI:
10.24191/scl.v20i2.6428

ABSTRACT

Melioidosis, an infection caused by *Burkholderia pseudomallei*, is one of the most common infectious diseases in conservation centres. Numerous cases of misdiagnosis associated with melioidosis have been reported because this disease presents a broad range of clinical symptoms, making laboratory diagnosis challenging. Given the promising results of lateral flow immunoassay antigen detection in improving melioidosis diagnosis, this study was conducted to evaluate the potential of CPS-specific CABT-SQ001 (Creative Diagnostic) monoclonal antibody as a rapid point-of-care (POC) detection kit for melioidosis in veterinary settings. A lateral flow immunoassay (LFIA) targeting the capsular polysaccharide (CPS) of *B. pseudomallei* was performed using the CABT-SQ001 monoclonal antibody (mAB, Creative Diagnostics) conjugated onto universal LFIA strips (ab270537). The evaluation of the sensitivity and specificity of the LFIA in the laboratory was performed using a total of 85 bacterial isolates spiked in simulated whole blood specimens and a culture-confirmed *B. pseudomallei* blood specimen flagged by the Automated BD BACTEC™ FX Blood Culture System. Subsequently, the reactivity of the LFIA strips was further evaluated with the blood samples of two orangutans collected from the Bukit Merah Orang Utan Island (BMOUI). The sensitivity and specificity of the assay in blood specimens were 46.84% (37/79) and 77.78% (7/9), respectively. The LFIA demonstrated false-positive results when tested with blood specimens from two orangutans during field

[1*] Corresponding author. *E-mail address*: nuruliman_mohamad@yahoo.com
https://doi.org/10.24191/scl.v20i2.6428

evaluation at BMOUI. Given the limited sample size and observed false-positive results, these findings should be interpreted as preliminary trial. The assay demonstrated moderate sensitivity and specificity, indicating that further optimisation and validation are required before field application. This study provides exploratory insights into the development of point-of-care diagnostic for melioidosis in wildlife settings rather than a definitive validation of diagnostic performance.

## INTRODUCTION

Commented [MI1]: AQ2: Carefully check the core content of the manuscript, including the Title, Results, Tables, and Figures, to ensure no formatting or data errors occurred during typesetting.

Melioidosis, a neglected tropical disease caused by the opportunistic soil-borne pathogen *Burkholderia pseudomallei*, poses a significant threat in Southeast Asia, including Malaysia. While this disease is often recognised for its human impact, it also poses a substantial risk to wildlife, particularly to species such as orangutans. In Malaysia, melioidosis cases in animals are considered underreported, with only approximately 10% of cases reported annually at BMOUI (Hayashi *et al.,* 2018). Due to their close interaction with soil and water reservoirs, orangutans in conservation centres are vulnerable to *B. pseudomallei*, making melioidosis one of the leading infectious causes of death in these animals (Sprague and Neubauer, 2004). Documented across more than 50 species worldwide, melioidosis impacts biodiversity and conservation efforts, particularly in regions where wildlife and natural habitats overlap closely with human environments (Rachlin *et al*., 2019). In line with this, recent studies at BMOUI have expanded beyond behavioural observations to include physiological and health monitoring, reflecting the importance of integrated surveillance in improving captive management, animal welfare, and conservation outcomes (Nozmi *et al*., 2025).

In human medicine, serological tests have been commonly used for melioidosis detection; however, these are often inadequate in conservation and veterinary settings. Detecting antibodies through serology can only indicate exposure to the pathogen but fails to confirm active infection, as IgG antibodies may persist for years after an initial infection (Sermswan *et al.,* 2000). This limitation underscores the need for rapid and sensitive diagnostic tools that can confirm active infection in real-time, especially for animals with variable symptom presentations compared to humans. Lateral flow immunoassays (LFIAs) have gained attention as point-of-care testing (POCT) tools due to their portability, speed, and ease of use in field settings. However, current LFIA technologies for melioidosis have been primarily validated on human samples and exhibit wide ranges of sensitivity and specificity, which can vary depending on the type of sample used (Nualnoi *et al*., 2024; Houghton *et al*., 2014). The potential for false positives results and the necessity for precise reagent preparation can limit the effectiveness of these tools in resource-limited conservation centres (Najomtein *et al*., 2024; Currie *et al*., 2022). Nonetheless, the LFIA format remains one of the most promising diagnostic options for melioidosis detection in conservation settings, owing to its adaptability to field conditions and rapid results.

To address these limitations, this study aimed to evaluate and optimise LFIA specifically for detecting *B. pseudomallei* capsular polysaccharides in orangutans at the BMOUI conservation centre in Malaysia. This evaluation considered the unique diagnostic needs of animals, with an emphasis on achieving high sensitivity and specificity to distinguish active infections in conservation settings. Adapting LFIAs for use in animals presents unique challenges. Animal species, such as orangutans, may exhibit melioidosis symptoms that differ significantly from those in humans, complicating diagnostic efforts and increasing the risk of misdiagnosis or delayed treatment (Shaw *et al.,* 2018). Additionally, research to validate LFIA in animals has been limited, and no rapid diagnostic tests are specifically approved for detecting melioidosis in wildlife or conservation settings. This research and development gap underscores the urgency of developing diagnostics that not only work in human health contexts but are also validated for use in animals, particularly in high-risk species such as orangutans. Such diagnostics are vital for the early detection and management of melioidosis, enabling conservation centres to implement timely preventive strategies. However, this study is intended as an exploratory evaluation, and findings should be interpreted within the limitations of a preliminary investigation rather than as a fully validated diagnostic approach.

https://doi.org/10.24191/scl.v20i2.6428

## EXPERIMENTAL

### Ethical statements

Animal ethics approval was obtained through the Committee on Animal Research and Ethics of Universiti Teknologi MARA (UiTM CARE:289/2019) and Department of Wildlife and National Parks under research permit (JPH&TN[IP]:100-34/1.24 Jld.15[36]). The consent approval from BMOUI Foundation was obtained prior to the blood sample collection.

### Bacterial strains

The clinical isolates of *B. pseudomallei* obtained from the Department of Medical Microbiology and Parasitology, School of Medical Science, and the environmental isolates were retrieved from the Institute for Research in Molecular Medicine (INFORMM) of Universiti Sains Malaysia. The selected strains of *B. pseudomallei* labelled as BUPS/12/14/A and BUPS/19/13/A were based on the predominant genotypes in Malaysia, namely ST54 and ST46, respectively (Zueter, 2016). Meanwhile, the *B. cepacia* complex used in this study was isolated from BMOUI.

### Preparation of bacterial whole cell lysates

A single colony of *B. pseudomallei* was grown overnight in 10 mL of brain heart infusion (BHI) broth at 37°C in a shaker incubator until $OD_{600}$=1.0. The optical density of the bacterial suspension was determined by BioPhotometer (Eppendorf). Then, the bacterial suspension was centrifuged at 3500 rpm for 10 minutes at 4°C and the supernatant was discarded. The pellet was dissolved in 100 µL 1× phosphate buffer saline (PBS) and kept on ice. Next, the cell suspension was heat-killed at 100°C for 1 hour. Subsequently, the sample was centrifuged at 12 000 rpm for 5 minutes. The supernatant was carefully transferred into a new tube and kept on ice before loading. The lysate was stored at -20°C until further use.

### Antigen capture Enzyme-Linked Immunoassay (ELISA)

The presence of CPS, which is the most encouraging target, was detected in *B. pseudomallei* whole lysate by indirect semi-quantitative ELISA (Houghton *et al.,* 2014). Using a standard ELISA procedure, 100 µL of *B. pseudomallei* whole cell lysate in PBS was suspended in a flat-bottom 96-well microtiter plate (TPP). The plate was sealed and incubated for 2 hours at room temperature before being incubated overnight at 4°C. Then, the coating buffer was tapped off from the plate and washed thrice with 300 µL of PBS-Tween20 (PBS containing 0.5% Tween 20) on a shaking platform. The plate was flipped onto a paper towel to remove excess washing buffer. Next, the wells were blocked with 300 µL of 5% skimmed milk for 1 hour at 37°C. Subsequently, 100 µL of rabbit anti-CPS (CABT-SQ001) primary monoclonal antibody diluted 1:1000 in 1×PBS was added into the 96-well plate. Then the plate was sealed and incubated for 2 hours at 37°C. Then, the washing step was repeated. After that, 100 µL of goat anti-rabbit IgG-HRP (whole molecule) secondary antibody diluted 1:2000 in 1×PBS was added into the 96-well plate. The plate was sealed and incubated for 2 hours at 37°C. The plate was washed five times again on a shaking platform. Lastly, 100 µL of tetramethylbenzidine substrate (TMB) was added into the wells and incubated in the dark for 30 minutes, then 100 µL of hydrogen peroxide stop solution was added into the well. The colour changes of the solution in the wells were observed. The absorbance was read at 450nm wavelength using a spectrophotometer.

### SDS-PAGE and Western Immunoblotting

Briefly, 100 µL of whole cell lysate was suspended in 100 µL of SDS-PAGE sample buffer containing 95 µL of Laemmli buffer and 5 µL of β-mercaptoethanol. The sample was heated at 100°C for 5 minutes before being centrifuged at 12 000 rpm for 5 minutes prior to electrophoresis on 10% Mini-Protean® TGX precast gel. Subsequently, 20 µL of the supernatant was loaded into the wells of two precast gels. A broad-range pre-stained protein ladder was used as a standard to estimate the molecular mass of the target antigen

https://doi.org/10.24191/scl.v20i2.6428

separated on SDS-PAGE gel. A constant current of 25 mA and 150 V was set to run the electrophoresis for approximately 45 minutes until the dye front reached the bottom of the gel. Coomassie Brilliant Blue staining of the gel was performed according to the standard protocol.

Semi-dry western blot was performed immediately using another gel from the previous SDS-PAGE. The CPS of *B. pseudomallei* was blotted onto a nitrocellulose membrane and detected by antibody coupling. The same size of gel, nitrocellulose membrane and thick-blot papers were immersed in cold transfer buffer for 30 minutes. Then, the gel, nitrocellulose membrane and thick-blot papers were sandwich-assembled on the pre-wetted anode-based platform followed by the transfer blot system. The protein bands from the electrophoresed gel were transferred to the nitrocellulose membrane using the Semi-Dry Transfer System (Bio-Rad Laboratories GmbH, Germany). A constant voltage was applied at 15V and the transfer blotting was run for 30 minutes.

The membrane was then transferred and washed under distilled water and pre-stained with Ponceau S solution after the transfer to confirm the presence of the target antigen. Then, the nitrocellulose membrane was rinsed with distilled water to remove the Ponceau S stain and blocked in 10 mL of 5% skimmed milk for 1 hour at room temperature to minimize non-specific binding. Following the blocking, the membrane was washed thrice with 1× PBS-T20 for 10 minutes each time. The blotted proteins on the nitrocellulose membrane were probed with a series of antibodies. The nitrocellulose membrane was incubated with gentle agitation overnight in 10 mL primary antibody diluted to 1:1000 in 1× PBS. The next day, the membrane was washed again with 1× PBS-T20. Then, the membrane was incubated in 10 mL HRP-conjugated secondary antibody diluted to 1:2000 in 1× PBS for 2 hours on a shaking platform. The membrane was washed again with 1× PBS-T20 as described previously and the membrane was immersed in 1× PBS for another 15 minutes with gentle agitation on a shaking platform to remove Tween 20 detergent. The probed membrane was air-dried on filter paper prior to proceeding with target CPS detection. The membrane was immersed in freshly prepared 4-chloro-2-naphtol substrate solution with gentle agitation for around 15 minutes until the desired band was fully developed.

**Sample preparation and LFIA testing**

The universal lateral flow assay kit purchased from the Abcam company (ab270537) was used to detect the presence of target *B. pseudomallei* CPS in blood samples. The kit was optimized to develop a customized sandwich lateral flow assay by utilizing Lightning-Link® and gold conjugation. Prior to the immunoassay procedure, the reagents were allowed to sit at room temperature. The protocol from the manufacturer was followed accordingly with slight modification. Prior to universal LFIA strip application, the 40 nm Gold-Biotin 10 OD provided in the kit was diluted to 1 OD in 1× universal running buffer and 0.1% BSA. The blood sample (analyte) was 1:10 diluted in 1× universal running buffer and 0.1% BSA. For a single strip, 5 µL of diluted Ulfa-Tag conjugated antibody, 5 µL of 6 OD 40 nm Gold-detection antibody, 5 µL of 1 OD 40 nm of Gold-Biotin solution and 75 µL of diluted blood sample were mixed together in a 1.5 mL Eppendorf tube and incubated for 5 minutes. Then, 80 µL of the mixture was loaded into a well of a 96-well plate and one universal strip was inserted into each tested well. The strip was handled from the wicking pad and avoided touching the nitrocellulose membrane pad. The test was run for 30 minutes. The T-line colour intensity was compared using a scorecard. The visual observation was performed by three independent observers. Positive control LFIA tests were performed using suspensions containing *B. pseudomallei* culture, while negative controls consisted of buffer-only solutions. The appearance of both test and control lines was used to confirm assay validity and proper strip function.

Prior to LFIA testing, a limit of detection (LOD) study was conducted to determine the lowest detectable concentration of *B. pseudomallei* that could be reliably identified by the LFIA strips. This established the working detection range of the assay for subsequent testing. The determination of lower LOD of the LFIA strips was performed by testing different concentrations of *B. pseudomallei* culture (BUPS/12/14/A) serially diluted in PBS. The visual LOD was observed by naked eye on the appearance of the test line on the LFIA strip and the test line colour intensity shown by the strips was compared to the

https://doi.org/10.24191/scl.v20i2.6428

score reported on the scoring card provided by the manufacturer. From the observation, the lowest dilution factor detected by the LFIA in this study was $10^4$ meanwhile the test line was not detected at $10^5$ dilution factors. Thus, the dilution that detected the lowest CFU/mL was used in the preparation of LFIA blood samples.

**Reactivity testing of LFIA with spiked blood and positive blood culture bottle**

A total of 85 bacterial isolates, including *B. pseudomallei* clinical isolates (n = 74), environmental isolates (n = 2) and non-*B. pseudomallei* isolates (n = 9), were spiked in simulated whole blood specimens. Briefly, 200 µL of the overnight bacterial suspension was serially diluted 10-fold to $10^4$ CFU/mL and spiked into 2 mL of sterile blood specimens from anonymous donors. All spiked blood specimens and one culture-confirmed *B. pseudomallei* blood specimen (n = 1) flagged by the Automated BD BACTEC™ FX Blood Culture System were tested with LFIA strips. A single-proportion formula was used to calculate the sample size in this study based on the estimated seroprevalence of animal melioidosis in Malaysia (5.7%) as reported by Musa *et al*., 2012. Using a 95% confidence level (Z = 1.96) and a margin of error of 5% (d = 0.05), the calculated sample size was 82.5, which was rounded up to 83 (Arifin, 2017). After accounting for a 5% dropout rate, the minimum required sample size was 88.

**Reactivity testing with blood specimens of Orangutans from BMOUI**

The developed LFIA underwent preliminary assessment using two blood specimens from orangutans collected at BMOUI, which were interpreted as case observations. Sampling was limited to a single conservation centre. It was subject to regulatory and ethical constraints in Malaysia since blood collection in orangutans requires sedation, which is not recommended unless clinically necessary. Therefore, samples were collected opportunistically during routine health check-ups conducted twice yearly at BMOUI. All procedures adhered to the guidelines from the World Health Organization (WHO, 2014) and International Union for Conservation of Nature (IUCN, 1989) with the help of a veterinary officer from BMOUI. For every procedure, the orangutans were given sedative Zoletil™ for intramuscular injection containing a combination of zolazepam and tiletamine, with a dosage of 2.7 mg/kg per body weight prior to blood collection for safety purposes. The collected blood was transferred into EDTA tubes. An aliquot of 100 µL from the blood was transferred into an Eppendorf tube for immediate lateral flow immunoassay testing. Then, the tubes were stored and shipped in the cold packs to keep the specimen at 4°C (WHO, 2014). In the laboratory, the collected blood was cultured on agar media to observe any bacterial and fungal growth. Then, the rest of the blood collected from the orangutans was transferred into blood culture bottles and was incubated at 37°C for five days in an automated BD BACTEC™ blood culture system to confirm any infection.

## RESULTS AND DISCUSSION

**ELISA for determination of antigen-antibody binding**

ELISA test was performed to confirm the antigen-antibody binding prior to detection of the CPS antigen of *B. pseudomallei* in the LFIA test. In this study, two concentrations of CPS mAb were tested. It showed that the optimal ratio for CPS mAb dilution was at 1:1000 and the results were demonstrated in Fig. 1. From the spectrophotometry analysis measured at 450nm wavelength, the mean value (± SD) of absorbance reading for each well were 0.0515 (± 0.0021), 0.287 (± 0.0113), 0.0615 (± 0.0064) and 0.582 (± 0.0481) respectively with the *p*-value of 0.001.

https://doi.org/10.24191/scl.v20i2.6428

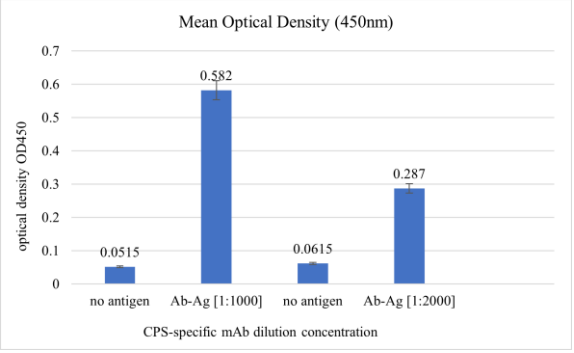


Fig. 1. Absorbance intensity at 450nm for ELISA plate using two concentrations of CPS-specific mAb.

The CPS was expected to be the major virulence determinant to be detected in melioidosis patients' samples. Numerous studies have shown that CPS can increase the lethal dose in a murine model, supporting its role as an antigenic determinant in this context (Burtnick *et al.,* 2018; Scott *et al.,* 2014). Multiple studies have been conducted in other centres to explore the potential of *B. pseudomallei* antigens, reporting a range of sensitivity and specificity values (Anandan *et al*., 2010). For example, Hii *et al*. (2017) reported that the sensitivity and specificity of an ELISA conducted in their study were high at 84.71% and 93.64%, respectively, when using the whole-cell antigen of *B. pseudomallei*.

**Analysis of Western Immunoblotting**

Western immunoblotting was performed to investigate the reactivity of mAb CPS against three strains of *B. pseudomallei* and two strains of relatively close *Burkholderia* species, namely *B. thailandensis* and *B. cepacia*. Fig. 2(A) demonstrates the presence of CPS antigen in three strains of *B. pseudomallei* lysates samples labelled as BUPS/19/13/A, BUPS/12/14/A and SP32285/S1 at approximately 200kDa. Fig. 2(B) confirmed the absence of CPS antigen in *B. cepacia* (lane 2) and *B. thailandensis* (lane 3) as only lane 1, which was loaded with whole lysate of *B. pseudomallei* showed the presence of CPS.

Multiple laddering bands were also observed on the SDS-PAGE gel, indicating the presence of other proteins in *B. pseudomallei* whole cell lysates. Meanwhile, the molecular weight of *B. pseudomallei* CPS was expected to range between 150 kDa and 300 kDa (Brett *et al*., 2000; Reckseidler-Zenteno *et al.,* 2005; Nualnoi *et al*., 2016). Nualnoi *et al.* (2024) evaluated the reactivity of an anti-CPS mAb produced in mice immunised with inactivated *B. pseudomallei* and observed the CPS at approximately 300 kDa on SDS-PAGE. Variation in strains or isolate types contributed to varying degrees of antigenic variability, which may affect the reactivity of the mAb against the same species (Zou *et al.,* 2008). Reckseidler-Zenteno *et al.* (2009) categorised the CPS of *B. pseudomallei* into four groups, namely CPS I, CPS II, CPS III and CPS IV. They stated that CPS I was observed only in *B. pseudomallei* and *B. mallei*. Five distinct structures of *B. pseudomallei* CPS were identified as having virulence potential, as observed in melioidosis patients (Nualnoi *et al*., 2016).

https://doi.org/10.24191/scl.v20i2.6428

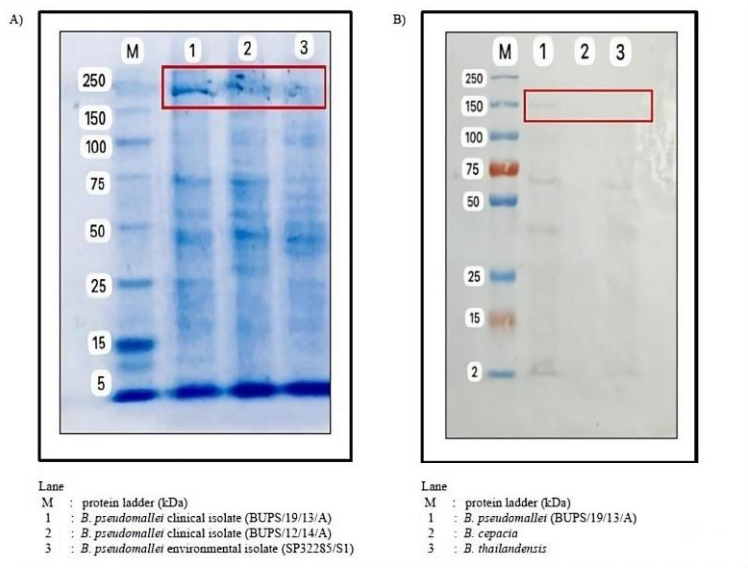


Fig. 2 Expression of protein samples stained with Coomassie blue electrophoresed on a 12% SDS-PAGE gel extracted from whole-cell lysates of *B. pseudomallei* isolates (A) and expression of CPS antigen in lane 1 using *B. pseudomallei* whole cell lysate (B) and the absence of CPS antigen in lanes 2 and 3 using *B. cepacia* and *B. thailandensis* whole cell lysates by Western immunoblotting. *Red box indicates the presence of CPS at approximately 200kDa

**Evaluation of LFIA sensitivity and specificity**

Following the successful detection of *B. pseudomallei* CPS by previous ELISA and Western blot, the anti-CPS mAb was used in the lateral flow immunoassay to detect the presence of *B. pseudomallei* in the samples. The sensitivity outcomes of the LFIA in this study are presented in Table 1. The LFIA evaluation showed a sensitivity of 46.84%, and results for the LFIA strips are shown in Fig. 3(A). Visual observation was performed to assess the colour intensity of both the test and control line and to compare them with the manufacturer's scorecard. The appearance of a red line at both the test and control lines, whether of strong or faint intensity, was considered a positive signal. An absent red line at the control line rendered the test invalid. All LFIA results showed complete agreement in visual interpretation among three independent observers, corresponding to perfect inter-observer agreement (Fleiss' $\kappa = 1.00$).

https://doi.org/10.24191/scl.v20i2.6428

Table 1. Evaluation of lateral flow immunoassay using spiked blood of *B. pseudomallei* clinical (n = 74), environmental (n = 2) isolates and positive melioidosis BACTEC blood culture (n = 1).

| No. | Type of sample | Isolate | Lateral flow result |
|---|---|---|---|
| 1 | Clinical *B. pseudomallei* spiked blood | BUPS/01/19/A | Negative |
| 2 | | BUPS/09/19/A | Negative |
| 3 | | BUPS/10/19/A | Positive |
| 4 | | BUPS/12/19/A | Positive |
| 5 | | BUPS/19/19/A | Positive |
| 6 | | BUPS/22/19/A | Negative |
| 7 | | BUPS/24/19/A | Positive |
| 8 | | BUPS/25/19/A | Negative |
| 9 | | BUPS/27/19/A | Positive |
| 10 | | BUPS/29/19/A | Positive |
| 11 | | BUPS/30/19/A | Positive |
| 12 | | BUPS/31/19/A | Positive |
| 13 | | BUPS/33/19/A | Positive |
| 14 | | BUPS/34/19/A | Positive |
| 15 | | BUPS/35/19/A | Positive |
| 16 | | BUPS/36/19/A | Positive |
| 17 | | BUPS/37/19/A | Positive |
| 18 | | BUPS/04/18/A | Negative |
| 19 | | BUPS/05/18/A | Negative |
| 20 | | BUPS/11/18/A | Positive |
| 21 | | BUPS/13/18/A | Negative |
| 22 | | BUPS/26/18/A | Negative |
| 23 | | BUPS/01/17/A | Negative |
| 24 | | BUPS/04/17/A | Negative |
| 25 | | BUPS/09/17/A | Positive |
| 26 | | BUPS/11/17/A | Negative |
| 27 | | BUPS/17/17/A | Negative |
| 28 | | BUPS/06/16/A | Positive |
| 29 | | BUPS/09/16/A | Positive |
| 30 | | BUPS/13/16/A | Negative |
| 31 | | BUPS/16/16/A | Positive |
| 32 | | BUPS/03/15/A | Negative |
| 33 | | BUPS/04/15/A | Positive |
| 34 | | BUPS/05/15/A | Positive |
| 35 | | BUPS/06/15/A | Negative |
| 36 | | BUPS/07/15/A | Negative |
| 37 | | BUPS/08/15/A | Positive |
| 38 | | BUPS/09/15/A | Negative |
| 39 | | BUPS/10/15/A | Negative |
| 40 | | BUPS/11/15/A | Negative |
| 41 | | BUPS/12/15/A | Negative |
| 42 | | BUPS/13/15/A | Negative |
| 43 | | BUPS/14/15/A | Negative |

https://doi.org/10.24191/scl.v20i2.6428

| | | | |
|---|---|---|---|
| 44 | | BUPS/15/15/A | Negative |
| 45 | | BUPS/83/15/A | Negative |
| 46 | | BUPS/07/14/A | Negative |
| 47 | | BUPS/11/14/A | Negative |
| 48 | | BUPS/12/14/A | Positive |
| 49 | | BUPS/14/14/A | Negative |
| 50 | | BUPS/17/14/A | Negative |
| 51 | | BUPS/23/14/A | Positive |
| 52 | | BUPS/25/14/A | Positive |
| 53 | | BUPS/29/14/A | Negative |
| 54 | | BUPS/31/14/A | Positive |
| 55 | | BUPS/32 /14/A | Negative |
| 56 | | BUPS/33/14/A | Negative |
| 57 | | BUPS/34/14/A | Negative |
| 58 | | BUPS/03/13/A | Negative |
| 59 | | BUPS/07/13/A | Positive |
| 60 | | BUPS/08/13/A | Positive |
| 61 | | BUPS/10/13/A | Negative |
| 62 | | BUPS/11/13/A | Positive |
| 63 | | BUPS/12/13/A | Positive |
| 64 | | BUPS/13/13/A | Negative |
| 65 | | BUPS/15/13/A | Positive |
| 66 | | BUPS/16/13/A | Positive |
| 67 | | BUPS/17/13/A | Positive |
| 68 | | BUPS/19/13/A | Positive |
| 69 | | BUPS/22/13/A | Negative |
| 70 | | BUPS/25/13/A | Negative |
| 71 | | BUPS/26/13/A | Negative |
| 72 | | BUPS/27/13/A | Negative |
| 73 | | BUPS/03/09/A | Negative |
| 74 | | BUPS/13/09/A | Negative |
| 75 | Environmental *B. pseudomallei* spiked blood | SP32285/S1 | Positive |
| 76 | | SP32285/S2 | Positive |
| 77 | Positive BACTEC blood culture | n/a | Negative |

*n/a: not available

Fig. 3(B) and Table 2 present the LFIA results tested with relatively close *Burkholderia* spp. and other bacterial species for specificity evaluation. The specificity evaluation was expected to demonstrate that all strips would test negative with blood specimens spiked with genetically related *Burkholderia* species and other related bacterial species, thereby yielding a 100% outcome. However, the LFIA in this study demonstrated 77.78% specificity, as two strips tested with blood spiked with both clinical and environmental strains of *B. thailandensis* showed two lines, indicating false positive results. Cross-reactivity with *B. thailandensis* has been frequently reported in the diagnosis of melioidosis (Rongkard *et al*., 2016).

https://doi.org/10.24191/scl.v20i2.6428

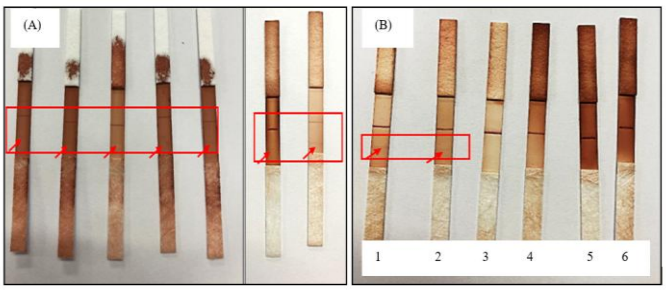


Fig. 3(A). The red arrow shows the appearance of a red line on the LFIA strips at the test line, indicating a positive signal tested with *B. pseudomallei* clinical isolates and environmental isolates. Fig. 3(B). The red arrow of the test line at Strip 1 and Strip 2 indicated a positive signal tested with *B. thailandensis* clinical (BUPS/06/18/A) and environmental (P969/S1) isolates, whilst negative for *B. cepacia* (Strip 3), *B. ubonensis* (Strip 4), *B. stagnalis* (Strip 5) and *P. fluorescen* (Strip 6).

It is important to determine the specificity of the mAb against several neighbouring species of *Burkholderia spp*. as a preliminary observation in the development of an antigen-capture immunoassay (Nualnoi *et al.,* 2017). Immunoassays that employ the unpurified crude antigens, including proteins, polysaccharides and lipopolysaccharides with intact epitopes, may cross-react with antibodies against other common infectious agents (Thepthai *et al.,* 2005). Baylis *et al*. (2017) reported that the CPS of *B. thailandensis* was structurally indistinguishable from the CPS of *B. pseudomallei*. They also reported that the anti-CPS mAb recognition of *B. thailandensis* was comparable to its recognition of CPS in *B. pseudomallei*. This structural similarity, therefore, accounts for the cross-reactivity observed between these two related species. Sim *et al.* (2010) reported similar findings, showing that the CPS of *B. thailandensis* shared more than 90% similarity in the 6-deoxy-D-manno-heptose backbone and 10% α-1,3 mannose structure with identical epitopes.

Table 2. Evaluation of lateral flow immunoassay using spiked blood of genetically relatives of *Burkholderia* (*n* = 7) and other bacteria species (*n* = 2).

| No. | Organism | Isolate | Lateral flow result |
|---|---|---|---|
| | Genetically relatives | | |
| 1 | *B. thailandensis* | BUPS/06/18/A | Positive |
| 2 | *B. thailandensis* | P969/S1 | Positive |
| 3 | *B. cepacia* | BUPS/04/19/A | Negative |
| 4 | *B. ubonensis* | B16050/S1 | Negative |
| 5 | *B. stagnalis* | P2469/S1 | Negative |
| 6 | *Pseudomonas fluorescens* | n/a | Negative |
| 7 | *Pseudomonas aeruginosa* | n/a | Negative |
| | Other type of bacteria | n/a | |
| 8 | *Klebsiella pneumoniae* | | Negative |
| 9 | *Escherichia coli* | n/a | Negative |

*n/a: not available

https://doi.org/10.24191/scl.v20i2.6428

**Evaluation of LFIA using blood specimens of Orangutans at BMOUI**

Further evaluation of the developed LFIA strips was conducted using two blood specimens from orangutans at BMOUI, and the results are presented in Fig. 4 and Table 3. Notably, both LFIA strips exhibited two lines indicating a positive result but demonstrated negative results when tested with the gold standard culture method and the BACTEC automated blood culture system. Due to the extremely limited sample size (n = 2), no statistical analysis or diagnostic performance metrics could be meaningfully derived from these field observations.

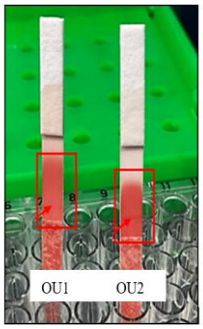


Fig. 4. The red arrow shows the appearance of a red line on the LFIA Strips at the test line, indicated positive signal tested with blood from two orangutans labelled as OU1 and OU2 at BMOUI.

Table 3. *B. pseudomallei* detection from blood specimens of orangutans collected from BMOUI (*n* = 2).

| No. | Sample ID | RESULT | | | Interpretation |
|---|---|---|---|---|---|
| | | LFI | Gold standard | | |
| | | | Culture | BACTEC | |
| 1 | OU1 | Positive | Negative | Negative | False positive |
| 2 | OU2 | Positive | Negative | Negative | False positive |

The findings of this study should be interpreted with caution as the overall design remains exploratory, particularly for field-based evaluation. The assessment of acceptable LFIA performance is relatively subjective and can vary based on the manufacturers, guidelines and specifications (Mistry *et al*., 2021). According to Fairley *et al.* (2021), the recommended acceptable ranges for LFIA sensitivity and specificity in melioidosis detection were 34.1% to 78.9% and 93.3% to 96.3%, respectively. Notably, the specificity achieved in this study (77.78%) falls below the recommended lower threshold of 93.3%, underscoring the need to optimise the assay further, particularly to reduce cross-reactivity with *B. thailandensis*. Components such as haemoglobin, immunoglobin, EDTA, heparin and lactoferrin naturally present in whole blood have been recognised as blood inhibitors that can influence the outcomes of lateral flow immunoassay (Noparatvarakorn *et al*., 2023; Bowen and Remaley, 2014; Musso and La Scola, 2013; Soud and Rådström, 2001). Furthermore, false negative or weakly positive results indicated by a faint band at the test line on the LFIA strips can be caused by a high antigen concentration during sample preparation. This phenomenon was known as the hook effect or prozone effect, in which antibody binding sites become saturated by the excess antigen in the sample, resulting in reduced signal intensity on the LFIA strip (Srisrattakarn *et al*., 2020). Furthermore, the concentration of CPS antigen in spiked specimens was not quantitatively determined and the volume of the bacterial culture diluted to spike the blood was determined according to the manufacturer's instruction, which limits the interpretation of false-negative results and potential hook (prozone) effects. Future work should include precise antigen quantification to better correlate analyte

https://doi.org/10.24191/scl.v20i2.6428

concentration with assay performance. Nualnoi *et al*. (2016) suggested that urine is the optimal specimen matrix for detecting melioidosis, however urine collection from the orangutan in this study is particularly challenging in captive wildlife settings due to the higher risk of sample cross-contamination.

While LFIA demonstrates potential as a rapid detection platform, the current assay performance (sensitivity 46.84%, specificity 77.78%) is insufficient to support its use as a reliable screening tool without further optimization. A recent review highlighted that although point-of-care diagnostic technologies for melioidosis have advanced considerably, further optimisation and validation of biomarkers are still required to improve the sensitivity and clinical utility of rapid diagnostic assays (Kusuma *et al*., 2026). Improving the accuracy of LFIA for melioidosis detection in conservation centres requires addressing its limitations in sensitivity and specificity. The observed false-positive results, particularly in samples containing *B. thailandensis* and in orangutan blood specimens, highlight limitations in both specificity and raise concerns regarding cross-reactivity. Recommendations for improvement include refining antibody reagents to increase pathogen specificity, thereby minimizing false-positive results and enhancing detection accuracy. Additionally, adapting the assay to account for variable blood matrix factors in different animal species may improve reliability across diverse wildlife applications. The development of multiplex LFIAs capable of detecting multiple pathogens commonly found in conservation settings would also enhance the tool's versatility and reduce the need for multiple separate tests. Ongoing validation of LFIA technology with a broader range of animal samples will be crucial to confirm its effectiveness and establish reliable testing protocols for conservation use. The use of LFIA testing for melioidosis in conservation centres is not only a diagnostic measure but also a preventive strategy. Early and accessible testing enables conservation staff to detect cases before symptoms become severe or spread to other animals, allowing targeted preventive measures such as isolation, supportive care, and treatment. This proactive approach helps mitigate the risk of outbreaks within high-density populations, which is vital for protecting vulnerable and endangered species such as orangutans. Therefore, integrating LFIA-based diagnostics into conservation centre protocols can strengthen overall disease management efforts and enhance the health resilience of animal populations, supporting both individual animal welfare and broader biodiversity conservation goals.

## CONCLUSION

The results of this study provide preliminary and exploratory insights into the use of a lateral flow immunoassay (LFIA) for melioidosis detection in wildlife. While the assay demonstrated moderate sensitivity (46.84%) and specificity (77.78%), false-positive results were observed, particularly in blood specimens from orangutans that were subsequently confirmed negative by culture methods. Given these performance limitations and the extremely limited number of field samples (n = 2), the findings should be interpreted with caution. At this stage, the LFIA is not sufficiently reliable for standalone screening or diagnostic use in conservation settings. This study should therefore be regarded as a proof-of-concept highlighting both the potential and current limitations of CPS-based LFIA for melioidosis detection. Nevertheless, this study provides a foundation for further development of LFIA-based diagnostics for melioidosis in wildlife. Future work should focus on improving assay sensitivity and specificity, incorporating quantitative antigen characterization, and conducting validation using larger and statistically robust sample sizes across diverse animal populations before considering integration into routine diagnostic or surveillance protocols in conservation environments.

**Acknowledgement**

We would like to offer our gratitude to Almarhum Tan Sri Datuk Mustapha Kamal Abu Bakar and the Board of Trustee of BMOUI Foundation who provided the opportunity to carry out research in BMOUI. This study was funded by Malaysian Ministry of Education in Fundamental Research Grant Scheme for Research Acculturation of Early Career Researchers: 600-IRMI/FRGS-RACER5/3(101/2109).

https://doi.org/10.24191/scl.v20i2.6428

**Conflict of Interest**

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

**Author Role Statement**

Nurul Iman Mohamad designed and carried out the research, wrote and revised the article. Sabapathy Dharmalingam conceptualised the central research idea and provided the theoretical framework. Nurul Aili Zakaria and Zakuan Zainy Deris supervised research progress; Farida Zuraina Mohd Yusof anchored the review, revisions and approved the article submission.

**REFERENCES**

Hayashi, M., Kawakami, F., Roslan, R., M., Hapiszudin, M. & Dharmalingam, S. (2018). Behavioural studies and veterinary management of orangutans at Bukit Merah Orang Utan Island, Perak, Malaysia. *Primates,* 59, 135-144.

Sprague, L. D., & Neubauer, H. (2004). Melioidosis in animals: a review on epizootiology, diagnosis and clinical presentation. *Journal of Veterinary Medicine,* 51, 305-320.

Rachlin, A., Shilton, C., Webb, J. R., Mayo, M., Kaestli, M., Kleinecke, M., Rigas, V., Benedict, S., Gurry, I., & Currie, B. J. (2019). Melioidosis fatalities in captive slender-tailed meerkats (*Suricata suricatta*): combining epidemiology, pathology and whole-genome sequencing supports variable mechanism of transmission with one health implications. *BMC Veterinary Research*, 15(458), 1-12.

Nozmi, N., Md Yusof, N. N., Ithnin, H., Ramli, M. F., & Mohd Yusof, F. Z. (2025). The relationship between reproductive hormones with general behaviour of captive female Bornean orangutans at Bukit Merah Orang Utan Island, Perak. *Science Letters,* 19(1), 75–87.

Sermswan, R. W., Wongratanacheewin, S., Anuntagool, N., & Sirisinha, S. (2000). Comparison of the polymerase chain reaction and serologic tests for diagnosis of septicemic melioidosis. *The American Journal of Tropical Medicine and Hygiene,* 63(3-4), 146-149.

Nualnoi, T., Wongwitwichot, P., Kaewmanee, S., Chanchay, P., Wongpanti, N., Ueangsuwan, T., Siangsanor, R., Chotirouangnapa, W., Saechin, T., Thungtin, S., Szekely, J., Wattanachant, C., & Saechan, V. (2024). Development of an Antigen Capture Lateral Flow Immunoassay for the Detection of *Burkholderia pseudomallei*. *Diagnostics (Basel, Switzerland)*, 14(10), 1033.

Houghton R.L., Reed, D.E., Hubbard, M.A., Dillon, M.J., Chen, H., Currie, B.J., Mayo, M., Sarovich, D.S., Theobald, V., Limmathurotsakul, D., Wongsuvan, G., Chantratita, N., Peacock, S.J., Hoffmaster, A.R., Duval, B., Brett, P.J., Burtnick, M.N., & AuCoin, D.P. (2014). Development of a prototype lateral flow immunoassay (LFI) for the rapid diagnosis of melioidosis. *PLoS Neglected Tropical Diseases,* 8(3), e2727.

Najomtien, P., Potisap, C., Chattagul, S., W. Sermswan, R., & Wongratanacheewin, S. (2024). Development of LAMP-Lateral Flow Immunoassay for diagnosis of melioidosis. *Srinagarind Medical Journal*, 39(2), 156–166.

Currie, B. J., Woerle, C., Mayo, M., Meumann, E. M., & Baird, R. W. (2022). What is the role of lateral immunoassay for the diagnosis of melioidosis? *Open Forum Infectious Diseases*, 9(5), ofac149.

**Commented [MI2]:** AQ3: Please ensure that all references are accurate and cited sequentially according to their appearance in the text.

**Commented [MI3R2]:** AQ4: To ensure the timeliness of the research, please integrate and cite more recent literature from the year 2026 into your manuscript.

https://doi.org/10.24191/scl.v20i2.6428

Shaw, T., Tellapragada, C., Ke, V., AuCoin, D. P., & Mukhopadhyay, C. (2018). Performance evaluation of Active Melioidosis Detect-Lateral Flow Assay (AMD-LFA) for diagnosis of melioidosis in endemic settings with limited resources. *PLoS ONE*, 13(3), e0194595.

Zueter, A.M., Abdul Rahman Z., Chan, Y.Y. & Harun, A. (2015). Brief communication genotyping of *Burkholderia pseudomallei* revealed high genetic variability among isolates from a single population group. *International Journal of Molecular Epidemiology and Genetics,* 6(1), 41-47.

World Health Organization. (2014). *A brief guide to emerging infectious diseases and zoonoses*. World Health Organization, Regional Office for South-East Asia. https://www.who.int/publications/i/item/9789290224587.

International Union for Conservation of Nature. (1989). *IUCN policy statement on research involving species at risk of extinction*. https://portals.iucn.org/library/efiles/documents/PP-003-En.pdf.

Burtnick, M. N., Shaffer, T. L., Ross, B. N., Muruato, L. A., Sbrana, E., DeShazar, D., Torres, A. G., & Brett, P. J. (2018). Development of subunit vaccines that provide high-level protection and sterilizing immunity against acute inhalation melioidosis. *Infection and Immunity*, 86(1), e00724-17.

Scott, A. E., Burtnick, M. N., Stokes, M. G. M., Whelan, A. O., Williamson, D., Atkins, T. P., Prior, J. L., & Brett, P. J. (2014). *Burkholderia pseudomallei* capsular polysaccharide conjugates provide protection against acute melioidosis. *Infection and Immunity*, 82(8), 3206-3213.

Anandan, S., Augustine, A., Mathai, E., & Jesudason, M. V. (2010). Evaluation of IgM ELISA using a sonicate and a lipopolysaccharide antigen for the serodiagnosis of melioidosis. *Indian Journal of Medical Microbiology*, 28(2), 158–161.

Hii, S. Y. F., Ali, N. A., Ahmad, N., & Amran, F. (2017). Comparison of in-house IgM and IgG ELISAs for the serodiagnosis of melioidosis in Malaysia. *Journal of Medical Microbiology*, 66(11), 1623–1627.

Reckseidler-Zenteno, S. L., De Vinney, R., & Woods, D. E., (2005). The capsular polysaccharide of *Burkholderia pseudomallei* contributes to survival in serum by reducing complement factor C3b deposition. *Infection and Immunity*, 73(2), 1106-1115.

Nualnoi, T., Kirosingh, A., Pandit, S. G., Thorkildson, P., Brett, P. J., Burtnick, M. N., & AuCoin, D. P. (2016). *In vivo* distribution and clearance of purified capsular polysaccharide from *Burkholderia pseudomallei* in a murine model. *PLOS Neglected Tropical Diseases*, 10(12), e0005217.

Zou, N., Tsai, S., Feng, S. H., Newsome, T., Kim, H. Y., Li, B., Zhang, S., & Lo, S. C. (2008). Relationship between antigenicity and pathogenicity for *Burkholderia pseudomallei* and *Burkholderia mallei* revealed by a large panel of mouse MAbs. *Hybridoma (2005)*, 27(4), 231–240.

Reckseidler-Zenteno, S. L., Moore, R., & Woods, D. E. (2009). Genetics and function of the capsules of *Burkholderia pseudomallei* and their potential as therapeutic targets. *Mini Reviews in Medicinal Chemistry*, 9(2), 265–271.

Rongkard, P., Hantrakun, V., Dittrich, S., Srilohasin, P., Amornchai, P., Langla, S., Lim, C., Day, N. P., AuCoin, D., Wuthiekanun, V., & Limmathurotsakul, D. (2016). Utility of a lateral flow immunoassay (LFI) to detect *Burkholderia pseudomallei* in soil samples. *PLoS Neglected Tropical Diseases*, 10(12), e0005204.

Nualnoi, T., Norris, M. H., Tuanyok, A., Brett, P. J., Burtnick, M. N., Keim, P. S., Settles, E. W., Allender, C. J., & AuCoin, D. P. (2017). Development of immunoassays for *Burkholderia pseudomallei* typical and atypical lipopolysaccharide strain typing. *The American Journal of Tropical Medicine and Hygiene*, 96(2), 358–367.

https://doi.org/10.24191/scl.v20i2.6428

Thepthai, C., Smithtikarn, S., Suksuwan, M., Songsivilai, S., & Dharakul, T. (2005). Serodiagnosis of melioidosis by a competitive enzyme-linked immunosorbent assay using a lipopolysaccharide-specific monoclonal antibody. *Asian Pacific Journal of Allergy and Immunology*, *23*(2-3), 127–132.

Bayliss, M., Donaldson, M. I., Nepogodiev, S. A., Pergolizzi, G., Scott, A. E., Harmer, N. J., Field, R. A., & Prior, J. L. (2017). Structural characterisation of the capsular polysaccharide expressed by *Burkholderia thailandensis* strain E555: wbiI (pKnock-KmR) and assessment of the significance of the 2-O-acetyl group in immune protection. *Carbohydrate Research*, 452, 17–24.

Sim, B. M., Chantratita, N., Ooi, W. F., Nandi, T., Tewhey, R., Wuthiekanun, V., Thaipadungpanit, J., Tumapa, S., Ariyaratne, P., Sung, W. K., Sem, X. H., Chua, H. H., Ramnarayanan, K., Lin, C. H., Liu, Y., Feil, E. J., Glass, M. B., Tan, G., Peacock, S. J., & Tan, P. (2010). Genomic acquisition of a capsular polysaccharide virulence cluster by non-pathogenic *Burkholderia* isolates. *Genome Biology*, 11(8), R89.

Mistry, D. A., Wang, J. Y., Moeser, M.-E., Starkey, T., & Lee, L. Y. W. (2021). *A systematic review of the sensitivity and specificity of lateral flow devices in the detection of SARS-CoV-2*. *BMC Infectious Diseases,* 21, 828.

Fairley, L., Smith, S., Maisrikrod, S., & Henning, L. (2021). Systematic review and meta-analysis of diagnostic tests for diagnosis of melioidosis. *Acta Tropica*, 214(105784), 1-9.

Noparatvarakorn, C., Sengyee, S., Yarasai, A., Phunpang, R., Dulsuk, A., Ottiwet, O., Janon, R., Morakot, C., Burtnick, M. N., Brett, P. J., West, T. E., & Chantratita, N. (2023). Prospective analysis of antibody diagnostic tests and TTS1 real-time PCR for diagnosis of melioidosis in areas where it is endemic. *Journal of Clinical Microbiology*, 61(3), e0160522.

Bowen, R. A., & Remaley, A. T. (2014). Interferences from blood collection tube components on clinical chemistry assays. *Biochemia Medica*, 24(1), 31–44.

Musso, D., & La Scola, B. (2013). Laboratory diagnosis of leptospirosis: a challenge. *Journal of Microbiology, Immunology, and Infection,* 46(4), 245–252.

Soud, W. A., & Rådström, P. (2001). Purification and characterization of PCR-inhibitory components in blood cells. *Journal of Clinical Microbiology*, 39(2), 485–493.

Srisrattakarn, A., Tippayawat, P., Chanawong, A., Tavichakorntrakool, R., Daduang, J., Wonglakorn, L., & Lulitanond, A. (2020). Development of a prototype lateral flow immunoassay for rapid detection of Staphylococcal Protein A in positive blood culture samples. *Diagnostics (Basel, Switzerland)*, 10(10), 794.

Kusuma, S. A. F., Anggraeni, S. R., Sari, Q. W., & Sofyana, N. T. (2026). *Current melioidosis diagnostic landscape and missed opportunities in biomarker development*. *Diagnostics, 16*(8), 1247.



https://doi.org/10.24191/scl.v20i2.6428