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Published in:

Journal of Medical Microbiology

DOI:

[10.1099/jmm.0.000098](https://doi.org/10.1099/jmm.0.000098)

Publication date:

2015

Link:

[Link to publication in PEARL](#)

Citation for published version (APA):

Robertson, G., Sorenson, A., Govan, B., Ketheesan, N., Houghton, R., Chen, H., Aucoin, D., Dillon, M., & Norton, R. (2015). Rapid diagnostics for melioidosis: a comparative study of a novel lateral flow antigen detection assay. *Journal of Medical Microbiology*, 64(8), 845-848. <https://doi.org/10.1099/jmm.0.000098>

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Article in *Journal of Medical Microbiology* · June 2015

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Rapid diagnostics for melioidosis: a comparative study of a novel lateral flow antigen detection assay

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The rapid diagnosis of septicaemic melioidosis will have an impact on reduction of mortality. Currently, this relies almost exclusively upon culture of the causative agent *Burkholderia pseudomallei* from clinical samples. In acute sepsis, blood is the preferred specimen for culture and therefore should be the target for a rapid diagnostic tool. A lateral flow immunoassay (LFI) for the detection of *B. pseudomallei* antigen has been developed. This was compared with molecular detection using the targets *T3SS1* and *IpxO*. Forty-five clinical samples of EDTA blood, which were culture-positive, were tested using both modalities. The LFI had a sensitivity of 40 %, whilst molecular detection had a sensitivity of 20 %. The poor performance of molecular detection has been described previously and is largely related to the use of whole-blood specimens collected into blood tubes containing EDTA. Whilst suboptimal, the LFI would be an adjunct in the rapid diagnosis of melioidosis.

Received 23 March 2015
Accepted 1 June 2015

INTRODUCTION

Burkholderia pseudomallei is a Gram-negative soil saprophyte which is the aetiological agent of melioidosis. This is a disease endemic to northern Australia and south-east Asia, and causes significant morbidity and mortality, with a wide spectrum of clinical presentations (Malczewski *et al.*, 2005; White, 2003). The gold standard for the diagnosis of melioidosis is culture from clinical specimens. This is, however, time-consuming and may not be easily available in endemic regions. Serology is of limited value as up to 50 % of blood culture-positive patients are seronegative by the indirect haemagglutination assay (IHA) at the time of presentation (Harris *et al.*, 2009). Molecular detection from clinical samples has been advocated with variable results. Most targets offer high specificity, but variable sensitivity (Couto *et al.*, 2009; Kunakorn *et al.*, 2000; Merritt *et al.*, 2006; Tomaso *et al.*, 2005). False-negative molecular detection of *B. pseudomallei* has been attributed to lower concentrations of the organism in blood (Supaprom *et al.*, 2007).

Whilst more complex methods, such as genomic transcription profiling using microarray technology have shown greater promise, this technology is generally neither available nor feasible in regions where this disease is endemic (Pankla *et al.*, 2009).

The recent development of a prototype lateral flow immunoassay (LFI) for antigen detection in melioidosis has renewed interest in rapid point-of-care testing for melioidosis (Houghton *et al.*, 2014). In the current study, a LFI prototype using clinical samples was trialled. Blood from culture-positive patients admitted with proven melioidosis was used for LFI and compared with a molecular detection assay using two different previously described primer sets (*T3SS1* cluster and *IpxO*). Serology using IHA was also performed.

Simple, rapid and reliable diagnostic tests for melioidosis will help identify cases earlier, leading to improved outcomes, given that culture and identification of *B. pseudomallei* can be delayed for up to 3 days. Furthermore, early diagnosis and selection of appropriate antimicrobial therapy help reduce the significant mortality associated with the disease.

METHODS

Ethics approval was granted by The Townsville Hospital and Health Services Ethics Committee (HREC/14/QTHS/13).

Clinical samples. In total, 45 clinical specimens from 30 patients were collected on admission and stored at -70°C . The 45 EDTA whole-blood samples were collected before, after or at the same time as specimens collected which were subsequently culture-positive for *B. pseudomallei*. All specimens were thawed and used as detailed below. EDTA blood samples which subsequently cultured *Pseudomonas aeruginosa*, coagulase-negative *Staphylococcus*, *Escherichia coli* or an unidentified Gram-negative bacillus (not *B. pseudomallei*) were used as negative controls.

LFI. The InBios Active Melioidosis Detect test is a membrane immunoassay designed to detect soluble capsular polysaccharide from *B. pseudomallei* in a range of sample types (Houghton *et al.*, 2014). For use with whole blood, 35 μl of specimen was added to the lateral flow strip under the indicator arrow. The strip was then transferred to a well loaded with chase buffer and the results recorded after 15 min. A positive, valid test was indicated by the presence of a red line in both the test and control regions.

Molecular detection. Quantitative real-time (qRT)-PCR was performed on the 45 clinical culture-positive samples, along with four controls using previously described molecular targets *T3SS1* and *IpxO* and protocols (Kaestli *et al.*, 2012; Merritt *et al.*, 2006; Meumann *et al.*, 2006).

Serology. The IHA was performed as described previously (Gilmore *et al.*, 2007) using sera collected at the same time as the positive blood cultures. A positive IHA was defined as a titre of $\geq 1:40$.

RESULTS AND DISCUSSION

Of the 45 EDTA blood samples, 27 were collected on the same day as the positive blood culture. Four were collected 1–7 days before and 14 were collected 1–4 days after collection of the positive blood culture. These specimens had been stored at -70°C for up to 72 months. The four control samples used in the study tested negative by both the LFI and molecular detection.

The results for LFI, *T3SS1*, *IpxO* PCRs and IHA are given in Table 1. These include the time frame (days) when the sample was collected in relation to the sample that yielded a positive *B. pseudomallei* culture. There were a total of 16 positive LFIs (40 %, 16/40) with five indeterminate assays. The indeterminate assays were due to the non-appearance of the control line and were probably related to a lack of sample flow. We did not repeat these due to a limited number of available LFI strips.

There were nine positive PCR results (20 %, 9/45). These included either or both targets used. When compared with culture, the LFI had a sensitivity of 40 % and the combined PCR targets had a sensitivity of 20 %. A summary of the yields of these diagnostic approaches based on the time difference between culture positivity and blood sample collection is given in Table 2.

The early diagnosis of melioidosis currently relies upon the culture and correct identification of *B. pseudomallei* from

Table 1. Comparison of the LFI with molecular detection and IHA

Sample ID	LFI	qRT-PCR		IHA	Collection of sample relative to day of positive blood culture*
		<i>T3SS1</i>	<i>IpxO</i>		
3	+	+	+	5	+2
6	+	+	-	<5	+2
8	+	-	-	<5	+2
11	+	-	-	10	0
12	+	-	-	80	0
16	+	-	-	320	0
17	+	-	-	<5	-1
19	+	-	-	<5	0
24	+	-	-	640	-3
25	+	+	-	<5	1+
26	+	-	-	<5	0
30	+	+	+	<5	-7
37	+	-	-	1280	+1
38	+	+	+	<5	+1
39	+	+	+	<5	0
40	+	-	-	<5	-3
2	IND	-	-	5	0
7	IND	-	-	<5	+1
9	IND	-	-	<5	-
15	IND	+	+	320	0
18	IND	-	-	<5	0
1	-	-	-	5	0
4	-	-	-	5	+1
5	-	-	-	<5	+4
10	-	-	-	>5120	0
13	-	-	-	<5	0
14	-	-	-	<5	0
20	-	-	-	160	+2
21	-	+	+	160	+2
22	-	-	-	160	+1
23	-	-	-	160	0
27	-	-	-	20	+2
28	-	-	-	20	+1
29	-	-	-	20	+2
31	-	-	-	40	0
32	-	-	-	<5	2+
33	-	-	-	5	0
34	-	-	-	<5	0
35	-	-	-	<5	+2
36	-	-	-	80	0
41	-	-	-	<5	0
42	-	-	-	<5	0
43	-	-	-	<5	0
44	-	+	+	<5	+1
45	-	-	-	<5	0
Control	-	-	-	NA	NA
Control	-	-	-	NA	NA
Control	-	-	-	NA	NA
Control	-	-	-	NA	NA

IND, indeterminate; NA, not applicable; *, blood sample collected on the same day as sample that yielded a positive *B. pseudomallei* culture; +, sample collected *n* days after the sample that yielded a positive culture; -, sample collected *n* days before the sample that yielded a positive culture.

Table 2. Comparison of time of collection of sample with the diagnostic validity of LFI and molecular detection

Sample collection	N (N*)	IHA-positive [n (%)]	LFI-positive [n (%)]	qRT-PCR-positive [n (%)]	
				<i>T3SS1</i>	<i>IpxO</i>
1–7 days before the positive culture	4 (3)	1 (25)	3 (75); 3 (100)	1 (25)	1 (25)
On the same day as the positive culture	27 (24)	3 (11)	9 (33.3); 9 (37.5)	3 (11.1)	7 (26)
1–4 days after the positive culture	14 (13)	3 (21.4)	4 (28.6); 4 (30.8)	5 (35.7)	4 (28.5)
Total	45 (40)	7 (15.5)	16 (35.5); 16 (40)	9 (20)	12 (26.7)

*N and italicized numbers based on 40 functional LFI tests.

clinical samples. Bacteraemic melioidosis carries a greater mortality than non-bacteraemic disease. Whilst previous studies have shown that molecular or antigen detection of *B. pseudomallei* from sputum or urine can be an effective diagnostic modality (Ekpo *et al.*, 2007; Meumann *et al.*, 2006; Pongsunk *et al.*, 1999), direct detection from blood remains problematic. The sensitivity of direct PCR detection of *B. pseudomallei* from blood has been reported to vary from 31 (Kunakorn *et al.*, 2000) to 56 % (Meumann *et al.*, 2006). One of these studies suggested that EDTA plasma was superior to EDTA blood for PCR assays (Richardson *et al.*, 2012). The primers chosen (*T3SS1* and *IpxO*) are well described and used routinely in the molecular confirmation of isolates. Their poor performance here may relate to the samples used. It is acknowledged that further work in determining ideal primer sets for the detection of *B. pseudomallei* from EDTA blood is warranted.

The development of the LFI has provided an opportunity to assess a new rapid diagnostic test for melioidosis. This study has shown that this assay may have a role as an adjunct in early diagnosis of melioidosis. It performed better than both molecular targets used. There was no difference in positivity related to the age of the specimen. Specimens from 2008 were just as likely to be positive as more recent specimens from 2014. Although the numbers were small, the LFI appeared to perform better with blood that had been collected 1–7 days prior to the collection of blood cultures that subsequently yielded positive cultures. It is likely that this reflects a lack of clinical recognition that the patient was septicemic at the time, with the failure to collect blood cultures accordingly.

We used four negative controls of blood collected from patients with bacteraemias. These included blood that subsequently cultured *E. coli*, coagulase-negative *Staphylococcus*, *P. aeruginosa* and an unidentified Gram-negative bacillus. The reason for this choice was to try to cover a range of organisms seen in a diagnostic laboratory. We specifically chose an unidentified Gram-negative bacillus to ‘blind’ the process. Although increasing the number of negative controls would have been ideal, we were limited by the number of LFI strips available for this work. We were unable to obtain sera from a patient with the closely related

Burkholderia cepacia bacteraemia because of the rarity of this condition.

A comparison was also made with the IHA performed on blood collected at the same time as the clinical specimens that were culture-positive. A total of 12 (40 %) patients of the 30 culture-positive patients were IHA-positive. Of the 16 LFI-positive samples, only four (25 %) were IHA-positive. Of the nine qRT-PCR (either *T3SS1* or *IpxO*)-positive, only two (22 %) were IHA-positive as well. This highlights the poor utility of the IHA as a diagnostic test for melioidosis (Harris *et al.*, 2009). There was little difference in IHA positivity between the LFI-positive samples based on time of collection (Table 2).

The main limitations of this study include the length of storage of the specimens used in the study and the limited number of samples. Another limitation was the exclusion of specimens other than EDTA whole blood, such as urine or sputum. This was done deliberately to simulate the clinical situations where septic patients require urgent diagnosis of bacteraemia. We specifically excluded urine and sputum to mimic this situation of severe sepsis where blood cultures are routinely collected. In contrast, urine may be negative if the source was not urinary, and sputum can be difficult to collect in this group. It was also decided not to use enriched blood cultures as this would introduce a delay in diagnosis. Nevertheless, this could be an aspect of rapid diagnosis of suspected melioidosis when a blood culture has signalled positive with Gram-negative bacilli in an endemic region.

This study examined the utility of the LFI as a rapid diagnostic tool. It performed better than molecular detection from blood. The early detection of sepsis due to *B. pseudomallei* remains an elusive goal. The development of the LFI provides a possible alternative.

REFERENCES

Couto, M. S., Cordeiro, R. A., Rocha, M. F., Grangeiro, T. B., Leitão Junior, N. P., Bandeira, T. J., Sidrim, J. J. & Brillhante, R. S. (2009). A diagnosis of *Burkholderia pseudomallei* directly in a bronchoalveolar

lavage by polymerase chain reaction. *Diagn Microbiol Infect Dis* 65, 73–75.

Ekpo, P., Rungpanich, U., Pongsunk, S., Naigowit, P. & Petkanchanapong, V. (2007). Use of protein-specific monoclonal antibody-based latex agglutination for rapid diagnosis of *Burkholderia pseudomallei* infection in patients with community-acquired septicemia. *Clin Vaccine Immunol* 14, 811–812.

Gilmore, G., Barnes, J., Ketheesan, N. & Norton, R. (2007). Use of antigens derived from *Burkholderia pseudomallei*, *B thailandensis* and *B. cepacia* in the indirect hemagglutination assay for melioidosis. *Clin Vaccine Immunol* 14, 1529–1531.

Harris, P., Owens, L., Ketheesan, N. & Norton, R. (2009). Clinical features that affect indirect-hemagglutination-assay responses to *Burkholderia pseudomallei*. *Clin Vaccine Immunol* 16, 924–930.

Houghton, R. L., Reed, D. E., Hubbard, M. A., Dillon, M. J., Chen, H., Currie, B. J., Mayo, M., Sarovich, D. S., Theobald, V. & other authors (2014). Development of a prototype lateral flow immunoassay (LFI) for the rapid diagnosis of melioidosis. *PLoS Negl Trop Dis* 8, e2727.

Kaestli, M., Richardson, L. J., Colman, R. E., Tuanyok, A., Price, E. P., Bowers, J. R., Mayo, M., Kelley, E., Seymour, M. L. & other authors (2012). Comparison of TaqMan PCR assays for detection of the melioidosis agent *Burkholderia pseudomallei* in clinical specimens. *J Clin Microbiol* 50, 2059–2062.

Kunakorn, M., Raksakait, K., Sethaudom, C., Sermswan, R. W. & Dharakul, T. (2000). Comparison of three PCR primer sets for diagnosis of septicemic melioidosis. *Acta Trop* 74, 247–251.

Malczewski, A. B., Oman, K. M., Norton, R. E. & Ketheesan, N. (2005). Clinical presentation of melioidosis in Queensland, Australia. *Trans R Soc Trop Med Hyg* 99, 856–860.

Merritt, A., Inglis, T. J., Chidlow, G. & Harnett, G. (2006). PCR-based identification of *Burkholderia pseudomallei*. *Rev Inst Med Trop Sao Paulo* 48, 239–244.

Meumann, E. M., Novak, R. T., Gal, D., Kaestli, M. E., Mayo, M., Hanson, J. P., Spencer, E., Glass, M. B., Gee, J. E. & other authors (2006). Clinical evaluation of a type III secretion system real-time PCR assay for diagnosing melioidosis. *J Clin Microbiol* 44, 3028–3030.

Pankla, R., Buddhisa, S., Berry, M., Blankenship, D. M., Bancroft, G. J., Banchereau, J., Lertmemongkolchai, G. & Chaussabel, D. (2009). Genomic transcriptional profiling identifies a candidate blood biomarker signature for the diagnosis of septicemic melioidosis. *Genome Biol* 10, R127.

Pongsunk, S., Thirawattanasuk, N., Piyasangthong, N. & Ekpo, P. (1999). Rapid identification of *Burkholderia pseudomallei* in blood cultures by a monoclonal antibody assay. *J Clin Microbiol* 37, 3662–3667.

Richardson, L. J., Kaestli, M., Mayo, M., Bowers, J. R., Tuanyok, A., Schupp, J., Engelthaler, D., Wagner, D. M., Keim, P. S. & Currie, B. J. (2012). Towards a rapid molecular diagnostic for melioidosis: comparison of DNA extraction methods from clinical specimens. *J Microbiol Methods* 88, 179–181.

Supaprom, C., Wang, D., Leelayuwat, C., Thaewpia, W., Susaengrat, W., Koh, V., Ooi, E. E., Lertmemongkolchai, G. & Liu, Y. (2007). Development of real-time PCR assays and evaluation of their potential use for rapid detection of *Burkholderia pseudomallei* in clinical blood specimens. *J Clin Microbiol* 45, 2894–2901.

Tomaso, H., Pitt, T. L., Landt, O., Al Dahouk, S., Scholz, H. C., Reisinger, E. C., Sprague, L. D., Rathmann, I. & Neubauer, H. (2005). Rapid presumptive identification of *Burkholderia pseudomallei* with real-time PCR assays using fluorescent hybridization probes. *Mol Cell Probes* 19, 9–20.

White, N. J. (2003). Melioidosis. *Lancet* 361, 1715–1722.