Validation and comparison of an extrapolysaccharide (EPS)-based in-house ELISA and the PanBio melioidosis rapid cassette test-kits for serodiagnosis of melioidosis in a non-endemic area

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**KEYWORDS**
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**Summary**
Detection of anti-\textit{Burkholderia pseudomallei} antibodies in sera from melioidosis patients still represents a keystone in the confirmation of the clinical diagnosis, especially in non-endemic areas. An in-house assay was compared to lateral flow assays for the rapid detection of melioidosis-specific IgG or IgM. Employing 50 positive sera from patients and 200 negative sera from blood donors, sensitivity of the ELISA, the IgG and IgM assay were 84.0%, 90.0% and 84.0%, respectively. Specificity ranged from 98.0% (ELISA) to 99.5% (IgM assay). The application of the described diagnostic assays is a suitable method for the serodiagnosis of melioidosis in a non-endemic area.

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**1. Introduction**
Melioidosis is an emerging tropical disease that causes diagnostic problems in endemic and especially non-endemic areas such as Germany or other European countries where a rising number of imported cases are reported.\textsuperscript{1} In the last decade, many efforts have been made to develop new molecular or immunological techniques for diagnostic purposes. The ELISA and immunoblot procedures were examined for their potential to replace indirect haemagglutination (IHA) or immunofluorescence tests (IFT) in melioidosis serology.\textsuperscript{2} Despite its poor sensitivity and specificity, IHA remains the most widely used test. Its application is problematic in areas of endemicity, particularly in Thailand, where rates of background seropositivity may be up to 30–47% in various populations.\textsuperscript{2} So far, all new serological assays using more defined, purified or recombinant antigens have been exclusively evaluated in endemic areas. This is also true for the combination of two rapid immunochromatographic tests for the diagnosis of melioidosis infection.

During and after the 2004 tsunami, the local population, European travellers, as well as deployed troops operating in humanitarian missions in Southeast Asia, were potentially exposed to \textit{Burkholderia pseudomallei} via contact with contaminated soil or water.\textsuperscript{3} This natural disaster clearly revealed the lack of appropriate diagnostic assays in most European countries. In this study we demonstrate our clinical experience with a newly introduced in-house ELISA and two standardized lateral flow assays, which have been previously evaluated in different endemic areas of melioidosis.\textsuperscript{4}

**2. Materials and methods**
Melioidosis Rapid Cassette Test kits [immunochromatographic test (ICT)] were supplied by PanBio (Windsor, Queensland, Australia), and sera were tested and reported according to the manufacturer’s instructions.\textsuperscript{4} Purified extrapolysaccharide antigen (EPS) extracted from \textit{B. pseudomallei} (strain IMB 123) was used as the specific antigen in a standard anti-human Ig sandwich ELISA.\textsuperscript{5} Cut-off, inter- and intra-assay variances were determined according to standard procedures. Thereafter, all assays were evaluated using 50 sera from melioidosis cases from Germany (4), Switzerland (1), Great Britain (1),...
Austria (4) and Malaysia (40) [culture proven (18) or clinically defined and IFT > 1:320 (32)] and 200 sera from healthy German blood donors. Finally, 172 clinical samples received from suspected melioidosis cases in Germany, Austria and Indonesia were analyzed using all three tests.

3. Results

The cut-off of the EPS-based ELISA was set to 0.283 (mean + 3SD of 242 sera from healthy German blood donors). Inter-assay variance (10 repeats) ranged from 8.6% (strong positive sample) to 15.9% (weak positive sample), whereas the intra-assay was slightly lower (<9% for all samples). The specificity of the ELISA was 98%. Regarding sensitivity, 15 out of 18 culture-proven cases showed positive results (83.3%). If culture and the combination of clinical symptoms and a positive IFT (titre above 1:320) were used as the gold standard, the sensitivity was 84.0% (42/50 samples). Within the same two groups of serum samples, specificities of the IgG and IgM rapid cassette tests were 98.5% and 99.5%, respectively. The sensitivity of the IgG assay was slightly higher (90.0%) when compared to the IgM test (84.0%).

We also analysed 172 serum samples [Austria (43), Germany (60), hospital ship Berlin, Banda Aceh (69)] sent to the Bundeswehr Institute of Microbiology, Munich, Germany for serological testing in 2005 and 2006. When we compared the results of the ELISA with those obtained with the combination of both ICTs, we observed a strong concordance (Table 1), although the rapid test combination gave a slightly higher number of positive samples. There was no opportunity to compare these results with another serological assay, but 26 out of 40 samples that gave positive results in both rapid tests (ELISA, and combined ICTs) were from patients showing a seroconversion or who were positive by culture.

Moreover, we could also demonstrate that the specificity of the diagnostic tests was notably higher in serum samples from European individuals (>98.0%) than in sera from inhabitants of endemic regions (69–90%). People from endemic regions often have raised background levels of IgG against melioidosis and therefore the interpretation of serological results is cumbersome. In non-endemic regions, where clinical diagnosis is often delayed, serological testing might prove to be more straightforward. A positive IgM titre might be indicative of an active infection or recent exposure, while an elevated IgG result, with or without corresponding IgM, should be more indicative of past exposure.

For the first time, serological diagnostic tests for melioidosis were evaluated in a non-endemic region. Our results compare favourably with the sensitivities and specificities that have previously been published using serological tests for melioidosis. These assays will be useful in indicating the presence or absence of specific antibodies against B. pseudomallei, thereby contributing to the laboratory confirmation of imported melioidosis.

Authors’ contributions: WDS and SDP prepared the concept and design of the study; WDS, SDP and DF obtained, analysed and interpreted the serological results; WDS and DF drafted the manuscript; SDP critically revised the article. All authors read and approved the final manuscript. WDS and DF are guarantors of the paper.

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References