Utility of a rapid lateral flow assay to resolve erroneous identification of Burkholderia pseudomallei as Burkholderia thailandensis by Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry

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Title: Utility of a rapid lateral flow assay to resolve erroneous identification of *Burkholderia pseudomallei* as *Burkholderia thailandensis* by MALDI-TOF Mass Spectrometry.

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Sir,

Timely identification of *Burkholderia pseudomallei* is important to optimise clinical management of this frequently lethal condition (1) and to prevent laboratory acquired infections.

A 53-year-old fisherman from Da Nang, Vietnam, presented to an Australian hospital with a ruptured mycotic aortic aneurysm between the celiac trunk and superior mesenteric artery. Past history was significant for type 2 diabetes mellitus and untreated chronic Hepatitis B.

After 36 hours of incubation, the aerobic peripheral blood culture (BACTEC™ BD, USA) flagged with a Gram-negative bacillus with bipolar staining. At 24 hours incubation at 35°C in atmospheric oxygen there was equal growth on horse blood and McConkey agar (bioMerieux, Australia) of moist, cream-coloured non-lactose fermenting, oxidase positive colonies. The bacteria were also cultured from aortic tissue. The isolate was identified by MALDI-TOF Mass Spectrometry (MS) (Bruker Daltonics) using the Standard database (version 7.0) as *Burkholderia thailandensis* with an identification score of 1.97 (genus-level identification 1.7-2.0, species-level identification >2.0). *B. pseudomallei* was not a listed organism within the probability results. Given the suggestive epidemiological and clinical features for *B. pseudomallei* the isolate was referred to the Menzies School of Health Research (Darwin, Australia) for definitive identification. A rapid lateral flow immunoassay (LFA) against the *B. pseudomallei* capsular polysaccharide antigen (CPS) (2) was positive (Figure 1) and likewise, the isolate was identified as *B. pseudomallei* by the VITEK®2 (93% probability) and API® 20E after 48 hours growth. A *B. pseudomallei* species specific PCR targeting the type III secretion system gene (3) was positive

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providing conclusive identification. Interestingly, repeat mass spectrometry using the bioMérieux VITEK MS with the standard database also incorrectly identified the isolate as *B. multivorans* (99.9% probability).

Increasingly MALDI-TOF MS is used as a rapid and reliable technique for bacterial identification; however, the absence of reference spectra for rare and high-risk pathogens such as *B. pseudomallei* within the standard Bruker and VITEK MS databases may lead to misidentification of *B. pseudomallei* as the genetically similar but much less pathogenic *B. thailandensis* (4-7). Use of in-house curated databases with *B. pseudomallei* isolates from a broad geographic distribution or commercial 'Security Relevant' databases is associated with improved diagnostic accuracies of up to 100% (4, 5, 8-10). However, these databases are not routinely available to most clinical laboratories and misidentifications can still occur if the 'Security Relevant' databases are not fully operational.

This case demonstrates the potential utility of the recently developed LFA against the *B. pseudomallei* CPS (2) as a rapid point-of-care and adjunct to MALDI-TOF MS in routine clinical microbiology laboratories. The LFA is highly sensitive (98.7%) and specific (97.2%). It is also rapid (15 minutes) and can be performed on bacterial cultures or direct clinical samples (2), although sensitivity is significantly lower (40%) on culture unenriched whole-blood (11). Although the LFA remains experimental and is yet to be FDA approved, its ability to resolve identification dilemmas renders it a highly useful diagnostic assay. The Rapid Latex Agglutination Assay (12) is another highly sensitive (99.1%) potential adjunctive test for *B. pseudomallei* identification.

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79 Word Count: 497

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136 Figure 1: Lateral Flow Immunoassay against *B. pseudomallei* capsular
137 polysaccharide: Arrow 1 - patient isolate, Arrow 2 - positive control.

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