Further Evaluation of a Rapid Diagnostic Test for Melioidosis in an Area of Endemicity

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Immunochromatographic test (ICT) kits for the rapid detection of immunoglobulin G (IgG) and IgM antibodies to Burkholderia pseudomallei were compared to the indirect hemagglutination (IHA) assay. In 138 culture-confirmed melioidosis cases, sensitivities were 80, 77, and 88% for IHA, ICT IgG, and ICT IgM, respectively. In a prospective study of 160 consecutive sera samples sent for melioidosis serology, respective specificities were 91, 90, and 69, positive predictive values were 41, 32, and 18, and negative predictive values were 99, 98, and 100%. ICT IgM kits are unreliable for diagnosis of melioidosis, but ICT IgG kits may be useful for diagnosing travelers presenting with possible melioidosis who return from regions where melioidosis is endemic.

Melioidosis is the infectious disease caused by the soil and water bacterium Burkholderia pseudomallei. Melioidosis is most commonly described from southeast Asia and northern Australia, but the area where this disease is endemic includes India and China, and imported cases are increasingly being recognized in Europe and the United States (2, 5, 12). Definitive diagnosis requires positive bacterial culture and confirmation of the organism, which usually takes several days. Furthermore, B. pseudomallei is resistant to many standard antibiotics used in empirical therapy for sepsis (12). Therefore, various antigen and nucleic acid detection tests and serology assays have been developed to expedite diagnosis (1, 6, 9, 10, 11). A commercially available immunochromatographic test (ICT) kit for the rapid determination of immunoglobulin M (IgM) and IgG antibodies to B. pseudomallei has been developed, with excellent sensitivity and specificity reported (4). We have evaluated this kit in an area of northern Australia where melioidosis is endemic.

Melioidosis Rapid Cassette Test kits were supplied by PanBio (Windsor, Queensland, Australia), and sera were tested and reported according to the manufacturer’s instructions, which have been slightly modified from the previously described methods (4). Briefly, 5 μl of serum was placed on each of the target areas of the separate IgG and IgM test cassettes. Three drops of kit buffer were then added, and after 15 min the results were read; any trace of a pink-purple line was recorded as a positive result. All sera were tested by standard B. pseudomallei indirect hemagglutination (IHA) assay, with a titer of ≥1:40 considered reactive in our examination. A definitive diagnosis of melioidosis was the culture of B. pseudomallei from patient clinical specimens by using standard bacterial identification methods (3).

We first analyzed sera from 138 culture-confirmed cases of melioidosis for which the sera had been collected within 5 days of admission and stored until tested at −70°C. Positive results were 110 for IHA (sensitivity, 79.7%), 121 for ICT IgG (sensitivity, 87.7%), and 106 for ICT IgG (sensitivity, 76.8%). Twenty of these patients had presented with chronic melioidosis, defined as symptoms being present for more than 2 months (3). In this subset sensitivities were 95, 100, and 95% for IHA, ICT IgM, and ICT IgG, respectively.

To ascertain the specificity and predictive values of the assays, we prospectively tested all patients who had sera sent for melioidosis serology at Royal Darwin Hospital over a 6-week period in early 2003, during the monsoonal wet season when most cases of melioidosis occur in our region (3). Sera from patients with past melioidosis were excluded from analysis, leaving 160 patients. Results are shown in Table 1. During that period, 10 new cases of melioidosis were confirmed by positive culture. For the other 150 patients the cultures for B. pseudomallei were negative, and none of these patients was treated as having culture-negative melioidosis or developed melioidosis over the subsequent 12 months, with active surveillance continued for those with positive serology.

While the ICT IgM had good sensitivity, false-positive results were common in the prospective study, with a specificity of only 68.7% and a positive predictive value of only 17.5%. These data are very different from the 95% specificity previously reported for the assay (4). In the earlier study specificity was calculated from the testing of a combination of laboratory and blood donor samples (4). False-positive IgM tests are well recognized for various infections, and it has been recommended that assessment of assay specificity and predictive values be undertaken with prospectively collected samples from the population for whom the assay is being used (7, 8). Furthermore, reported sensitivities and specificities have been
found to be higher when studies of new serological assays have not followed such recommendations. We believe sample selection, and not technical differences in performing the assay, accounts for the difference between our results and those of the earlier study. By prospectively testing all patients referred for melioidosis serology, we conclude that the current ICT IgM test is not reliable for predicting melioidosis, having a low positive predictive value.

The ICT IgG test gave results very similar to those of IHA, which remains the most widely used serology assay for melioidosis (10). For both assays a level of background seropositivity is expected because of prior exposure to B. pseudomallei in areas where melioidosis is endemic (12), and this may well account for the low positive predictive value for active disease (melioidosis) in our region. However, the specificities determined in this study of 90 and 91.3%, respectively, suggest serology remains useful for selecting patients for more intensive culturing for B. pseudomallei. Negative initial serology in acute melioidosis is well recognized, and sensitivities in this study demonstrate that negative serology cannot be used to exclude melioidosis, especially early in acute disease. False-negative serology is less common with chronic melioidosis, occurring in only 1 of 20 patients in this study.

The ICT IgG cassette kit has the advantages of being transportable, user friendly, and able to produce an immediate result. It could be useful in hospital laboratories in areas where the disease is not endemic for rapid single-sample testing of patients with possible imported melioidosis. In these situations background seropositivity is less likely, especially in returning travelers. While patients presenting with acute melioidosis may initially have a negative ICT IgG result, the positive and negative predictive values should be especially high for those presenting with chronic symptoms consistent with melioidosis in areas where the disease is not endemic. This is an increasingly common clinical scenario, as more people with risk factors from the United States, Europe, and other locations where melioidosis is not endemic travel to regions where melioidosis is endemic (2). Nevertheless, culture of B. pseudomallei remains the gold standard for the diagnosis of melioidosis. A positive ICT IgG result could suggest the need for further appropriate cultures in laboratories not experienced with isolating and identifying B. pseudomallei. Cultures in selective media of throat and rectal swabs and any skin lesions are recommended, as is careful attention to correct identification of any gram-negative organisms isolated from blood and sterile sites.

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