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FAST-TRACK COMMUNICATION

Importance of Using Bruker's Security-Relevant Library for Biotyper Identification of *Burkholderia pseudomallei*, *Brucella* Species, and *Francisella tularensis*

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is an ideal platform for rapid and accurate identification of bacteria in clinical microbiology (1). Expeditious and inerrant identification of potentially hazardous agents protects laboratory staff from inadvertent exposure and enables prompt recognition of potential select agents. With the exception of experienced laboratorians who frequently encounter such agents (e.g., veterinary, public health, and reference laboratory staff), most laboratorians rely on excluding more commonly encountered bacteria before considering less common and potentially hazardous isolates. The ensuing testing may take several days, placing personnel at risk of laboratory-acquired infection.

Although the standard Biotyper reference library (Bruker, Billerica, MA) does not include select agents, several groups have demonstrated the high accuracy of MALDI-TOF MS in identification of multiple species of potentially hazardous bacteria when using appropriately constructed spectral libraries. Using a supplemented *Burkholderia* library, Inglis et al. identified *Burkholderia pseudomallei* isolated from blood cultures, reducing time to identification by 24 h (2). Using a supplemented library covering *Brucella* species, Ferreira et al. used MALDI-TOF MS to rapidly identify clinical isolates of *Brucella* species (3), and using a custom-made *Brucella* library, Lista et al. were able to identify *Brucella* species to the species level using MALDI-TOF MS (4). Finally, using a supplemented library, Seibold et al. reported use of MALDI-TOF MS for differentiation of species and even subspecies of *Francisella* (5). Although library supplementation enhances the performance of commercial MALDI-TOF MS libraries, given challenges obtaining and working with select agents in the United States, this is not an easy endeavor. Although the Biotyper reference library does not contain select agents, Bruker's security-relevant (SR) library does. The SR library can be obtained by users and searched simultaneously with the Biotyper reference library (6).

As a large reference laboratory, we regularly encounter bacteria classified as select agents. We have historically used phenotypic tests and 16S rRNA gene sequencing to identify *Brucella* species, *Francisella tularensis*, and *B. pseudomallei*. Recent implementation of the Biotyper system provided an opportunity to evaluate identification of select agents with the MALDI Biotyper reference library (version 3.3.1.2; 4,612 entries) alone and with concomitant analysis against the SR library (version 1.0; 107 entries).

We studied 20 isolates, including *Brucella melitensis* ($n = 6$), *Brucella suis* ($n = 3$), *Francisella tularensis* ($n = 9$), and *Burkholderia pseudomallei* ($n = 2$). Isolates were extracted in a biological safety cabinet prior to MALDI-TOF MS. Extraction was performed as previously described (7), except that the 70% ethanol exposure time was extended to 10 min to sterilize the samples (8). (This strategy will not sterilize spore-forming bacteria such as *Bacillus anthracis*.) MALDI-TOF MS was performed on a microflex LT mass spectrometer using MALDI Biotyper version 3.0 software. Spectra generated from a single analysis were queued with the Biotyper reference library with and without SR library supplementation.

Using the Biotyper reference library alone, 18 isolates returned high-quality spectra with results of "no reliable identification" (i.e., scores of <1.700). Spectra from the two *B. pseudomallei* isolates matched *Burkholderia thailandensis*, with scores of 1.954 and 1.962, which are considered valid scores for genus-level identification (i.e., *Burkholderia* species); the report was accompanied by a comment indicating that *B. thailandensis* is closely related to *B. pseudomallei*.

With the SR library in addition to the Biotyper reference library, seven *F. tularensis* isolates were identified as *F. tularensis* and two as *Francisella* species. Five *Brucella* isolates (including one *Brucella suis* isolate) were identified as *B. melitensis* and four as *Brucella* species. Identification of *Brucella* species to the genus level without species identification provides an actionable result for laboratory safety and public health. Finally, one *B. pseudomallei* isolate yielded a top match of *Burkholderia mallei* (score, 2.306), with a second-best match of *B. pseudomallei* (score, 2.300), and the second yielded a top match of *B. pseudomallei* (score, 2.304), with a second-best match of *B. mallei* (score, 2.301); based on criteria established in our laboratory requiring more than 10% score separation, we were unable to determine whether these isolates were *B. pseudomallei* or *B. mallei* using the SR library (1).

With the caveat that a limited number of isolates were studied, results of this study suggest the following. The Biotyper reference library appears not to misidentify select agents, as has been reported with other phenotypic identification systems (9, 10). However, it is likely to result in "no reliable identification" of *Brucella* species and *F. tularensis*; this has the potential to lead to further work-up of isolates and exposure risk. Concomitant searching of the Biotyper reference library and the SR library should enable

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identification of *Brucella* species and *F. tularensis* (at least to the genus level). An identification of *B. thailandensis* using the Biotyper reference library should suggest the possibility of *B. pseudomallei*; with the SR library, it may not be possible to distinguish *B. pseudomallei* from *B. mallei*. In our opinion, direct colony testing of suspected *Brucella* species, *F. tularensis*, or *B. pseudomallei* (or *B. mallei*) should be avoided. For ideal patient care, laboratory safety, and protection of the public's health, agents such as *Brucella* species, *F. tularensis*, and *B. pseudomallei* should be included in standard MALDI-TOF MS libraries used in clinical laboratories.

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