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Standard Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Reagents May Inactivate Potentially Hazardous Bacteria

Scott A. Cunningham,[a] Robin Patel[a,b]

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,[a] and Division of Infectious Diseases, Department of Medicine,[b] Mayo Clinic, Rochester, Minnesota, USA

Rapid and accurate identification of bacterial colonies is important in providing high-quality patient care. If not properly handled, however, colonies such as those of *Neisseria meningitidis* and *Brucella* species, pose an exposure risk to those working in clinical laboratories. As a reference laboratory, we do not uncommonly encounter such organisms; between November 2011 and December 2014, we identified 24 *Brucella* isolates, 12 *Francisella tularensis* isolates, 72 *Neisseria meningitidis* isolates, and 3 *Burkholderia pseudomallei* isolates (data courtesy of Stefanea Rucinski). With the advent of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), laboratories can rapidly identify a range of bacteria, including those that pose a risk if incorrectly handled (1). The ionization chamber and flight tube of the mass spectrometer are under vacuum, with air expelled out of the instrument. Without prior inactivation, there is a theoretical risk of dispersing viable bacteria via the exhaust. Although bacteria can be inactivated by using tube-based protein extraction preparatory to MALDI-TOF MS (2), most laboratories routinely perform direct colony testing, with tube-based protein extraction being outside everyday laboratory workflow. Further, even when performing tube-based protein extraction, the steps that need to be performed in a biological safety cabinet (BSC) when handling hazardous bacteria need definition. The presence of a hazardous bacterium is often not recognized until after it has been through the identification process, forcing laboratories to create procedures to work up colonies that are isolated from suspicious cases or morphologically resemble those of hazardous bacteria by using tube-based protein extraction performed in a BSC, adding cost and decreasing turnaround time. A practical solution is needed to work with potentially hazardous bacteria in the era of MALDI-TOF MS. We evaluated methods to safely work with such organisms by using MALDI-TOF MS, assessing bacterial inactivation times in 70% ethanol (EtOH) and bactericidal activities of 70% formic acid and MALDI-TOF MS matrix.

EtOH exposure times were assessed as previously described (3, 4). Briefly, >4.0 McFarland suspensions of the following species were prepared in 1 ml of 70% EtOH: *Bacillus cereus* (Minnesota Department of Health [MDH] laboratory preparedness survey isolate, a surrogate for *Bacillus anthracis*), *Clostridium sporogenes* (MDH isolate, a surrogate for *Clostridium botulinum*), *Francisella philomiragia* (MDH isolate, a surrogate for *F. tularensis*), *Oligella ureolytica* (Mayo Clinic isolate, a surrogate for *Brucella* species), and *Burkholderia thailandensis* (MDH isolate, a surrogate for *B. mallei*/*pseudomallei*). To promote spore formation, *B. cereus* was cultivated on esculin agar and *C. sporogenes* was acclimated to room air for 4 h; spores were confirmed by Gram staining. Five drops of EtOH suspensions of bacterium were transferred into thioglycolate broth (prereduced and enriched for anaerobic bacteria [10 ml] or supplemented with rabbit serum for the aerobic bacteria [5 ml]) at postinoculation intervals of 10 and 30 s and 1, 5, and 10 min. Organism growth and sterility controls were included. Cultures were incubated for 5 days and monitored daily for visible growth (Table 1). As expected, only non-spore-forming bacteria were inactivated with 70% EtOH, with exposure times of ≤5 min.

We also evaluated the bactericidal activity of 70% formic acid and MALDI-TOF MS matrix (α-cyano-4-hydroxycinnamic acid [Bruker Daltonics, Billerica, MA] dissolved in 50% acetonitrile and water) by using the isolates described above, as well as *Yersinia pseudotuberculosis* ATCC 907 (a surrogate for *Yersinia pestis*), *Neisseria meningitidis* serogroups B and Y, *F. tularensis*, and *Brucella melitensis* (Mayo Clinic isolates). Select agents were handled in accordance with http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=b9126e9fba23e3e7933354a1d2630d72&ty=HTML&h=L&n=9y1.0.1.5.58&r=PART. For each isolate, two autoclaved 9-mm-diameter stainless steel discs (Mesa Labs, Lakewood, CO) were prepared as mimics of MALDI-TOF MS plates. Material from a bacterial colony was combined with 1 μl of 70% formic acid and dried on one disk. Material from a bacterial colony was smeared onto the other disk, overlaid with 2 μl of MALDI-TOF MS matrix, and dried. The former was modeled to simulate on-plate formic acid-based extraction, and the latter was modeled to simulate direct colony testing. Each disk was placed into 5 ml brain heart infusion broth (BBL Becton, Dickinson, Sparks, MD) or (for *C. sporogenes*) prereduced, enriched anaerobic thioglycolate, which were incubated for 7 days at 35°C. Organism growth and sterility controls were included. Seventy percent formic acid and MALDI-TOF MS matrix each inactivated all of the bacteria tested. This suggests that laboratories may safely identify potentially hazardous bacteria by MALDI-TOF MS by using either on-plate formic acid-based extraction or direct colony testing, provided that processing is performed in a BSC and that all bacterial material is "treated" with 70% formic acid and/or MALDI-TOF MS matrix before MALDI-TOF MS plates are removed from the BSC.

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Address correspondence to Robin Patel, patel.robin@mayo.edu.

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TABLE 1 Bacterial EtOH inactivation data

Organism	Grow after EtOH exposure for[a]:																									
	10 s					30 s					1 min					5 min					10 min				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
B. cereus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. thailandensis	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−
C. sporogenes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F. philomiragia	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
O. urealytica	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] +, growth; −, no growth.

Our results also suggest that when using tube-based extraction, non-spore-forming bacteria may be treated with 70% EtOH for 5 min in a BSC, following which the rest of the procedure can be performed outside the BSC, but spore-forming bacteria tested by using tube-based extraction should remain inside a BSC until treated with formic acid/acetonitrile.

These results are encouraging in terms of safety, because they suggest that special treatment of potentially hazardous bacterial cells, including tube-based extraction, is unnecessary. Use of common MALDI-TOF MS reagents with appropriate steps performed in a BSC, in conjunction with appropriately supplemented databases (1) and interpretive guidelines (5), provides an accurate and likely safe tool for rapid identification of potentially hazardous bacteria by MALDI-TOF MS using on-plate testing. A larger data set, evaluating additional strains and species at multiple centers, would be helpful.

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