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LETTER TO THE EDITOR

Comparison of Mechanical Disruption Techniques for Rapid Inactivation of *Mycobacterium* and *Nocardia* Species before Identification Using Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) Mass Spectrometry

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Prior to protein extraction for identification by Vitek MS, mycobacterial and *Nocardia* samples must be inactivated for safe handling outside the biological safety cabinet (1, 2). Previous studies have shown bead-beating samples for 5 min in 70% ethanol (EtOH) with glass beads followed by a room temperature incubation for 10 min to be bactericidal (3). In laboratories that lack access to a Mini-Beadbeater-24 (BioSpec Products, Bartlesville, OK), the use of a vortex adapter for Vortex-Genie 2 (product no. 270677; bioMérieux SA, Durham, NC) is a less noisy, space-saving, and more cost-effective alternative that takes advantage of a common piece of laboratory equipment. This study compared sample inactivation using a Beadbeater homogenizer for 5 min and a vortex-type mixer with an adapter vortexing at maximum speed for 15 min on a panel of 28 strains of 13 *Mycobacterium* species and 23 strains of 5 clinically relevant *Nocardia* species (3, 4). Inactivation was measured by plating samples after application of each technique and monitoring for growth for 42 days for mycobacteria and 21 days for *Nocardia*. Both disruption techniques, as previously described, successfully inactivated *Nocardia* and mycobacterial samples prepared from solid media (3).

The study was performed by resuspending a 1-μl loopful of colonies grown on solid medium into a vial containing 0.5-mm-diameter glass beads and 500 μl of 70% EtOH. To ensure inactivation during routine testing, additional inactivation studies were performed for mycobacteria using a higher biomass (average population of 1.3×10^9 CFU per 10-μl loopful) than normally encountered in a clinical microbiology laboratory (3). Quantifications of 1-μl and 10-μl mycobacterial samples were previously determined (3). The samples underwent mechanical disruption either by bead-beating for 5 min or by vortexing at maximum speed on a vortex adapter for 15 min. After disruption, samples were incubated for 10 min at room temperature in the upright position. Cells were pelleted by centrifugation to remove the EtOH and resuspended in sterile water for plating. Positive controls were prepared in duplicate for each strain tested by plating a 1-μl loopful of colonies resuspended in sterile water. All plates were monitored for growth. Growth was observed for all positive controls. Since no growth was observed on culture plates after the allotted incubation period, both disruption techniques were bactericidal for mycobacterial and *Nocardia* test strains at either cell density (Tables 1 and 2).

Although this study was performed using solid medium, the 10-μl sample size exceeds the reported biomass recovered from liquid medium samples at the time of positivity, making this inactivation method acceptable for solid and liquid medium samples (5–7). While the Beadbeater provided a more rapid mechan-

TABLE 1 Mycobacterial species inactivated by mechanical disruption techniques

Mycobacterial species tested	No. of strains inactivated by:	
	Beadbeater	Vortex adapter
Mycobacterium tuberculosis		
Antibiotic susceptible	2	2
Antibiotic resistant	3	3
Mycobacterium fortuitum	3	3
Mycobacterium senegalense	1	1
Mycobacterium abscessus	3	3
Mycobacterium intracellulare	3	3
Mycobacterium kansasii	3	3
Mycobacterium avium	2	2
Mycobacterium chelonae	2	2
Mycobacterium gordonae	1	1
Mycobacterium scrofulaceum	1	1
Mycobacterium smegmatis	1	1
Mycobacterium genavense	2	2
Mycobacterium haemophilum	1	1
Total	28	28

TABLE 2 *Nocardia* species inactivated by mechanical disruption techniques

Nocardia species tested	No. of strains inactivated by:	
	Beadbeater	Vortex adapter
Nocardia cyriacigeorgica	9	9
Nocardia farcinica	4	4
Nocardia kruczakiae	2	2
Nocardia nova	7	7
Nocardia otitidiscavarum	1	1
Total	23	23

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ical disruption of the sample, the equipment may be cumbersome for routine applications in a small laboratory setting. The vortex adapter may require three times the disruption time as the Bead-beater but takes advantage of a common piece of laboratory equipment which could be used in a biological safety cabinet.

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