MALDI-TOF Identification of Bacteria Using 70% Formic Acid On-plate Direct Extraction

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Introduction

Rapid identification of suspected pathogens is a critical function of the microbiology laboratory and may lead to better patient management. Most methods for organism identification, including many commercial systems, depend on microbial growth or extended incubation (hours to several days). Extra testing, such as Gram stains and simple biochemical tests, may also be required to complete the identification algorithm.

Analysis of cellular protein patterns by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology allows for rapid identification of organisms. Briefly, a single colony is placed onto the MALDI target plate, an extraction matrix is added, and the target plate is placed into the MALDI mass spectrometer instrument. Laser pulses directed at the isolate-matrix ionize the sample, and the resulting ions are accelerated in a flight tube due to high voltage. These ions (proteins) are then separated according to mass, and the spectral protein profile (mainly ribosomal proteins) is compared to a reference database to obtain the identification.

The entire process is completed within minutes and does not require any incubation. However, a significant number of isolates may not be successfully identified with this direct spot method and may require extraction procedures. These time-consuming tube-based extraction procedures require 70% formic acid and acetonitrile.

We compared the accuracy of the MALDI Biotyper® (Bruker Corporation) direct spot MALDI-TOF method with a method that uses on-plate direct 70% formic acid extraction followed by matrix overlay for identification of bacteria.

Method

1. Isolates (N=190) were freshly grown on agar media under atmospheric and temperature conditions that were appropriate for growth of the specific organism.
2. All initial testing was performed according to the manufacturer's instructions.
   a. Direct Identification: A pure isolate bacterial spot was placed in duplicate on a target plate and air dried. 1 µL of matrix was then added and allowed to air dry, followed by identification with the Biotyper.

Method (cont)

3. 70% formic acid on-plate extraction method:
   a. Pure isolates were directly spotted onto the MALDI target plate in duplicate.
   b. 1 µL of 70% formic acid was added to each spot and allowed to air dry.
   c. 1 µL of extraction matrix was added to each spot and allowed to air dry.
4. A Bruker Bacterial Test Standard for calibration and quality control was included with each run.
5. The inoculated target was placed in the Bruker Biotyper instrument and analyzed using MALDI-TOF to obtain the organism profiles. The MALDI-TOF identifications were compared with the known genus and species identification, which was either obtained from the American Type Culture Collection (ATCC) or previously identified in our laboratories using current methods (including commercial automated systems, conventional testing, or molecular methods).
6. Identification scores were assigned as in Table 1.

Table 1. Bruker Biotyper Score and Identification Level

Score Range	Identification Level
≥2.000	Genus and species
1.700-1.999	Genus only
<1.700	No reliable identification

Results

1. 190 isolates were tested by both the direct spot and 70% formic acid extraction method.
   a. 139 (73.2%) had the same identification category with both methods (Table 2).
   b. Of the 139 isolates, 79 (41.6% of total) had Biotyper scores of ≥2.000, 30 isolates had scores of 1.700-1.999, and another 30 isolates had scores <1.700 (both 15.8% of total).

Table 2. Number of Isolates with Various Biotyper Scores Using the Direct Spot Method and the 70% Formic Acid On-plate Extraction Method

	70% Formic Acid On-plate Extraction		
Direct Spot	≥2.000	≥1.700-1.999	<1.700
≥2.000	79	4	2
≥1.700-1.999	**23**	30	5
<1.700	**5**	**12**	30

Results

2. Forty isolates (21% of the total) showed a category improvement in identification with the on-plate 70% formic acid extraction method compared with the direct spot method.
   a. 30 of these 40 isolates were Gram-positive and 10 were Gram-negative (corrected from abstract; Table 3).
   b. Genus and species identifications were obtained for 28 of the 40 isolates.
   c. 12 isolates that had scores <1.700 with the direct spot method had higher (genus-level) scores of 1.700-1.999 with the on-plate 70% formic acid extraction method. All 12 were correctly identified to the genus and species level, despite the lower genus-level Biotyper scores.
   d. 11 isolates had lower scores with the on-plate extraction method than with the direct spot method. Seven (3.7%) of these 70% formic acid–extracted isolates had scores of <1.700 (no reliable identification). Genus and species were correctly identified for the remaining 4 isolates, all of which had Biotyper scores >1.900 with the 70% formic acid extraction method.

Table 3. Identification with Increased Biotyper Scores Due to 70% Formic Acid Direct Extraction

Biotyper Score Improvement to >2.000 (Genus and Species Identification)	
Aggregatibacter actinomycetemcomitans (2)	*Granulicatella adiacens*
Achromobacter xylosoxidans	*Kochuria rhizophilia*
Actinomyces europaeus	*Lactobacillus rhamnosus* (2)
Actinomyces odontolyticus	*Leuconostoc pseudomesenteroides*
Actinomyces species (2)	*Moraxella osloensis*
Bacillus cereus	*Neisseria gonorrhoeae*
Bacillus licheniformis	*Staphylococcus caprae*
Bacillus subtilis	*Staphylococcus lugdunensis*
Clostridium sporogenes	*Streptococcus anginosus*
Corynebacterium ureolyticum	*Streptococcus constellatus*
Corynebacterium xerosis	*Streptococcus intermedius*
Enterococcus raffinosus (2)	*Streptococcus pneumoniae*

Biotyper Score Improvement to >1.700 (Genus Identification*)	
Bacillus sonorensis	*Gordonia/Rhodococcus*
Capnocytophaga canimorus	*Moraxella atlantae*
Cardiobacterium hominis	*Neisseria elongata*
Corynebacterium afermentans	*Streptococcus anginosus* (2)
Corynebacterium tuberculostericum	*Vibrio albensis*
Corynebacterium ureolyticum	

*All genus only–level identifications were correct to both genus and species level.

Conclusions

- Direct on-plate extraction of isolates using 70% formic acid improved identification by >20% compared with that obtained using the direct spot method.
- On-plate formic acid extraction has the further advantage of not requiring an additional tube extraction step, which is time consuming and cumbersome, and requires larger-scale use of solvents (ethanol, formic acid, acetonitrile).

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