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# Letters to the Editor

## Improved Performance of Bacterium and Yeast Identification by a Commercial Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System in the Clinical Microbiology Laboratory[▽]

Bizzini et al. described the use of a commercial matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) system as a reliable, fast, and efficient method for the identification (ID) of bacteria and yeasts (1). Among 1,371 isolates identified by conventional methods, 1,037 (75.6%) isolates were identified by MALDI-TOF to either the species or genus level by direct analysis of a colony. A small amount of colony was smeared onto a gridded steel plate, allowed to dry briefly, and overlaid with 1 μl of HCCA (α-cyano-4-hydroxycinnamic acid) matrix before analysis in a Bruker Microflex MS system. For isolates which are not identified by this direct method, Bizzini et al. used an extraction method in which colonies were suspended in water and alcohol and centrifuged and bacterial proteins in the deposit were extracted with formic acid and acetonitrile before analysis (1).

We have been using a similar approach in the diagnostic laboratory at the Royal London Hospital, a tertiary referral hospital which serves a population of 2 million people in East London. Here we show that by a minor modification of the direct analysis method, it is possible to increase the number of successfully identified isolates without recourse to the alcohol extraction protocol, which, although simple, significantly increases the processing time.

We analyzed 2,020 isolates drawn from a routine diagnostic microbiology laboratory by the recommended direct smear method on a Bruker Microflex MS with the Biotyper software (version 2.0, database 3,740) and automation control. Approximately 42% of these isolates were Gram-negative bacilli, 35% staphylococci, 16% streptococci, 2% Gram-positive bacilli, 2% Gram-negative cocci, and 3% yeasts. Of the 2,020 isolates analyzed directly, 1,784 (88.3%) were acceptably identified to at least the genus level by the direct smear method. We accepted all scores of >1.7 as a valid identification to the genus level, based on the manufacturer's recommendations and our experience during the initial validation of this technology. The 236 isolates which did not give an acceptable ID (with scores of <1.7), mainly Gram-positive bacilli, coagulase-negative staphylococci, yeasts, and anaerobes, were then retested, with the additional step of adding 1 μl of neat formic acid to the air-dried bacterial spot. This was then allowed to air dry before the addition of the matrix and analyzed in the usual way. This approach yielded a further 220 (10.9%) reliable identifications, leaving only 16 (0.8%) without a reliable ID, and these were then analyzed by alcohol extraction. Seven isolates did not give a satisfactory identification by alcohol extraction; two of these were regarded as being of little clinical significance, and no attempt was made to identify them further. The remaining five isolates were identified as *Fusobacterium nucleatum*, *Prevotella oris*, *Paenibacillus barengoltzii*, *Actinomyces lignae*, *Actinomyces odontolyticus*, and *Pseudomonas aeruginosa* by sequencing 1,250- to 1,300-bp fragments of the 16S rRNA gene. Neither of the two anaerobic Gram-negative bacilli, *F. nucleatum* and *P. oris*, grew well, and the failure to obtain an ID by MALDI-TOF was probably due to lack of adequate growth. The protein spectra of at least one isolate each of *P. barengoltzii*, *A. lignae*, and *A. odontolyticus* are present in the MALDI-TOF database used, but this may not represent the range of different protein patterns which are present in members of that species. The failure to identify *P. aeruginosa* is more surprising, but the isolate, from a patient with cystic fibrosis, was extremely mucoid, and repeated alcohol extractions and analysis were never successful.

With the addition of *in situ* cell lysis by formic acid, we have shown that it is possible to increase the successful identification rate to 99.2% using a direct smear method without having to resort to the more time-consuming alcohol extraction method. In our experience, yeasts and Gram-positive bacilli, which are least likely to give a valid result by direct smear analysis, should be processed immediately with the formic acid step, thereby reducing processing time.

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### REFERENCE

1. **Bizzini, A., C. Durussel, J. Bille, G. Greub, and G. Prod'hom.** 2010. Performance of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. J. Clin. Microbiol. **48:**1549–1554.

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