

MALDI-TOF Mass Spectrometry for Routine Identification of Medically Relevant Bacteria

H. DUSCH, K. BURREN, H.P. HINRIKSON
Unilabs Mittelland, Berne, Switzerland

BACKGROUND

MALDI-TOF MS (matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry) of ribosomal proteins is assumed to provide faster and more accurate identification of bacterial pathogens compared to conventional microbiological investigations. However, experience with this new technology in routine diagnostic settings is still limited.

MATERIALS & METHODS

A total of 829 strains comprising clinical isolates and reference strains were investigated to assess the identification performance of MALDI-TOF MS (Microflex instrument and Biotyper reference database version 2.0 by Bruker Daltonics Inc.) compared to established routine procedures. These included selective and chromogenic media (for enteric bacteria, enterococci and Candida species), catalase, oxidase, plasma-coagulase, slide agglutination (Salmonella, Shigella, beta-hemolytic streptococci), indol spot, API strips and Vitek cards. Discrepant results between MALDI-TOF MS and routine methods were further analyzed by comparative 16S rDNA sequence analysis.

RESULTS

Investigations by MALDI-TOF MS required significantly less biomass and time-to-result compared to conventional testing. Minute parts of a single colony provided sufficient material for identification of whole cells in less than 30 minutes. The following major pathogens were always correctly identified: *Escherichia coli* (n=79), *Pseudomonas aeruginosa* (n=65), *Burkholderia cepacia*-complex (n=35), *Staphylococcus aureus* (n=21), *Enterococcus faecium* (n=12), *Campylobacter jejuni* (n=17) and *Campylobacter coli* (n=3). In contrary, MALDI-TOF MS repeatedly misidentified *Shigella* species as *Escherichia coli*, *Streptococcus mitis* as *Streptococcus pneumoniae*, and *Propionibacterium acnes* as *Eubacterium brachy*. The overall identification rate of MALDI-TOF MS at species level was 94.8 % for enteric bacteria, 81.9 % for Gram-negative nonfermenters, 100 % for Gram-positive catalase-positive cocci, 90.8 % for Gram-positive catalase-negative rods, 73.4 % for Gram-positive catalase-negative cocci and 77.1 % for obligate anaerobes (97.1% when *Propionibacterium acnes* was excluded from analysis).

CONCLUSIONS

The MALDI-TOF MS-based identifications were obtained much faster than those derived from commercial biochemical test kits such as API strips and Vitek cards.

The MALDI-TOF MS-based approach did not require prior knowledge of the type of bacteria to be tested unlike established routine identification methods.

The MALDI-TOF MS procedure required significantly less biomass and reagents compared to established routine identification methods.

The MALDI-TOF MS is projected to replace most conventional biochemical testing, and thus may allow significant savings in terms of reagent costs and turn-around-time.

The MALDI-TOF MS cannot currently identify some important human pathogens such as *Shigella* species and *Streptococcus pneumoniae* which still require additional confirmatory testing.

Table 1 Gram-positive cocci (n=159)

For 145 strains the complete identification (genus + species) was available, 14 strains were known only at genus level

	fully correct	correct genus	wrong genus	no result
catalase-positive				
<i>Kocuria</i> sp. (1)	-	0	0	1
<i>Micrococcus luteus</i> (1)	1	0	0	0
<i>Staphylococcus aureus</i> (21)	21	0	0	0
<i>Staphylococcus epidermidis</i> (15)	15	0	0	0
<i>Staphylococcus capitis</i> (6)	6	0	0	1
<i>Staphylococcus haemolyticus</i> (4)	4	0	0	1
<i>Staphylococcus hominis</i> (9)	9	0	0	0
<i>Staphylococcus lugdunensis</i> (4)	4	0	0	1
<i>Staphylococcus saccharolyticus</i> (2)	2	0	0	0
<i>Staphylococcus saprophyticus</i> (4)	4	0	0	0
<i>Staphylococcus coagulans</i> (4)	64	3	0	5
catalase-negative				
<i>Aerococcus sanguinicola</i> (5)	5	0	0	1
<i>Aerococcus urinae</i> (6)	1	1	0	4
<i>Enterococcus faecalis</i> (4)	4	0	0	0
<i>Enterococcus faecium</i> (12)	12	0	0	0
<i>Enterococcus</i> sp. (6)	0	0	0	0
<i>Facklamia hominis</i> (1)	0	0	0	1
<i>Helicobacterium</i> sp. (1)	0	0	0	0
<i>Streptococcus agalactiae</i> (6)	6	0	0	0
<i>Streptococcus anginosus</i> (4)	3	1	0	0
<i>Streptococcus constellatus</i> (4)	2	2	0	0
<i>Streptococcus intermedius</i> (4)	2	2	0	0
<i>Streptococcus bovis</i>	0	3	0	0
<i>Streptococcus pasteurianus</i> (1)	0	1	0	0
<i>Streptococcus dysgalactiae</i> (1)	0	1	0	0
<i>Streptococcus equinus</i> (1)	0	1	0	0
<i>Streptococcus gordonii</i> (2)	1	1	0	0
<i>Streptococcus mitis</i> (5)	0	5	0	0
<i>Streptococcus oralis</i> / <i>mitis</i> (3)	1	2	0	0
<i>Streptococcus mutans</i> (1)	0	0	0	0
<i>Streptococcus oralis</i> (2)	1	1	0	0
<i>Streptococcus pneumoniae</i> (4)	0	0	0	0
<i>Streptococcus pyogenes</i> (4)	0	0	0	0
<i>Streptococcus salivarius</i> (3)	0	0	0	0
<i>Streptococcus sanguinis</i> (1)	0	0	0	0
<i>Streptococcus</i> sp. (3)	-	0	0	2
	51	28	0	8

Legend :

- 1) on repetition correct result
- 2) reported *Aerococcus sanguinicola*
- 3) reported *Streptococcus intermedius*
- 4) reported *Streptococcus anginosus*
- 5) reported *Streptococcus galloyticus*
- 6) reported *Streptococcus pneumoniae*
- 7) *Streptococcus pneumoniae* (1), *Streptococcus* sp. (1)
- 8) reported *Streptococcus canis*
- 9) reported *Streptococcus* sp.

catalase-positives:

valid MALDI-results 67 / 72 = 92.9 %
 same result as routine tests 67 / 67 = 100.0 %

catalase-negatives :

valid MALDI-results 79 / 87 = 90.8 %
 same result as routine tests 58 / 79 = 73.4 %

Table 2 Enteric bacteria (n=211)

For 192 strains the complete identification (genus + species) was available, 19 strains were known only at genus level

	fully correct	correct genus	wrong genus	no result
<i>Cedecea davisae</i> (1)	1	0	0	0
<i>Citrobacter amalonaticus</i> (1)	0	0	0	0
<i>Citrobacter braakii</i> (1)	0	0	0	0
<i>Citrobacter freundii</i> (9)	0	0	0	0
<i>Citrobacter koseri</i> (5)	0	0	0	0
<i>Citrobacter</i> sp. (2)	-	0	0	0
<i>Enterobacter aerogenes</i> (4)	4	0	0	0
<i>Enterobacter cloacae</i> (4)	4	0	0	0
<i>Enterobacter</i> sp. (3)	-	3	0	0
<i>Erwinia persicina</i> (2)	2	0	0	0
<i>Escherichia coli</i> (79)	79	0	0	0
<i>Escherichia hermannii</i> (3)	0	0	0	0
<i>Escherichia</i> sp. (3)	0	0	0	0
<i>Ewingella americana</i> (1)	0	0	0	0
<i>Halofila alvei</i> (11)	11	0	0	0
<i>Klebsiella oxytoca</i> (13)	13	0	0	0
<i>Klebsiella pneumoniae</i> (13)	12	1	0	0
<i>Morganella morganii</i> (4)	4	0	0	0
<i>Mantoea agglomerans</i> (1)	0	0	0	0
<i>Prateus mirabilis</i> (7)	7	0	0	0
<i>Prateus vulgaris</i> (2)	2	0	0	0
<i>Providencia alcalifaciens</i> (2)	2	0	0	0
<i>Rahnella aquatilis</i> (1)	0	0	0	0
<i>Raoultella ornithinolytica</i> (7)	0	0	0	0
<i>Salmonella</i> sp. (11)	-	0	0	0
<i>Serratia liquefaciens</i> (1)	1	0	0	0
<i>Serratia marcescens</i> (13)	13	0	0	0
<i>Shigella boydii</i> (3)	0	0	0	0
<i>Shigella sonnei</i> (1)	0	0	0	0
<i>Yersinia enterocolitica</i> (3)	3	0	0	0
	187	17	7	0

Legend :

- 1) reported *Raoultella ornithinolytica*
- 2) reported *Klebsiella pneumoniae*
- 3) reported *Escherichia coli*

valid MALDI-results 211 / 211 = 100.0 %
 same result as routine tests 200 / 211 = 94.8 %

Table 3 Gram-negative nonfermentative rods (n= 229)

For 197 strains the complete identification (genus + species) was available, 32 strains were known only at genus level

	fully correct	correct genus	wrong genus	no result
<i>Achromobacter denitrificans</i> (7)	7	0	0	0
<i>Achromobacter</i> sp. (6)	-	0	0	0
<i>Achromobacter xylosoxidans</i> (30)	17	11	2	1
<i>Acinetobacter baumannii</i> (3)	3	0	0	0
<i>Acinetobacter johnsonii</i> (1)	0	1	0	0
<i>Acinetobacter junii</i> (1)	0	0	0	0
<i>Acinetobacter lawffii</i> (6)	6	0	0	0
<i>Acinetobacter</i> sp. (13)	-	0	0	0
<i>Alcaligenes faecalis</i> (2)	2	0	0	0
<i>Bordetella petrii</i> (1)	0	1	0	0
<i>Burkholderia cenocepacia</i> (18)	18	0	0	0
<i>Burkholderia cepacia</i> -Komplex (5)	5	0	0	0
<i>Burkholderia gladioli</i> (8)	8	0	0	0
<i>Burkholderia glathei</i> (1)	1	0	0	0
<i>Burkholderia multivorans</i> (4)	4	0	0	0
<i>Brevundimonas</i> sp. (2)	-	0	0	1
<i>Chryseobacterium indologenes</i> (2)	2	0	0	0
<i>Chryseobacterium</i> sp. (3)	0	0	0	3
<i>Comamonas</i> sp. (2)	0	0	0	2
<i>Ochrobactrum anthropi</i> (3)	3	0	0	0
<i>Ochrobactrum intermedium</i> (3)	1	2	0	0
<i>Ochrobactrum</i> sp.	-	0	0	0
<i>Oligella urethralis</i> (3)	3	0	0	0
<i>Pandoraea apista</i> (1)	1	0	0	0
<i>Pseudomonas aeruginosa</i> (65)	65	0	0	0
<i>Pseudomonas chloraphis</i> (3)	0	3	0	0
<i>Pseudomonas fluorescens</i> (2)	2	0	0	0
<i>Pseudomonas mendocina</i> (4)	3	0	0	1
<i>Pseudomonas putida</i> (6)	0	6	0	0
<i>Ralstonia pickettii</i> (1)	0	0	0	0
<i>Rhizobium radiobacter</i> (1)	1	0	0	0
<i>Sphingobacterium multivorum</i> (1)	1	0	0	0
<i>Sphingobacterium spiritivorum</i> (1)	1	0	0	0
<i>Sphingomonas paucimobilis</i> (4)	4	0	0	0
<i>Stenotrophomonas maltophilia</i> (15)	10	1	0	4
	160	55	6	8

Legend:

- 1) *Achromobacter piechaudii* (6), *Achromobacter spanius* (1)
- 2) *Achromobacter piechaudii* (1), *Achromobacter ruhländii* (9), *Achromobacter spanius* (1)
- 3) *Bordetella bronchiseptica*
- 4) *Bordetella bronchiseptica*
- 5) *Ochrobactrum grignonense*
- 6) *Pseudomonas aurantiaca*
- 7) *Alcaligenes* sp.
- 8) *Pseudomonas hisibicola* (3) and *Pseudomonas beteli* (1), both are old names for *Stenotrophomonas maltophilia*

valid MALDI-results 221 / 229 = 96.5 %
 same result as routine tests 181 / 221 = 81.9 %

Table 5 Other Gram-negative rods / Campylobacter (n=47)

For 66 strains the complete identification (genus + species) was available, 2 strains were known only at genus level

	fully correct	correct genus	wrong genus	no result
<i>Aeromonas caviae</i> (2)	2	0	0	0
<i>Aeromonas hydrophila</i> (1)	1	0	0	0
<i>Aeromonas veronii</i> bv. <i>sabria</i> (1)	0	1	0	0
<i>Aggregatibacter aphrophilus</i> (4)	3	0	0	1
<i>Campylobacter coli</i> (3)	3	0	0	0
<i>Campylobacter fetus</i> (1)	1	0	0	0
<i>Campylobacter jejuni</i> (17)	17	0	0	0
<i>Capnocytophaga canimorsus</i> (2)	2	0	0	0
<i>Eikenella corrodens</i> (1)	1	0	0	0
<i>Haemophilus influenzae</i> (8)	8	0	0	0
<i>Haemophilus parainfluenzae</i> (3)	3	0	0	0
<i>Kingella denitrificans</i> (1)	1	0	0	0
<i>Legionella pneumophila</i> (1)	1	0	0	0
<i>Maraxella catarhalis</i> (3)	3	0	0	0
<i>Neisseria animalaris</i> / <i>zoodegmatidis</i> (1)	0	0	0	1
<i>Neisseria flavescens</i> (1)	1	0	0	0
<i>Neisseria gonorrhoeae</i> (6)	6	0	0	0
<i>Neisseria</i> sp. (2)	-	0	0	2
<i>Pasteurella multocida</i> (4)	4	0	0	0
<i>Plesiomonas shigelloides</i> (3)	3	0	0	0
<i>Vibrio alginolyticus</i> (1)	1	0	0	0
<i>Vibrio metschnikovii</i> (1)	1	0	0	0
<i>Vibrio parahaemolyticus</i> (1)	1	0	0	0
	63	2	1	2

Legend :

- 1) reported as *Haemophilus influenzae*, on repetition correct result

valid MALDI-results 66 / 68 = 97.1 %
 same result as routine tests 64 / 66 = 97.0 %

Table 4 Gram-positive rods and Actinomycetes sp. (n= 103)

For 81 strains the complete identification (genus + species) was available, 22 strains were known only at genus level

	fully correct	correct genus	wrong genus	no result
<i>Actinobaculum schaalii</i> (5)	5	0	0	1
<i>Actinomyces europaeus</i> (1)	1	0	0	0
<i>Actinomyces graevenitzii</i> (4)	0	4	0	0
<i>Actinomyces israelii</i> (1)	0	0	0	1
<i>Actinomyces meyeri</i> (1)	0	0	0	0
<i>Actinomyces neuii</i> (4)	4	0	0	0
<i>Actinomyces odontolyticus</i> (1)	0	0	0	1
<i>Actinomyces</i> sp. (2)	-	0	0	0
<i>Actinomyces turicensis</i> (1)	1	0	0	0
<i>Arcanobacterium bernardiae</i> (2)	2	0	0	0
<i>Arcanobacterium haemolyticum</i> (2)	2	0	0	0
<i>Bacillus pumilus</i> (4)	4	0	0	1
<i>Bacillus</i> sp. (6)	-	0	0	3
<i>Bacillus subtilis</i> (2)	2	0	0	1
<i>Brachybacterium</i> sp. (2)	-	0	0	2
<i>Brevibacterium</i> sp. (1)	1	0	0	1
<i>Corynebacterium afermentans</i> (1)	1	0	0	0
<i>Corynebacterium striatum</i> / <i>amycolatum</i> (6)	6	0	0	0
<i>Corynebacterium coyleae</i> (1)	1	0	0	0
<i>Corynebacterium diphtheriae</i> (3)	3	0	0	0
<i>Corynebacterium</i> sp. (5)	-	0	0	0
<i>Corynebacterium tuberculoostearicum</i> (4)	4	0	0	2
<i>Dermabacter hominis</i> (1)	1	0	0	0
<i>Erysipelothrix rhusiopathiae</i> (1)	1	0	0	0
<i>Gardnerella vaginalis</i> (9)	9	0	0	2
<i>Lactobacillus rhamnosus</i> (3)	3	0	0	0
<i>Lactobacillus</i> sp. (2)	-	0	0	0