

D-1285 Large Scale Performance Evaluation of MALDI-ToF MS in a Central Reference Laboratory Setting

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Introduction

MALDI-ToF is a reliable and rapid method for identification of bacteria and is fast becoming the preferred method in clinical laboratories [1- 4]. Since its installation in our laboratory in July 2010 we have analysed and identified over twelve thousand clinical isolates using the MALDI-ToF MicroFlex Biotyper developed by Bruker Daltonik, GmbH. The data presented herein constitutes the largest performance validation of the Bruker Biotyper to date.

Methodology

Isolates

The 12000 clinical isolates were from recent veterinary and human disease from both community and nosocomial origins covering over 100 hospitals and 40 countries worldwide. Of these 12000 isolates a total of 9723 had been previously identified by collecting hospitals to species level using conventional identification methods. Clinical pathologies for the test isolates included bacteraemia, urinary-tract infections, respiratory-tract infections and skin and soft tissue infections.

Sample preparation

The study was conducted using the Bruker procedure for bacterial identification using a Microflex MALDI-ToF (Bruker Daltonik GmbH) mass spectrometer [3]. Samples for analysis were prepared by direct smear method involving the application of a small amount of biological material directly on a 96-well MALDI target plate in a thin smear. The thin microbial film was overlaid with 1µL of a saturated alpha-cyano-4-hydroxycinnamic acid matrix solution and allowed to air dry.

Identification by MALDI-ToF MS

Mass spectra were acquired using a Microflex MALDI-ToF mass spectrometer. Measured mass range of spectra was from 2000 to 20,000 Da. Spectra acquisition was performed using Bruker Biotyper data library v2.0 build 223.8 (3741 entries). A primary and secondary ID and score value are produced for each test isolate. The primary ID is the best identification match to the reference library and the corresponding primary score is a confidence value representing the similarity match of a test isolate to reference strain mass spectra. The secondary ID and score value represent the second best ID match and confidence value for the test isolate against the reference library. Results were reported as the primary ID and corresponding score value. Score values are ranked on an exponential scale from 0 – 3 with the following ID confidence acceptance criteria;



Results

Table 1.

Order	Biotyper Identification	N	N Species	MALDI Biotyper ID Log score Range	N (%) of Isolates with MALDI Biotyper ID Log score				Conventional ID Concordance to Biotyper			
					Very good (2.20)	Good (2.19 - 2.00)	Acceptable (1.99 - 1.70)	Poor (≤1.69)	genus N %	species N %	genus N %	species N %
Actinomycetales	<i>Corynebacterium</i>	12	3	1.99 - 2.45	10 83.3%	1 8.3%	1 8.3%	0 0.0%	10 83.3%	9 75.0%	10 83.3%	9 75.0%
	<i>Micrococcus</i>	4	1	1.85 - 2.16	0 0.0%	2 50.0%	2 50.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
Aeromonadales	<i>Aeromonas</i>	1	1	2.25	1 100.0%	0 0.0%	0 0.0%	0 0.0%	1 100.0%	0 0.0%	1 100.0%	0 0.0%
Bacillales	<i>Staphylococcus aureus</i>	2429	1	1.71 - 2.48	1722 70.9%	556 22.9%	151 6.2%	0 0.0%	2428 100.0%	2428 100.0%	2428 100.0%	2428 100.0%
	Coagulase-negative Staphylococci	257	14	1.73 - 2.49	80 31.1%	143 55.6%	34 13.2%	0 0.0%	252 98.1%	237 92.2%	252 98.1%	237 92.2%
	<i>Bacillus</i>	2	2	1.93 - 2.40	1 50.0%	0 0.0%	1 50.0%	0 0.0%	2 100.0%	2 100.0%	2 100.0%	2 100.0%
	<i>Paenibacillus</i>	1	1	2.29	1 100.0%	0 0.0%	0 0.0%	0 0.0%	1 100.0%	0 0.0%	1 100.0%	0 0.0%
Bacteroidales	<i>Bacteroides</i>	1	1	2.41	1 100.0%	0 0.0%	0 0.0%	0 0.0%	1 100.0%	1 100.0%	1 100.0%	1 100.0%
Burkholderiales	<i>Neisseria</i>	32	6	1.72 - 2.58	17 53.1%	10 31.3%	5 15.6%	0 0.0%	24 75.0%	19 59.4%	24 75.0%	19 59.4%
	<i>Bordetella</i>	2	1	2.20 - 2.30	1 50.0%	1 50.0%	0 0.0%	0 0.0%	2 100.0%	1 50.0%	2 100.0%	1 50.0%
	<i>Burkholderia</i>	2	2	2.39 - 2.51	2 100.0%	0 0.0%	0 0.0%	0 0.0%	1 50.0%	1 50.0%	1 50.0%	1 50.0%
	<i>Achromobacter</i>	1	1	2.10	0 0.0%	1 100.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
	<i>Cupriavidus</i>	1	1	1.84	0 0.0%	0 0.0%	1 100.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
Campylobacteriales	<i>Campylobacter</i>	65	2	1.47 - 2.40	30 46.2%	18 27.7%	8 12.3%	9 13.8%	64 98.5%	61 93.8%	64 98.5%	61 93.8%
Clostridiales	<i>Clostridium</i>	2	2	2.10 - 2.41	1 50.0%	1 50.0%	0 0.0%	0 0.0%	2 100.0%	2 100.0%	2 100.0%	2 100.0%
Enterobacteriales	<i>Escherichia coli</i>	1146	1	1.63 - 2.63	885 77.2%	208 18.2%	50 4.4%	3 0.3%	1118 97.6%	1117 97.5%	1118 97.6%	1117 97.5%
	<i>Klebsiella</i>	696	3	1.72 - 2.61	497 71.4%	138 19.8%	61 8.8%	0 0.0%	664 95.4%	638 91.7%	664 95.4%	638 91.7%
	<i>Enterobacter</i>	442	8	1.71 - 2.56	290 65.6%	121 27.4%	31 7.0%	0 0.0%	418 94.6%	355 80.3%	418 94.6%	355 80.3%
	<i>Citrobacter</i>	246	6	1.75 - 2.64	194 78.9%	41 16.7%	11 4.5%	0 0.0%	229 93.1%	223 90.7%	229 93.1%	223 90.7%
	<i>Morganella</i>	222	1	1.80 - 2.72	213 95.9%	7 3.2%	2 0.9%	0 0.0%	209 94.1%	209 94.1%	209 94.1%	209 94.1%
	<i>Serratia</i>	221	3	1.71 - 2.48	125 56.6%	70 31.7%	26 11.8%	0 0.0%	206 93.2%	200 90.5%	206 93.2%	200 90.5%
	<i>Proteus</i>	62	2	1.83 - 2.53	51 82.3%	9 14.5%	2 3.2%	0 0.0%	57 91.9%	57 91.9%	57 91.9%	57 91.9%
	<i>Raoultella</i>	13	2	1.99 - 2.55	9 69.2%	2 15.4%	2 15.4%	0 0.0%	13 100.0%	3 23.1%	13 100.0%	3 23.1%
	<i>Salmonella</i>	4	1	2.05 - 2.47	3 75.0%	1 25.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
	<i>Providencia</i>	2	2	2.12 - 2.17	0 0.0%	2 100.0%	0 0.0%	0 0.0%	1 50.0%	1 50.0%	1 50.0%	1 50.0%
	<i>Buttiauxella</i>	1	1	2.15	0 0.0%	1 100.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
	<i>Leclercia</i>	1	1	2.47	1 100.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
	<i>Pantoea</i>	1	1	2.05	0 0.0%	1 100.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
	<i>Yersinia</i>	1	1	2.29	1 100.0%	0 0.0%	0 0.0%	0 0.0%	1 100.0%	1 100.0%	1 100.0%	1 100.0%
Flavobacteriales	<i>Bergeyella</i>	1	1	2.07	0 0.0%	1 100.0%	0 0.0%	0 0.0%	1 100.0%	1 100.0%	1 100.0%	1 100.0%
Lactobacillales	α-haemolytic Streptococci	1061	7	1.70 - 2.57	751 70.8%	212 20.0%	98 9.2%	0 0.0%	1056 99.5%	1042 98.2%	1056 99.5%	1042 98.2%
	β-haemolytic Streptococci	128	7	1.73 - 2.43	39 30.5%	64 50.0%	25 19.5%	0 0.0%	124 96.9%	80 62.5%	124 96.9%	80 62.5%
	<i>Streptococcus pneumoniae</i>	248	1	1.71 - 2.41	76 30.6%	92 37.1%	80 32.3%	0 0.0%	247 99.6%	211 85.1%	247 99.6%	211 85.1%
	<i>Enterococcus</i>	478	11	1.57 - 2.57	341 71.3%	111 23.2%	24 5.0%	2 0.4%	473 99.0%	412 86.2%	473 99.0%	412 86.2%
	<i>Aerococcus</i>	1	1	1.82	0 0.0%	0 0.0%	1 100.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
Legionellales	<i>Legionella</i>	7	1	2.15 - 2.37	4 57.1%	3 42.9%	0 0.0%	0 0.0%	7 100.0%	7 100.0%	7 100.0%	7 100.0%
	<i>Malassezia</i>	1	1	2.09	0 0.0%	1 100.0%	0 0.0%	0 0.0%	1 100.0%	1 100.0%	1 100.0%	1 100.0%
	<i>Haemophilus</i>	701	4	1.71 - 2.53	537 76.6%	120 17.1%	44 6.3%	0 0.0%	700 99.9%	665 94.9%	700 99.9%	665 94.9%
	<i>Pasteurella</i>	12	3	2.17 - 2.37	10 83.3%	2 16.7%	0 0.0%	0 0.0%	12 100.0%	10 83.3%	12 100.0%	10 83.3%
	<i>Actinobacillus</i>	11	3	2.12 - 2.31	4 36.4%	7 63.6%	0 0.0%	0 0.0%	10 90.9%	10 90.9%	10 90.9%	10 90.9%
Pasteurellales	<i>Histophilus</i>	2	1	1.84 - 1.94	0 0.0%	0 0.0%	2 100.0%	0 0.0%	2 100.0%	2 100.0%	2 100.0%	2 100.0%
	<i>Aggregatibacter</i>	1	1	2.03	0 0.0%	1 100.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
	<i>Pseudomonas</i>	659	4	2.00 - 2.48	555 84.2%	104 15.8%	0 0.0%	0 0.0%	651 98.8%	648 98.3%	651 98.8%	648 98.3%
Pseudomonadales	<i>Moraxella</i>	376	3	1.72 - 2.46	265 70.5%	87 23.1%	24 6.4%	0 0.0%	367 97.6%	366 97.3%	367 97.6%	366 97.3%
	<i>Acinetobacter</i>	155	8	1.81 - 2.55	117 75.5%	30 19.4%	8 5.2%	0 0.0%	146 94.2%	143 92.3%	146 94.2%	143 92.3%
	<i>Saccharomycetales Candida</i>	4	3	1.80 - 2.53	2 50.0%	0 0.0%	2 50.0%	0 0.0%	4 100.0%	4 100.0%	4 100.0%	4 100.0%
Xanthomonadales	<i>Stenotrophomonas</i>	7	1	1.71 - 2.27	1 14.3%	5 71.4%	1 14.3%	0 0.0%	5 71.4%	5 71.4%	5 71.4%	5 71.4%
Total		9723	133	1.31 - 2.72	6838 70.3%	2174 22.4%	697 7.2%	14 0.1%	9509 97.8%	9172 94.3%	9509 97.8%	9172 94.3%

Results & Discussion

The Bruker MALDI-ToF Biotyper performed well in our laboratory with results very similar to published literature. Our data (Table 1) show 97.8% and 94.3% identification to genus and species levels respectively. Sogawa *et al*, 2011 [5] report 97.0% and 91.7% respectively (N=468), Eigner *et al*, 2009 [1] report 95.2% identification to the species level (N=1062) and Seng *et al*, 2009 [2] report 95.4% correct identification (N=1660).

Seng *et al*, 2009 also reported accuracy to species level for the following as Enterobacteriaceae (95.5%), non-fermenting rods (79.7%), staphylococci (99.5%), enterococci (100%) and streptococci (93.7%).

Our data appear very similar for Enterobacteriaceae (92.2%), streptococci (92.8%) and staphylococci (99.2%). Slightly higher results were seen for non-fermenting rods (97.2%) and slightly lower results for enterococci (86.2%). However, in published literature the sample size tested is considerably smaller than the analysis presented here.

Conclusions

- The data presented herein constitutes the largest performance evaluation of the Bruker Biotyper to date.
- The Biotyper provides a rapid and reliable identification of clinically relevant bacteria with reproducible results.
- High differentiation power and fast turnaround time support the use of MALDI-ToF technology for our bacterial identification at Quotient Bioresearch Ltd.
- We suggest that MALDI-ToF technology may well replace conventional bacterial identification in reference and clinical microbiology laboratories in the future.

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References

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