Use of mass spectometry for the identification of anaerobic bacteria, change anaerobic bacteriology?

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Workflow MALDI-TOF MS

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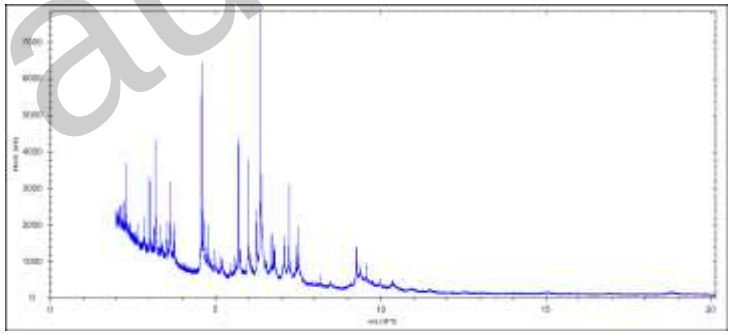
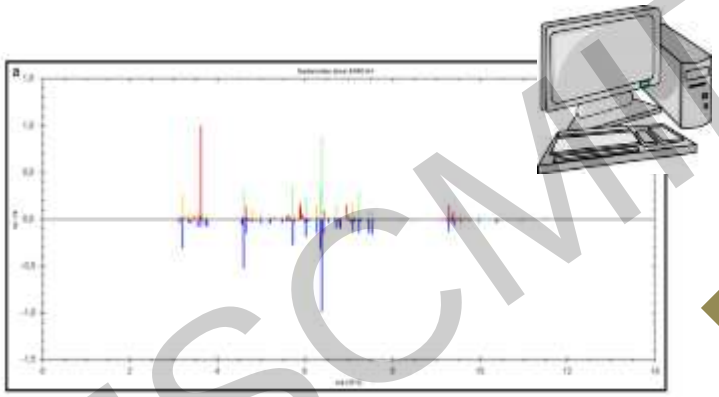
Direct spotting of bacteria on target using a toothpick

Add HCCA matrix

Data acquisition



Data analyses



Log score:	
<1.7	no reliable identification
1.7 – 2.0	identification with low confidence
≥ 2.0	identification with high confidence

Anaerobic culture

Phenotypic

primary incubation
2-7 days

pure culture
2 days

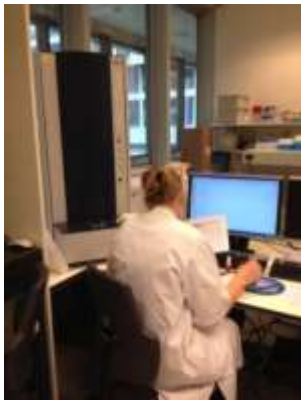
aerotolerance
1 day

identification
2-14 days

MALDI-TOF MS

primary incubation
2-7 days

MALDI-TOF MS testing
minutes



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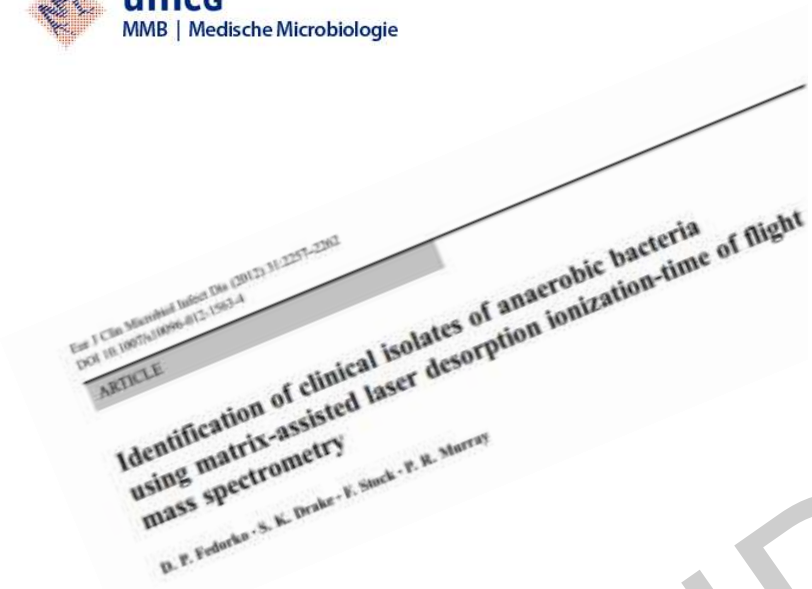

Eur J Clin Microbiol Infect Dis (2012) 31:2257–2262
DOI 10.1007/s10096-012-1583-4

ARTICLE

Identification of clinical isolates of anaerobic bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry

D. P. Fedorko · S. K. Drake · F. Stock · P. R. Murray

Anaerobe 22 (2013) 123–125

Contents lists available at SciVerse ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Note

Improving the identification of anaerobes in the clinical microbiology laboratory through MALDI-TOF mass spectrometry

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ABSTRACT

In this study, 1325 anaerobes were analyzed by MALDI-TOF MS. Of these, 92.5% were correctly identified at the species level. One unidentified species and several uncommon and rare species were identified. These results show that this technique has become the new gold standard for the routine identification of clinical anaerobes.

Journal of Medical Microbiology (2012), 61, 1393–1400

DOI 10.1099/jmm.0.043927-0

The value of MALDI-TOF MS for the identification of clinically relevant anaerobic bacteria in routine laboratories

Elisabeth Nagy,[1] Simone Becker,[2] Markus Kostrzewa,[2] Noémi Barta[1] and Edit Urbán[1]

The reliability of the identification obtained with MALDI-TOF MS is superior over phenotypic methods

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Situation 2012/2013

1. ± 60-70 % of the anaerobes can be identified by MALDI-TOF MS

2. Performance differs per species/genus

3. MALDI-TOF MS database needs optimization for the identification of anaerobic bacteria

   - addition of spectra to existing database increases performance
   - reference strains
   - clinical isolates

Example from literature

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Species identification of clinical *Prevotella* isolates by Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry

Wybo et al. J. Clin. Microbiol. 2012; 50:1415-1418

Commercial reference database:
- **63 % correct species identification**
- **11 % correct genus identification**
- **26 % no identification**

Commercial database + house made MSPs:
- **83 % correct species identification**
- **6 % correct genus identification**
- **11 % no identification**

14 % due to the addition of MSPs of species not yet present in database

6 % due to the addition of MSPs of species already present in database

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ENRIA

European Network for the Rapid Identification of Anaerobes

Lead:
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In collaboration with:
Dr. W. Pusch (Bruker, Germany)
Dr. M. Kostrzewa (Bruker Germany)

ENRIA project

Expertise laboratories:

- **UMCG, Groningen, The Netherlands**
- **University of Szeged, Szeged, Hungary**
- **Odense University Hospital, Odense, Denmark**
- **University Hospital Brussels, Brussels, Belgium**
- **Health Protection Agency, London, England**
- **Praticien Hospitalier, Montpellier, France**
- **Public Health Wales, Cardiff, Wales**

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Accomplished so far:

Collected: ± 650 strains representing 258 species

Optimization for the identification of gram-positive anaerobic cocci (GPAC)

Present: 43 MSP's

Added: 110 MSP's

16 new species were added

Species	No. of MSP's present	added	total
Peptostreptococcus stomatis	0	5	5
Peptostreptococcus anaerobius	4	3	7
Peptostreptococcus canis	0	2	2
Peptococcus niger	1	5	6
Finegoldia magna	11	0	11
Murdochiella asaccharolyticus	0	3	3
Parvimonas micra	7	0	7
Anaerococcus murdochii	1	8	9
Anaerococcus degeneri	0	2	2
Anaerococcus lactolyticus	1	4	5
Anaerococcus tetradius	2	4	6
Anaerococcus prevotii	2	2	4
Anaerococcus vaginalis	1	12	13
Anaerococcus hydrogenalis	2	0	2
Anaerococcus senegalensis	0	1	1
Anaerococcus obesiensis	0	1	1
Anaerococcus octavius	2	0	2
Anaerococcus provenciensis	0	2	2
Anaerococcus nagyae	0	3	3
Peptoniphilus grossensis	0	7	7
Peptoniphilus tyrelliae/senegalensis	0	1	1
Peptoniphilus rhinitidis	0	3	3
Peptoniphilus harei	4	8	12
Peptoniphilus gorbachii	1	5	6
Peptoniphilus timonensis	0	1	1
Peptoniphilus olsenii	0	4	4
Peptoniphilus lacrimalis	0	5	5
Peptoniphilus koenoeneniae	0	2	2
Peptoniphilus duerdenii	0	5	5
Peptoniphilus indolicus	2	0	2
Peptoniphilus asaccharolyticus	1	0	1
Peptoniphilus ivorii	1	4	5
Peptoniphilus coxii	0	8	8

Validation optimized GPAC db

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species ID	Bruker database			Bruker database + ENRIA GPAC database			
	Species ID	Genus ID	No ID	Species ID	Genus ID	No ID	higher score
P. harei (n=28)	19	8	1	28	0	0	26
F. magna (n=25)	16	8	1	16	8	1	0
P. micra (n=27)	25	2	0	25	2	0	0
A. vaginalis (n=8)	0	2	6	8	0	0	8
A. murdochii (n=5)	4	1	0	5	0	0	1
A. hydrogenalis (n=2)	0	0	2	0	2	0	2
A. obesiensis (n=2)	0	0	2	0	0	2	0
P. anaerobius (n=8)	7	1	0	8	0	0	8
P. stomatis (n=2)	0	0	2	1	1	0	2
M. asaccharolytica (n=2)	0	0	2	2	0	0	2
P. lacrimalis (n=2)	0	0	2	2	0	0	2
P. grossensis (n=2)	0	1	1	1	1	0	2
different GPAC species (n=7)	0	1	6	7	0	0	7
GPAC (9)	2	0	7	2	1	6	2
Total (% of all strains)	73	24	32	105	15	9	62
% of all strains	**56,6%**	18,6%	24,8%	**81,4%**	11,6%	7,0%	48,1%

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ENRIA MSPs added to the V6 db

Genus	MSPs (n)	no. of species represented
Acidaminococcus spp.	7	2
Actinomyces spp.	16	7
Alistipes spp.	3	3
Anaerococcus spp.	17	3
Atopobium spp.	4	3
Bacteroides spp.	31	14
Bifidobacterium spp.	6	4
Clostridium spp.	21	12
Desulfovibrio spp.	6	3
Dialister spp.	5	2
Fusobacterium spp.	8	3
Leptotrichia spp.	11	4
Parabacteroides spp.	10	4
Peptoniphilus spp.	25	8
Peptostreptococcus spp.	5	2
Porphyromonas spp.	8	4
Prevotella spp.	48	18
Propionibacterium spp.	2	2
Sutterella spp.	5	2

Including MSPs of 26 different species belonging to different genera

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Does the quality /reliability of the identification of anaerobic bacteria only depend on the MALDI-TOF MS database?

Which pre-analytical factors influence the quality of the spectrum of anaerobic bacteria?

- **Reproducibility of spotting**
- **Incubation time**
- **Exposure to oxygen**

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Reproducibility spotting results

Strain	Person 1 range	SD	no reliable ID (n)[a]	genus ID (n)	species ID (n)	Person 2 range	SD	no reliable ID (n)[a]	genus ID (n)	species ID (n)
Gram-negative bacteria										
B. thetaiotaomicron	2.031-2.198	0.052	0	10	10	2.080-2.257	0.052	0	10	10
P. intermedia	1.958-2.121	0.050	0	10	8	1.976-2.092	0.042	0	10	8
F. necrophorum	2.263-2.387	0.050	0	10	10	2.187-2.418	0.072	0	10	10
F. nucleatum	1.596-2.108	0.152	1	9	4	1.192-2.107	0.269	1	9	5
C. ureolyticus	0.962-2.012	0.859	8	2	1	1.700-2.043	0.826	2	8	2
V. parvula	2.306-2.402	0.039	0	10	10	2.182-2.416	0.071	0	10	10
Gram-positive bacteria										
P. micra	1.687-2.338	0.233	1	9	6	2.202-2.399	0.058	0	10	10
F. magna	0.959-2.116	0.344	1	9	5	1.831-2.108	0.075	0	10	7
P. ivorii[b]	1.255-1.743	0.162	7	3	0	1.162-1.637	0.137	10	0	0
A. minutum[b]	1.831-2.528	1.244	4	6	5	2.183-2.481	0.105	0	10	10
C. butyricum	2.054-2.244	0.070	0	10	10	2.009-2.265	0.086	0	10	10
A. israelii[b]	1.114-1.314	0.639	10	0	0	1.968-2.006	0.838	8	2	1
A. graevenitzii[b]	1.338-2.191	0.926	5	5	2	1.851-2.250	1.082	4	6	4
A. meyeri[b]	1.946-2.239	1.116	6	4	3	1.308-2.217	0.961	6	4	2
B. dentium[b]	1.912-2.304	0.908	2	8	7	2.163-2.388	0.971	2	8	8
B. longum[b]	1.780-2.229	0.150	0	10	6	2.018-2.218	0.071	0	10	10
P. acnes[b]	2.014-2.177	0.713	1	8	8	2.058-2.316	0.088	0	10	10
E. lenta[b]	1.263-1.851	0.833	8	2	0	2.104-2.311	0.929	2	8	8

The influence of incubation time, sample preparation and exposure to oxygen on the quality of the MALDI-TOF MS spectrum of anaerobic bacteria. Veloo AC *et al*. Clin Microbiol Infect 2014; 20:O1091-1097

Exposure to oxygen

Species	Exposure to oxygen (hours) 0	1	6	24	48
Gram-negative bacteria					
Bacteroides thetaiotaomicon	2.14	2.24	2.10	2.17	2.35
Bacteroides stercoris	2.19	2.14	2.18	2.25	2.27
Parabacteroides johnsonii	2.23	2.32	2.29	2.31	2.28
Fusobacterium necrophorum	2.08	2.30	2.19	<1.7[a]	<1.7[a]
Fusobacterium nucleatum[b]	2.08	2.27	2.32	2.19	2.08
Prevotella intermedia	1.92	1.93	1.86	1.95[c]	<1.7[a]
Prevotella oris	2.08	2.17	2.01	2.35	2.29
Alistipes onderdonkii	2.21	2.24	2.29	2.29	2.21
Veillonella parvula	2.25	2.13	2.2	2.20	2.16
% reliable species ID direct spotting	89	89	89	78	78
% reliable species ID total	89	89	89	78	78
Gram-positive bacteria					
Finegoldia magna	2.24	2.05[c]	2.34	2.52	2.50
Peptoniphilus harei	2.15	2.00	2.13	2.23[b]	2.19
Peptoniphilus ivorii	<1.7[d]	1.81[e]	<1.7[d]	1.76[e]	1.80[e]
Clostridium hathewayii	2.36	2.23	2.29	2.07	2.19
Clostridium ramosum	2.03	2.19	2.27	2.06	2.17
Actinomyces graevenitzii	2.05[c]	2.03[e]	2.11	2.06[e]	<1.7[d]
Actinomyces meyeri	2.03	2.27	2.35	2.19	2.25
Bifidobacterium longum	2.00[c]	2.12[c]	1.99[c]	2.15	2.17
Propionibacterium acnes	2.18	2.14[c]	2.12	2.13	2.13
Eggerthella lenta	2.11	2.30	2.27	2.16	2.02[e]
Collinsella aerofaciens	2.26[c]	2.16	2.23[c]	2.32[c]	2.28[c]
% reliable species ID direct spotting	64	55	73	64	64
% reliable species ID total	91	82	82	91	82

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Recommendations for good quality spectrum

The fact whether an unknown anaerobic bacterium can be identified using MALDI-TOF MS mainly depends on:

-Inter examiner variation

-The type of colony

-Amount of bacteria spotted

-An average incubation time of 48 hours

-If sufficient MSPs are present in the MALDI-TOF MS database

The influence of incubation time, sample preparation and exposure to oxygen on the quality of the MALDI-TOF MS spectrum of anaerobic bacteria.
Veloo AC *et al*. Clin Microbiol Infect 2014; 20:O1091-1097

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Multi-center ring trial

10 unknown strains were sent to the core laboratoria

- ***Veillonella parvula***
- ***Solobacterium moorei***
- ***Bacteroides fragilis***
- ***Actinomyces israellii***
- ***Anaerococcus murdochii***
- ***Campylobacter ureolyticus***
- ***Robinsoniella peoriensis ****
- ***Peptoniphilus coxii ****
- ***Fusobacterium nucleatum***
- ***Propionibacterium acnes***

Direct spotting
On target extraction
Full extraction

*** not present in maldi db**

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Interpretation results

	score						
Strain	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
Veillonella parvula	1,941	2,172	1,982	1,897	1,858	1,98	2,135
Solobacterium moorei	2,162	2,203	2,021	2,13	2,014	1,839	1,936
Bacteroides fragilis	2,268	2,319	2,309	2,134	2,128	2,279	2,396
Actinomyces israelii	<1,7	<1,7	1,708	<1,7	<1,7	-	1,71
Anaerococcus murdochii	1,925	1,752	1,805	1,831	1,788	-	2,022
Campylobacter ureolyticus	<1,7	<1,7	<1,7	<1,7	<1,7	<1,7	<1,7
Peptoniphilus coxii	2,036	2,105	1,911	<1,7	<1,7	1,804	2,131
Robinsoniella peoriensis	1,939	1,887	1,827	<1,7	<1,7	1,913	2,004
Fusobacterium nucleatum	1,99	2,381	2,216	2,042	2,015	1,983	2,169
Propionibacterium acnes	1,9	2,057	2,076	2,018	<1,7	2,197	1,985

Correct species ID = 1 point
Correct genus ID = 0,5 point

Performance of the different laboratories

	Number of points						
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
Direct spotting							
Log score ≥2	3	6	4	4	3	2	6
Log score ≥1,7 and <2	2,5	1	2,5	1	1	2,5	1,5
Total	5,5	7	6,5	5	4	4,5	7,5
On target extraction							
Log score ≥2	3	5	6	4	3	5	6
Log score ≥1,7 and <2	2,5	0,5	1,5	1	2	1	0,5
Total	5,5	5,5	7,5	5	5	6	6,5
Full extraction							
Log score ≥2	8	8	7	6	4	8	7
Log score ≥1,7 and <2	0	0	0,5	1	1,5	0	0
Total	8	8	7,5	7	5,5	8	7
Total	**19**	**20,5**	**21,5**	**17**	**14,5**	**18,5**	**21**
Average	**6,3**	**6,8**	**7,2**	**5,7**	**4,8**	**6,2**	**7,0**

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What happens after several updates of the db?

	Number of points						
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
Direct spotting							
Log score ≥2	6	8	8	8	5	7	8
Log score ≥1,7 and <2	1,5	0	0,5	0,5	1,5	0	0,5
Total	7,5	8	8,5	8,5	6,5	7	8,5
On target extraction							
Log score ≥2	8	8	8	8	7	6	7
Log score ≥1,7 and <2	0	0	0,5	0,5	0,5	0,5	0
Log score ≥1,7 and <2	0,5	0	0,5	0	1	0	0
Total	8,5	8	8,5	7	7	8	7
Total	**24**	**24**	**25,5**	**24**	**21**	**21,5**	**22,5**
Average	**8**	**8**	**8,5**	**8**	**7**	**7,2**	**7,5**

An optimized database not only results in a higher number of strains which can be identified using MALDI-TOF MS, but also corrects for differences in performance between laboratories

db6903

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Changes in anaerobic bacteriology

easier

reliable

faster

cheaper

Better insight in the clinical relevance of anaerobic species

Examples:

- **Differentiation between *P. asaccharolyticus* and *P. harei***
- ***Fenollaria massiliensis***

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Thank you core laboratoria!!!!

A foundation is being laid for anaerobic diagnostics

Initiated by “Neglected Infections”of the EurSafety Health-net, which is financially supported by the EU, Niedersachsen, NordrheinWestfalen and the Dutch border Provinces.

ENRIA European Network for the Rapid Identification of Anaerobes

ESGAI ESCMID STUDY GROUP FOR ANAEROBIC INFECTIONS European Society of Clinical Microbiology and Infectious Diseases

ESGEM ESCMID STUDY GROUP FOR EPIDEMIOLOGICAL MARKERS European Society of Clinical Microbiology and Infectious Diseases

BRUKER

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