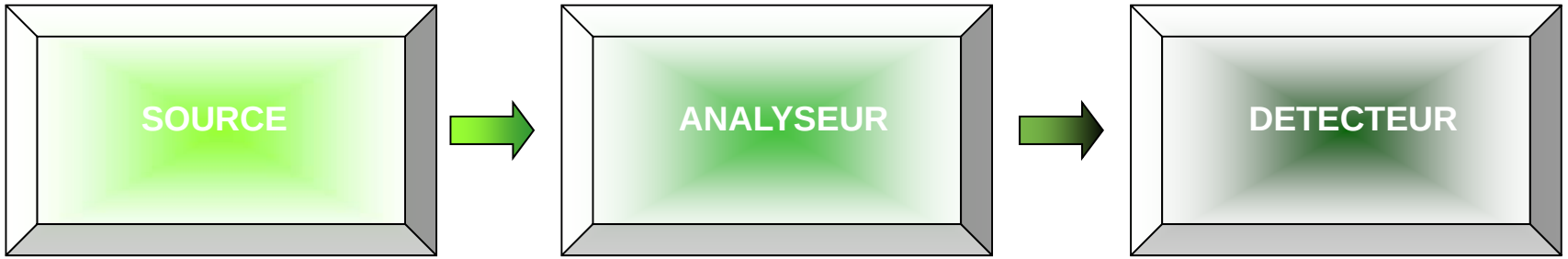
Apports de la spectromètrie de masse en pathologie infectieuse

Michel Drancourt, MD, PhD
URMITE UMR CNRS 6236 IRD 198 IFR 48
Institut Hospitalier et Universitaire POLMIT
Université de la Méditerranée, Marseille

12es JNI TOULOUSE 8-10 JUIN 2011

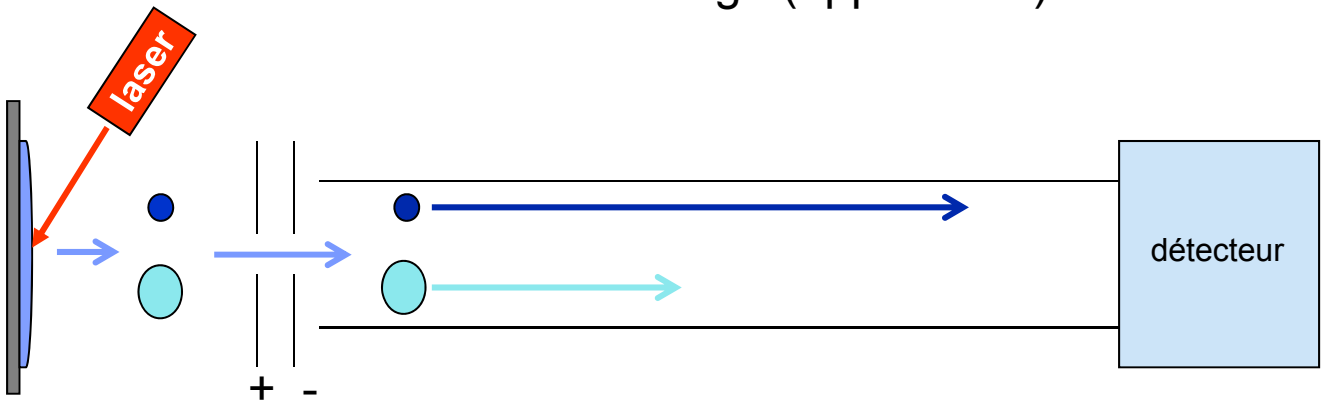
Structure générale d'un spectromètre de masse



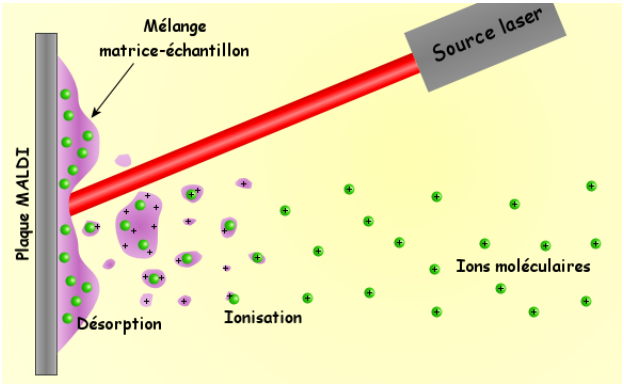
Pour volatiliser et ioniser les molécules

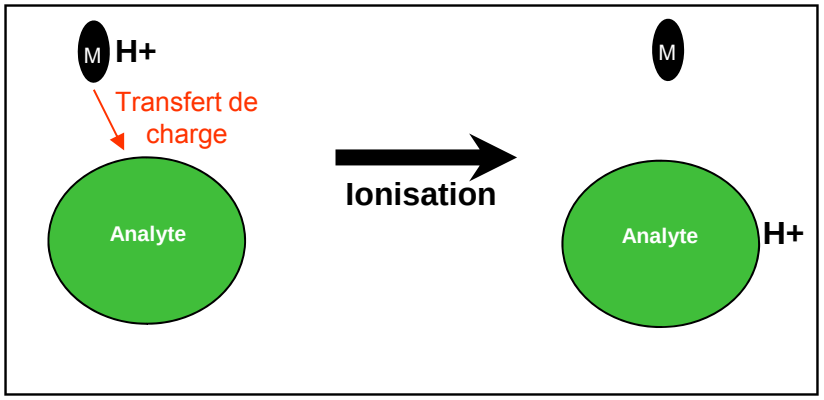
Il mesure les valeurs du rapport: masse / nb de charge (appelé m/z)

Détection des ions



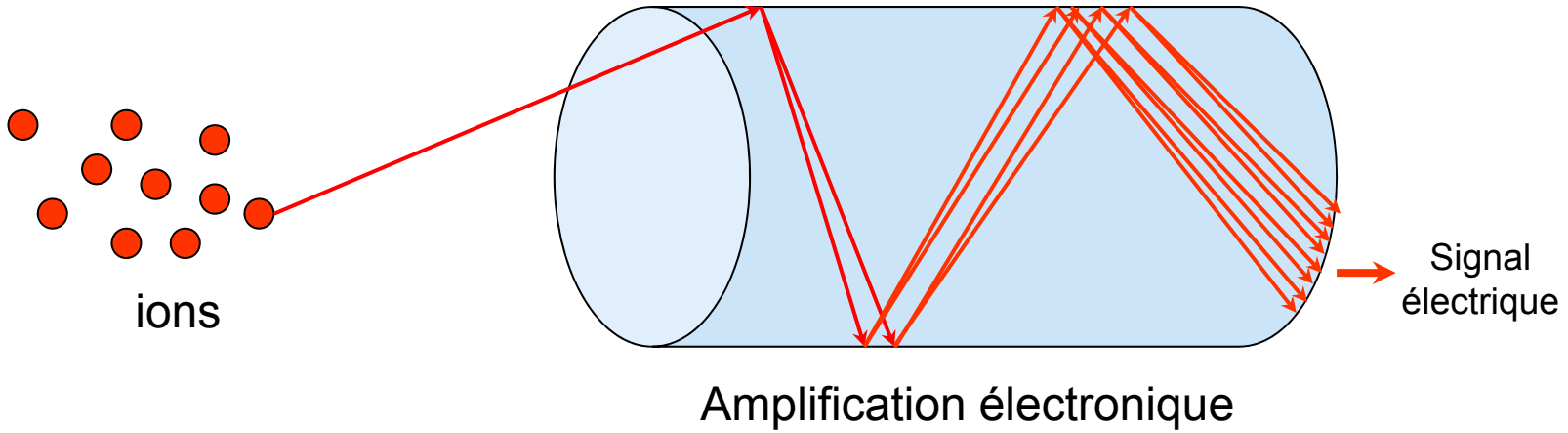
Principe de la source MALDI





- Etapes d'ionisation
  - 1- Co-crystallisation de l'échantillon et de la matrice photosensible
  - 2- Excitation des molécules de matrices par l'impulsion laser
  - 3- explosion de la matrice et ionisation de la matrice
  - 4- transfert de charge et ionisation de l'échantillon

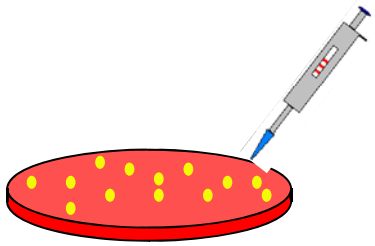
Le détecteur



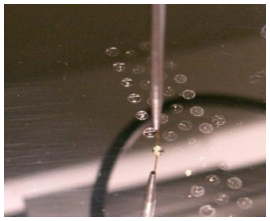
Principe d'amplification

- 1- un ion frappe le détecteur et arrache des électrons
- 2- les électrons arrachés frappent le détecteur et arrachent d'autres électrons, et ainsi de suite
- 3- l'ensemble des électrons arrachés vont forme un courant électrique

L’identification des colonies bactériennes et levures



Pick-up colony

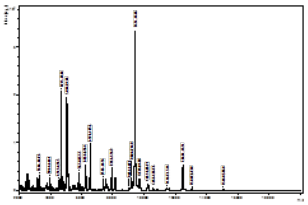


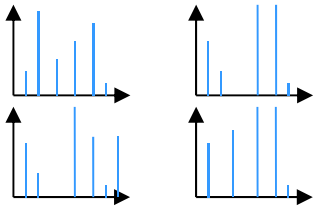
Deposit colony on plate

MALDI-TOF plate loading / matrix overlaying

MALDI-TOF processing

Data collection, interpretation and reporting





Profil

Database

Identification

Inactivation des pathogènes NSB3 compatible avec l'analyse MALDI-TOF

Bacillus anthracis, spores	80% TFA, 30-min	Lasch P, 2008
	Autoclaving	Castantia E, 2006
	Y-irradiation	Krishnamurthy T, 1996
Brucella melitensis	Y-irradiation	Krishnamurthy T, 1996
Burkholderia spp.	80% TFA, 30-min	Lasch P, 2008
Francisella tularensis	Y-irradiation	Krishnamurthy T, 1996
Mycobacterium tuberculosis	95°C, 30-min	Saleeb PG, 2011
	95°C, 60-min	Drancourt M, unpublished
	70% ethanol, 10-min	Lotz, 2010
Yersinia pestis	Y-irradiation	Krishnamurthy T, 1996
	70% ethanol, 60-min	Ayyadurai S, 2010
	80% TFA, 30-min	Lasch P, 2010 ; Lasch, 2008

MALDI-TOF

- **Currently we routinely test any colony: 200 colonies dayly**
- **Time for technician and cost equivalent to that from Gram staining (No reagent)**
- **ID in 98% of cases within 15-30'**
- **May detect toxins, antibiotics resistance, clones!**

Ongoing Revolution in Bacteriology: Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

Piseth Seng,[a] Michel Drancourt,[a] Frédérique Gouriet, Bernard La Scola, Pierre-Edouard Fournier, Jean Marc Rolain, and Didier Raoult

Pôle des Maladies Infectieuses, Assistance Publique–Hôpitaux de Marseille et Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, Unité Mixte de Recherche, Centre National de la Recherche Scientifique–Institut pour la Recherche et le Développement 6236, Institut Fédératif de Recherche 48, Faculté de Médecine, Université de la Méditerranée, Marseille, France

(See the editorial commentary by Nassif on pages 552–3)

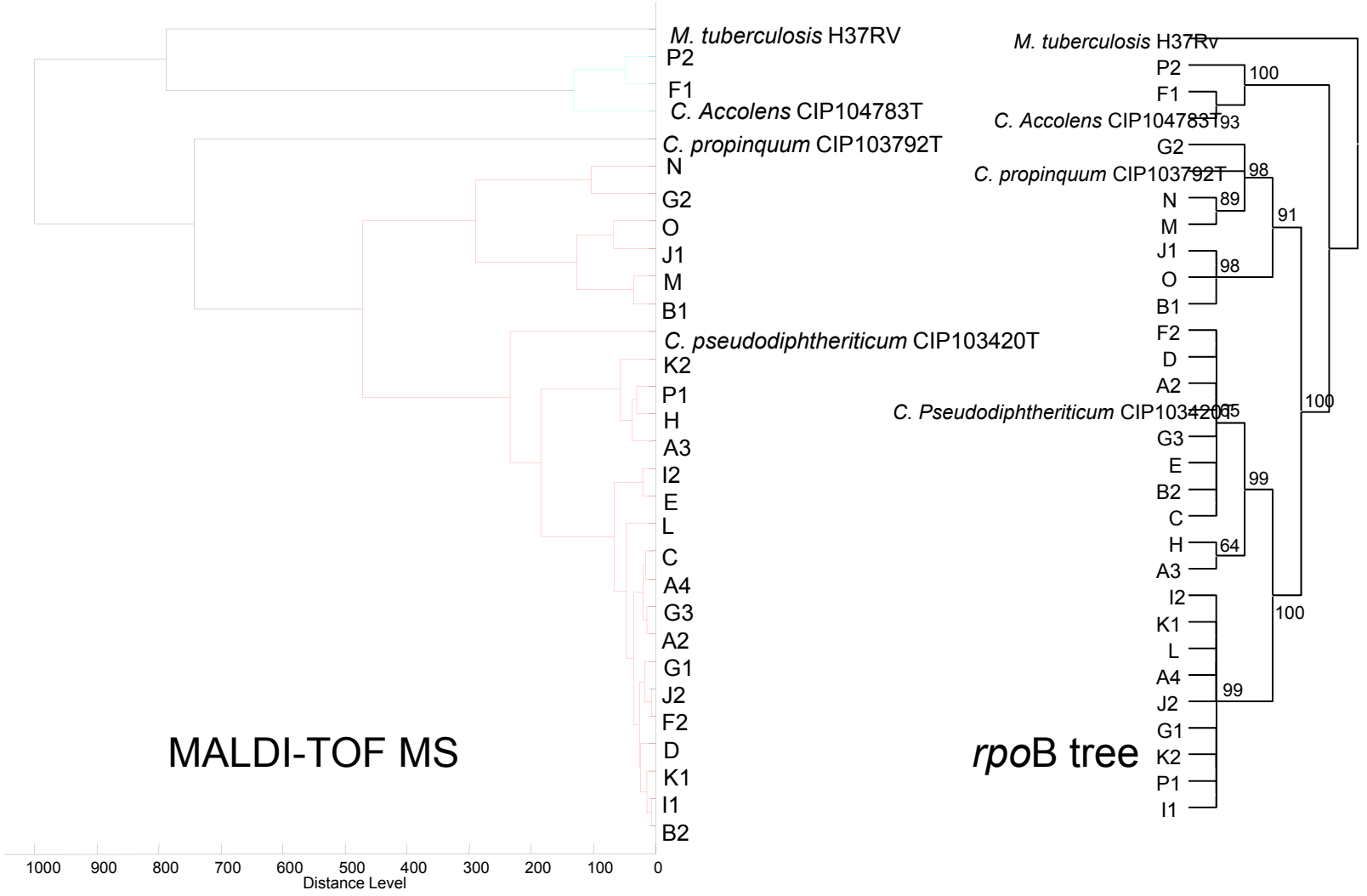
Background. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry accurately identifies both selected bacteria and bacteria in select clinical situations. It has not been evaluated for routine use in the clinic.

Methods. We prospectively analyzed routine MALDI-TOF mass spectrometry identification in parallel with conventional phenotypic identification of bacteria regardless of phylum or source of isolation. Discrepancies were resolved by 16S ribosomal RNA and *rpo*B gene sequence–based molecular identification. Colonies (4 spots per isolate directly deposited on the MALDI-TOF plate) were analyzed using an Autoflex II Bruker Daltonik mass spectrometer. Peptidic spectra were compared with the Bruker BioTyper database, version 2.0, and the identification score was noted. Delays and costs of identification were measured.

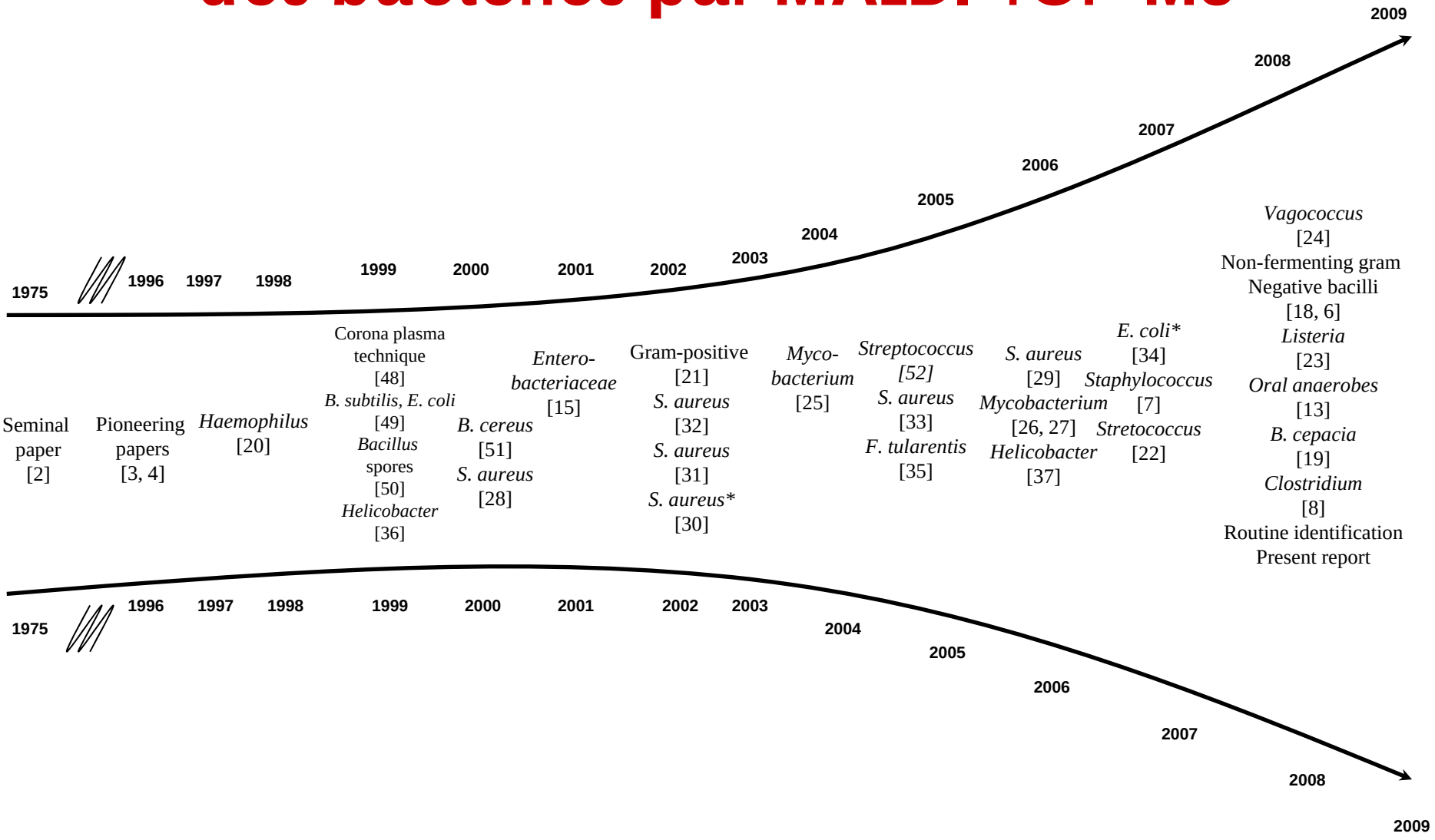
Results. Of 1660 bacterial isolates analyzed, 95.4% were correctly identified by MALDI-TOF mass spectrometry; 84.1% were identified at the species level, and 11.3% were identified at the genus level. In most cases, absence of identification (2.8% of isolates) and erroneous identification (1.7% of isolates) were due to improper database entries. Accurate MALDI-TOF mass spectrometry identification was significantly correlated with having 10 reference spectra in the database ($P = .01$). The mean time required for MALDI-TOF mass spectrometry identification of 1 isolate was 6 minutes for an estimated 22%–32% cost of current methods of identification.

Conclusions. MALDI-TOF mass spectrometry is a cost-effective, accurate method for routine identification of bacterial isolates in <1 h using a database comprising ≥10 reference spectra per bacterial species and a ≥1.9 identification score (Brucker system). It may replace Gram staining and biochemical identification in the near future.

Crawling outbreak of Corynebacterium pseudodiphtheriticum in CF patients



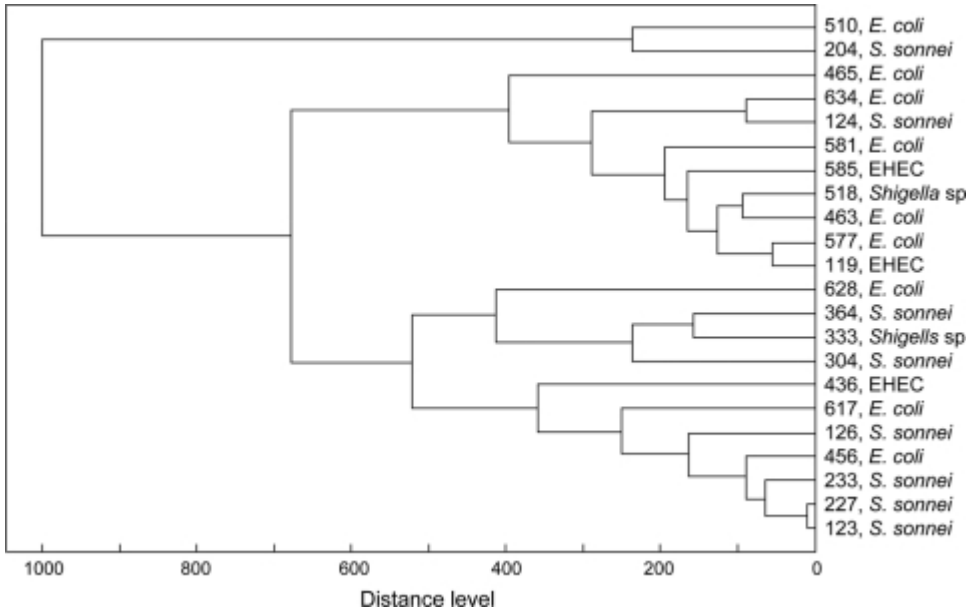
Un tiers de siècle d'identification des bactéries par MALDI-TOF-MS



Limites actuelles de l'identification des bactéries

- ***Streptococcus pneumoniae* ≠ *Streptococcus mitis***
- ***Shigella spp.* ≠ *Escherichia coli***
- **Inoculum ~ 10^8 cfu**

Log (score)-derived MSP dendrogram from *Shigella*, EHEC, and *E. coli* using the MALDI-TOF MS-based Biotyper system. The dendrogram was generated with the distance set at Euclidian and linkage set at completion (23).



He Y. et al. JCM 2010; 48:3888-92.

Performances of MALDI-TOF for yeast identification

Study (clinical)	n= isolates	n= genus/species	correct identification (%) genus	species	ref.
Marklein	267	7 / 25		92.5%	J Clin Micro 2009
Van Veen	61	7 / 12		85.2%	J Clin Micro 2010
Stevenson	194	6 / 23		99%	J Clin Micro 2010
Bader	1192	18 / 33		95.1%	Clin Micro Inf 2010
Putignani	303	5 / 19		84.8%	Mol Biosys 2011

Performances of MALDI-TOF for yeast identification

Impact of genus and species

	Putignani		Bader		Stevenson (clinical)		Marklein	
C.albicans	131/134	97.7%	512/512	100%	20/20	100%	87/87	100%
C.parapsilosis	56/59	94.9%	105/105	100%	46/46	100%	29/29	100%
C.glabrata	23/23	100%	272/272	100%	11/11	100%	52/52	100%
C.guilliermondii	14/15	93%	23		15/15	100%	1/1	
C.tropicalis	13/15	87%	88/88	100%	8/8		35/35	100%
C.krusei	7/7	100%	53		9/9		18/18	100%
C.cerevisiae	7/10		20/20		-		2/2	
C.neoformans	2/3		7/7		5/6		3/3	
Trichosporon					8/8		0/3	?

CHUV

Performances of MALDI-TOF for yeast identification

Correct id at species level

(5 studies combined)

> 95 %	***C.albicans***
	C.glabrata
	C.tropicalis
	C.krusei
	C.parapsilosis

Performances of MALDI-TOF for yeast identification

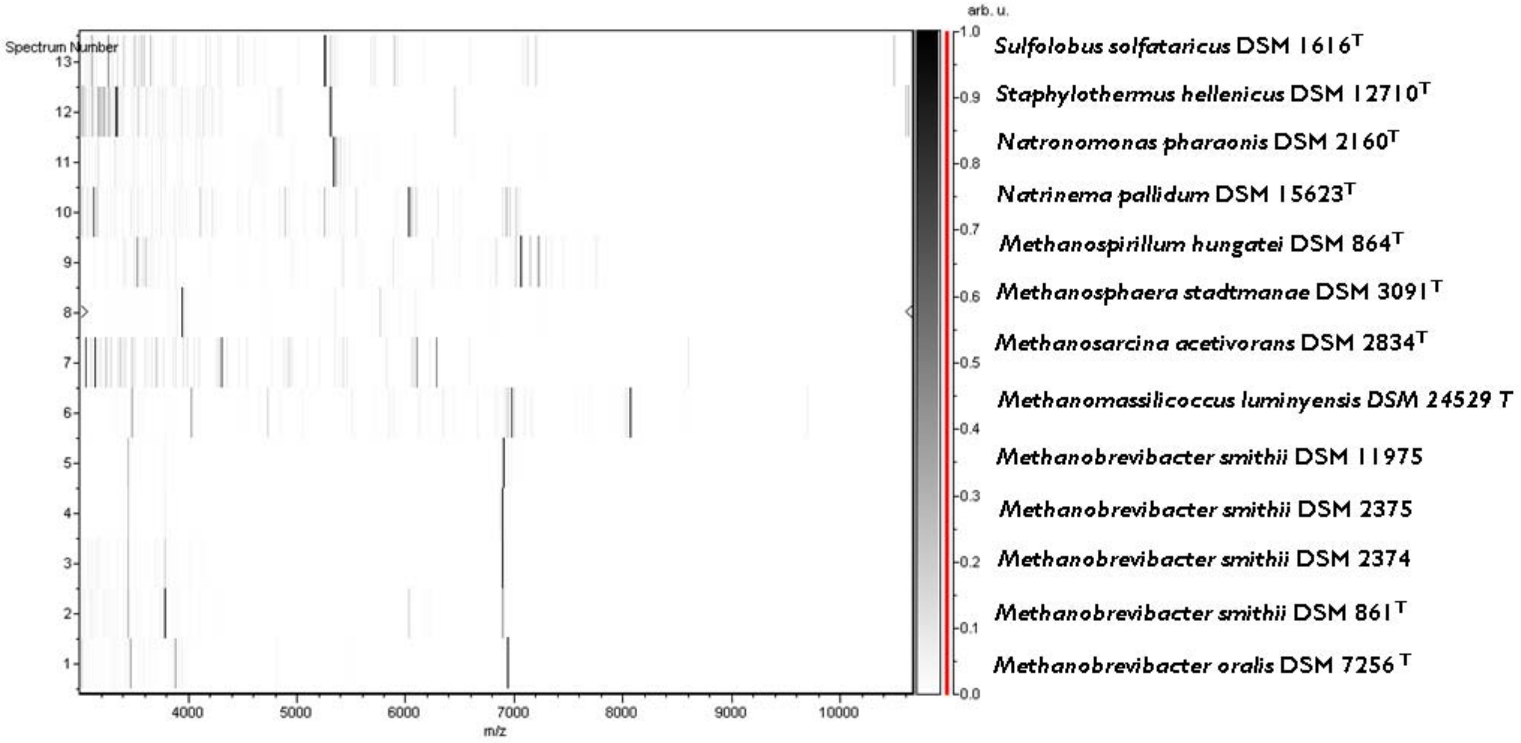
Weaknesses –	Strenghts +
extraction mandatory	speed
absence of species in database	cost
	accuracy
mis-classifications	recognition of new species
Pichia	very few mis-id
	Impact on patients

Performances of MALDI-TOF for filamentous fungi identification

Published studies

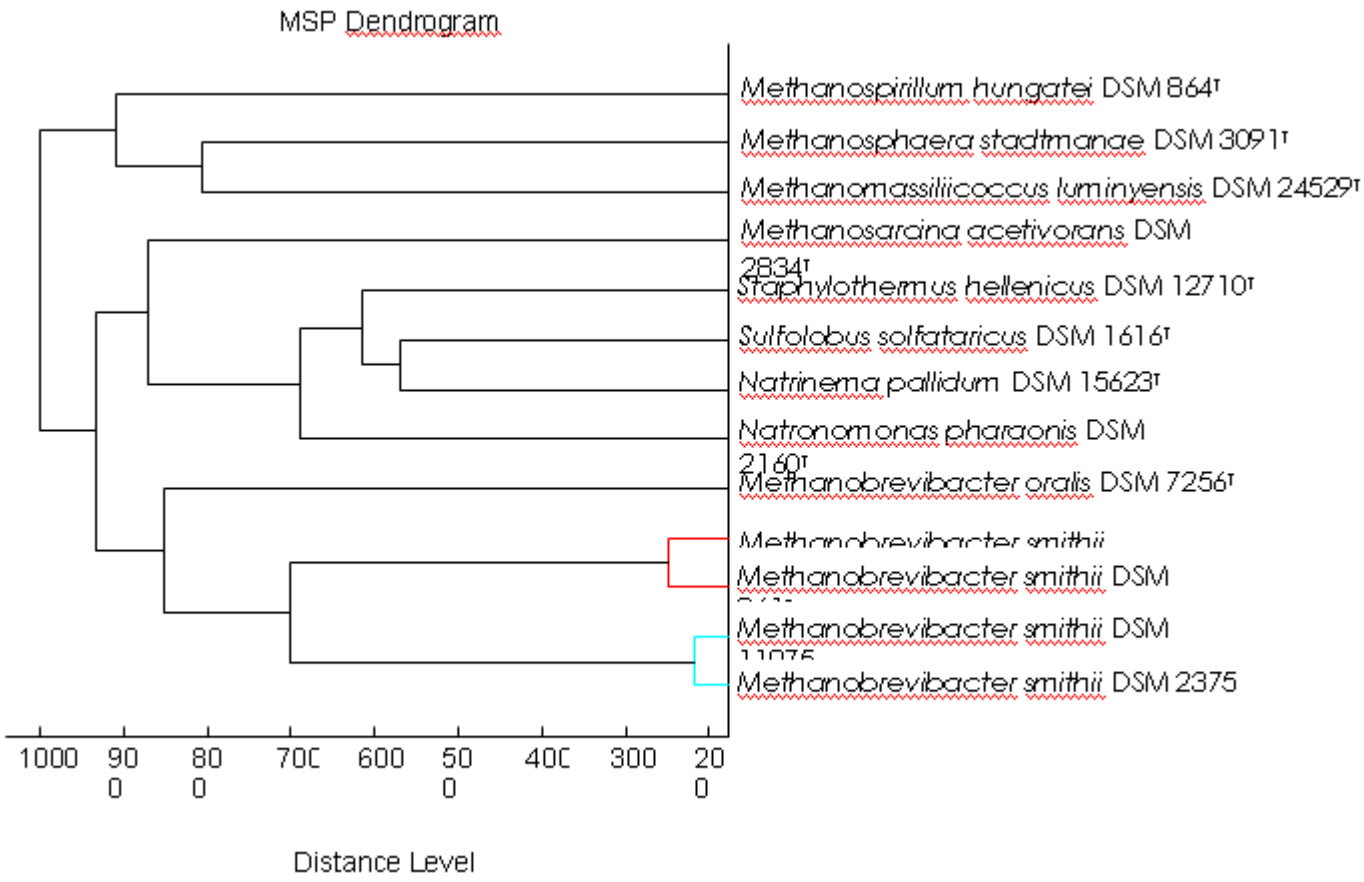
	Genus	n=	sp	correct id.(%)	incorrect	not in database	
Alanio	*Aspergillus*	140	28	138 (98.6%)	0	2	CMI 2010
Marinach-Patrice	*Fusarium*	62	9	57 (92%)	1	4	CMI 2009
Coulibaly	*Scedosporium*	25	7	25 (100%)			Medical Mycol 2011
Erhard	Dermatophytes	20	6	20 (100%)			Exp Derm 2008

Identification of *Archaea*



Dridi B, Drancourt M, unpublished data

Identification of *Archaea*



Dridi B, Drancourt M, unpublished data

Identification of viruses

Intervirology

Intervirology 2010;53:344–353
DOI: 10.1159/000312919

Published online: June 15, 2010

Tentative Characterization of New Environmental Giant Viruses by MALDI-TOF Mass Spectrometry

Bernard La Scola Angélique Campocasso Rolande N'Dong Ghislain Fournous
Lina Barrassi Christophe Flaudrops Didier Raoult

Pôle des Maladies Infectieuses, Assistance Publique-Hôpitaux de Marseille and URMITE UMR CNRS-IRD 6236, IFR48, Faculté de Médecine, Université de la Méditerranée, Marseille, France

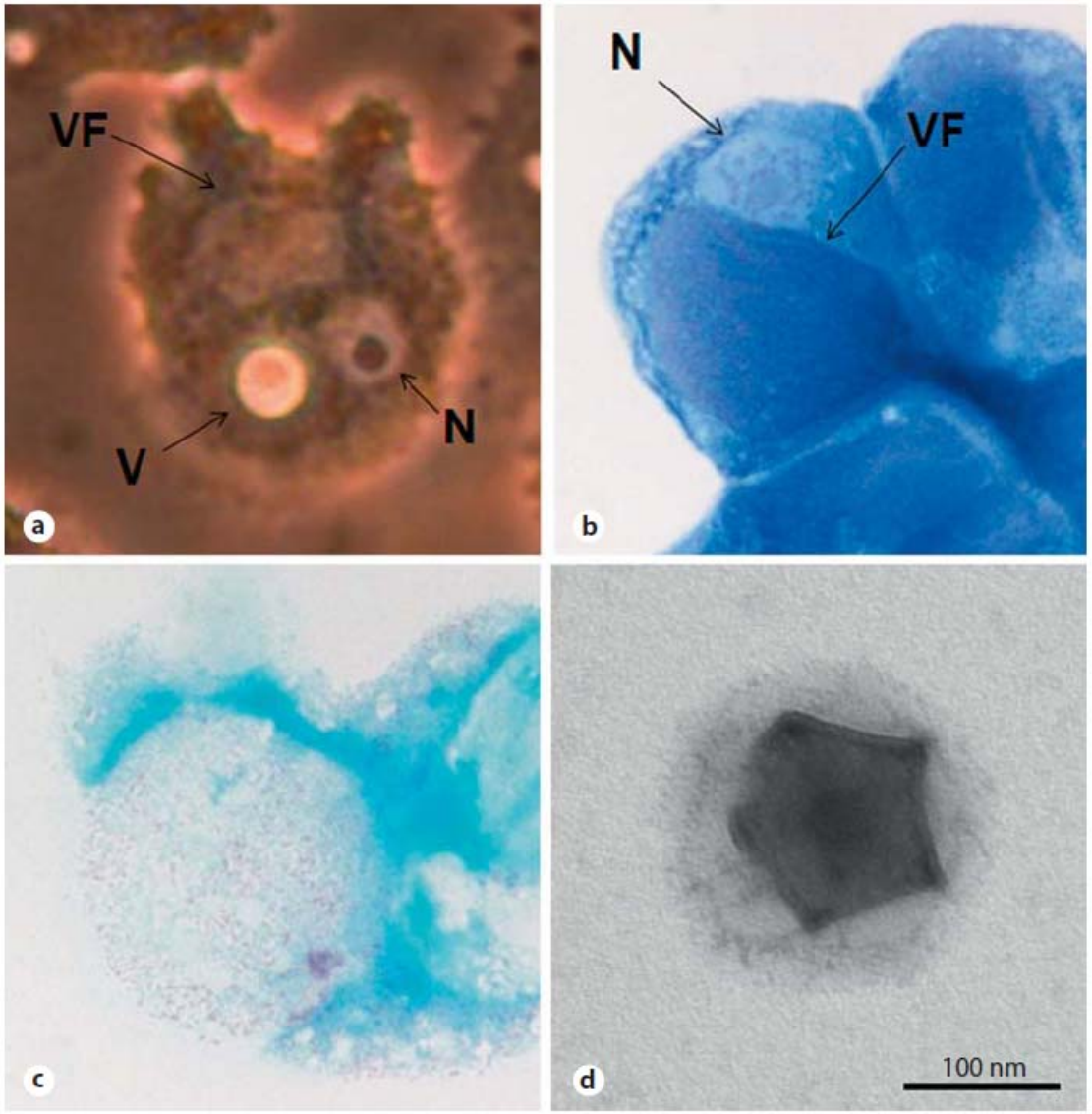
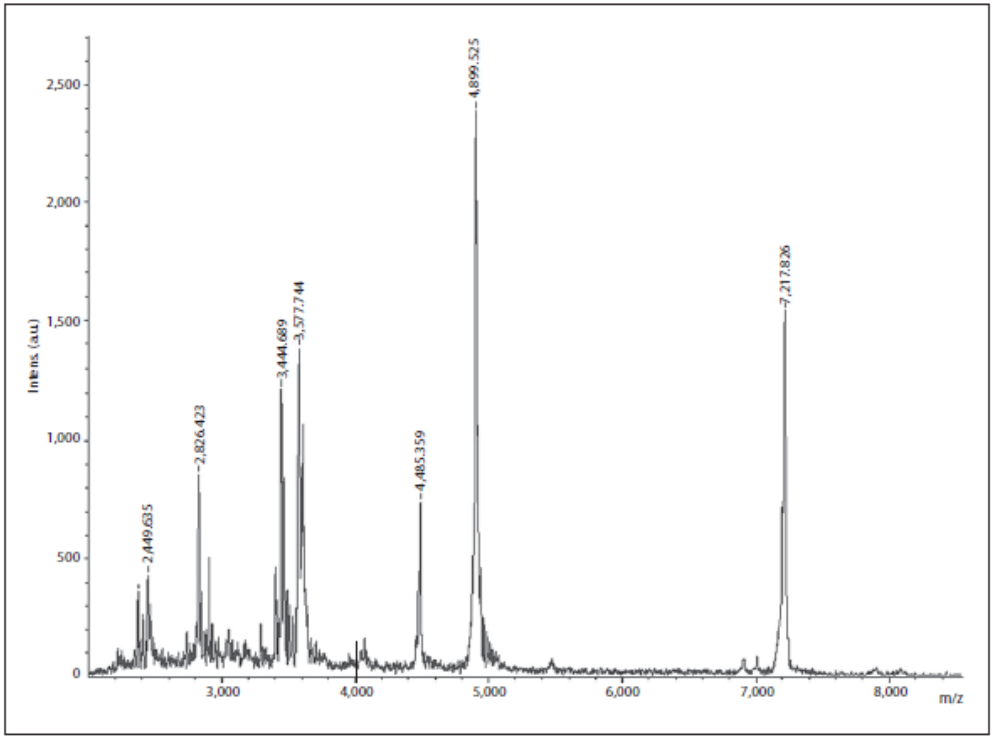


Fig. 1. Morphologic analysis of virus Cannes9 growing in *A. polyphaga*, as seen before lysis of the amoeba. **a** Inverted microscopic observation of viral infection. **b** Methylene blue staining: the virus factory appears as a pink-stained structure; the nucleus appears as a light structure. **c** On Gimenez staining, some viral particles appear in pink. **d** Negative staining electron microscopy of Cannes9 virus with its icosahedral particle surrounded by fibrils. VF = Virus factory; N = nucleus; V = vacuola.



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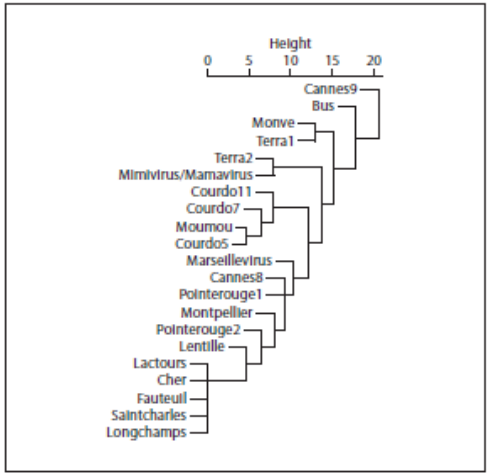

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Fig. 5. Example of the spectrum profile of a giant virus (here, Moumou) obtained by using MALDI-TOF MS.
Fig. 6. Dendrogram built by using MALDI-TOF MS data and based on the presence/absence of majors peaks in the profiles of each giant virus spectrum.

PLoS one

Direct Identification of Bacteria in Positive Blood Culture Bottles by Matrix-Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry

Bernard La Scola*, Didier Raoult

Pôle des Maladies Infectieuses, Assistance Publique-Hôpitaux de Marseille and URMITE UMR CNRS-IRD 6236, IFR48, Faculté de Médecine, Université de la Méditerranée, Marseille, France

Abstract

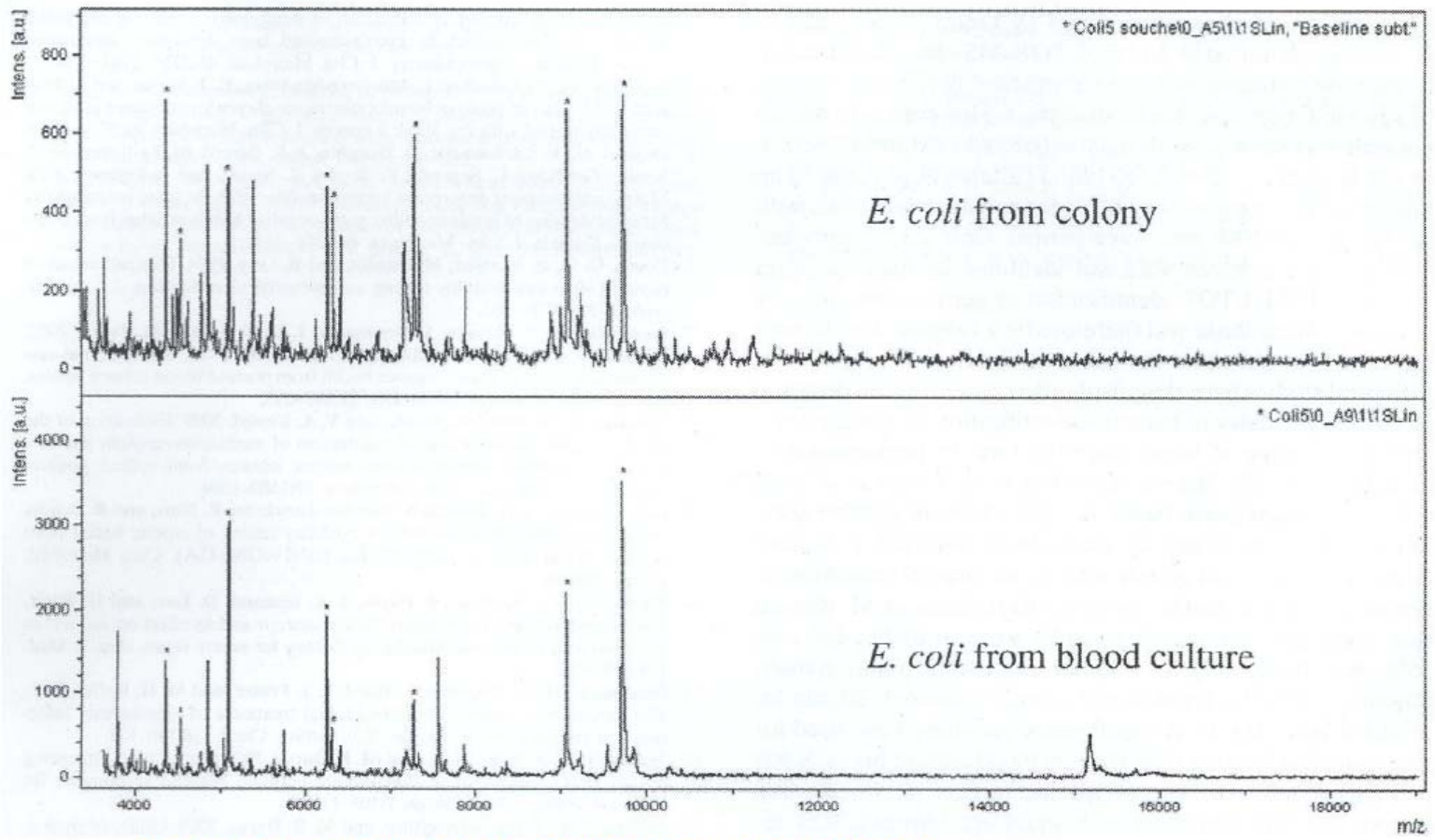
Background: With long delays observed between sampling and availability of results, the usefulness of blood cultures in the context of emergency infectious diseases has recently been questioned. Among methods that allow quicker bacterial identification from growing colonies, matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry was demonstrated to accurately identify bacteria routinely isolated in a clinical biology laboratory. In order to speed up the identification process, in the present work we attempted bacterial identification directly from blood culture bottles detected positive by the automate.

Methodology/Principal Findings: We prospectively analysed routine MALDI-TOF identification of bacteria detected in blood culture by two different protocols involving successive centrifugations and then lysis by trifluoroacetic acid or formic acid. Of the 562 blood culture broths detected as positive by the automate and containing one bacterial species, 370 (66%) were correctly identified. Changing the protocol from trifluoroacetic acid to formic acid improved identification of *Staphylococci*, and overall correct identification increased from 59% to 76%. Lack of identification was observed mostly with viridans streptococci, and only one false positive was observed. In the 22 positive blood culture broths that contained two or more different species, only one of the species was identified in 18 samples, no species were identified in two samples and false species identifications were obtained in two cases. The positive predictive value of bacterial identification using this procedure was 99.2%.

Conclusions/Significance: MALDI-TOF MS is an efficient method for direct routine identification of bacterial isolates in blood culture, with the exception of polymicrobial samples and viridans streptococci. It may replace routine identification performed on colonies, provided improvement for the specificity of blood culture broths growing viridans streptococci is obtained in the near future.

Direct Testing of Positive Blood Cultures by MALDI-TOF

Interpretation of spectra



From Ferroni A. et al., *J Clin Microbiol* (2010).

REVIEW 10.1111/j.1469-0691.2010.03290.x

Detection of microorganisms in blood specimens using matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a review

M. Drancourt

Unité de Recherche sur les Maladies Infectieuses Emergentes (URMITE) UMR CNRS 6236, IRD 198, IFR48, Université de la Méditerranée, Marseille, France

Clin Microbiol Inf 2010, 16:1620-1625

TABLE 1. Performance in identifying bacteria in positive blood culture broth

Nature of specimen (*n* = number of specimens)	Organism	Percentage of interpretable spectra (%)	Percentage of correct identification: genus level (%)	Percentage of correction identification: species level (%)	Major identification failures	References
Positive blood culture broth (*n* = 599)	Bacteria	94	76	76	*Streptococcus* spp.	16
Positive blood culture broth (*n* = 126)	Bacteria	97	79	57	*Streptococcus pneumoniae*	17
Positive blood culture broth (*n* = 179)	Bacteria	100[a]	80[a]	80[a]	*S. pneumoniae*	18
Spiked bottles (*n* = 33)					*Propionibacterium acnes*	
Spiked bottles (*n* = 312)	Bacteria	98	98	89	*S. pneumoniae*	
Positive blood culture broth (*n* = 388)	*Candida* spp.	96	98	91	*S. pneumoniae*	19
Positive blood culture broth (*n* = 304)	Bacteria	94.7	87	87	Uncommon species	20
Spiked bottles (*n* = 48)	*Candida* spp.	100	100	100	–	
Positive blood culture broth (*n* = 1)	*Candida albicans*	100	100	100	–	21

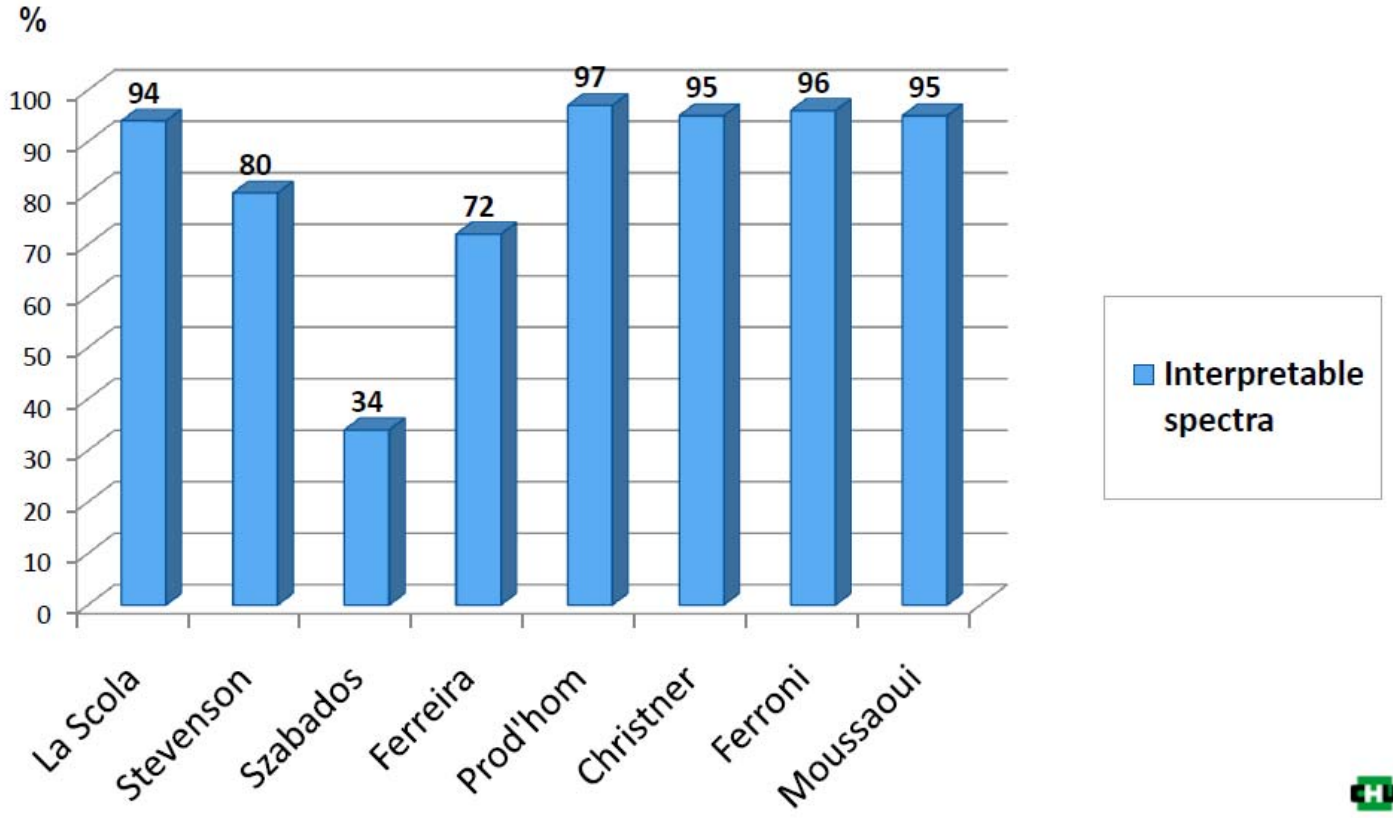
[a]Including spiked bottles.

Direct Testing of Positive Blood Cultures by MALDI-TOF

General performance

Percentage of interpretable spectra

(8 published studies of mono-microbial bacteremia)

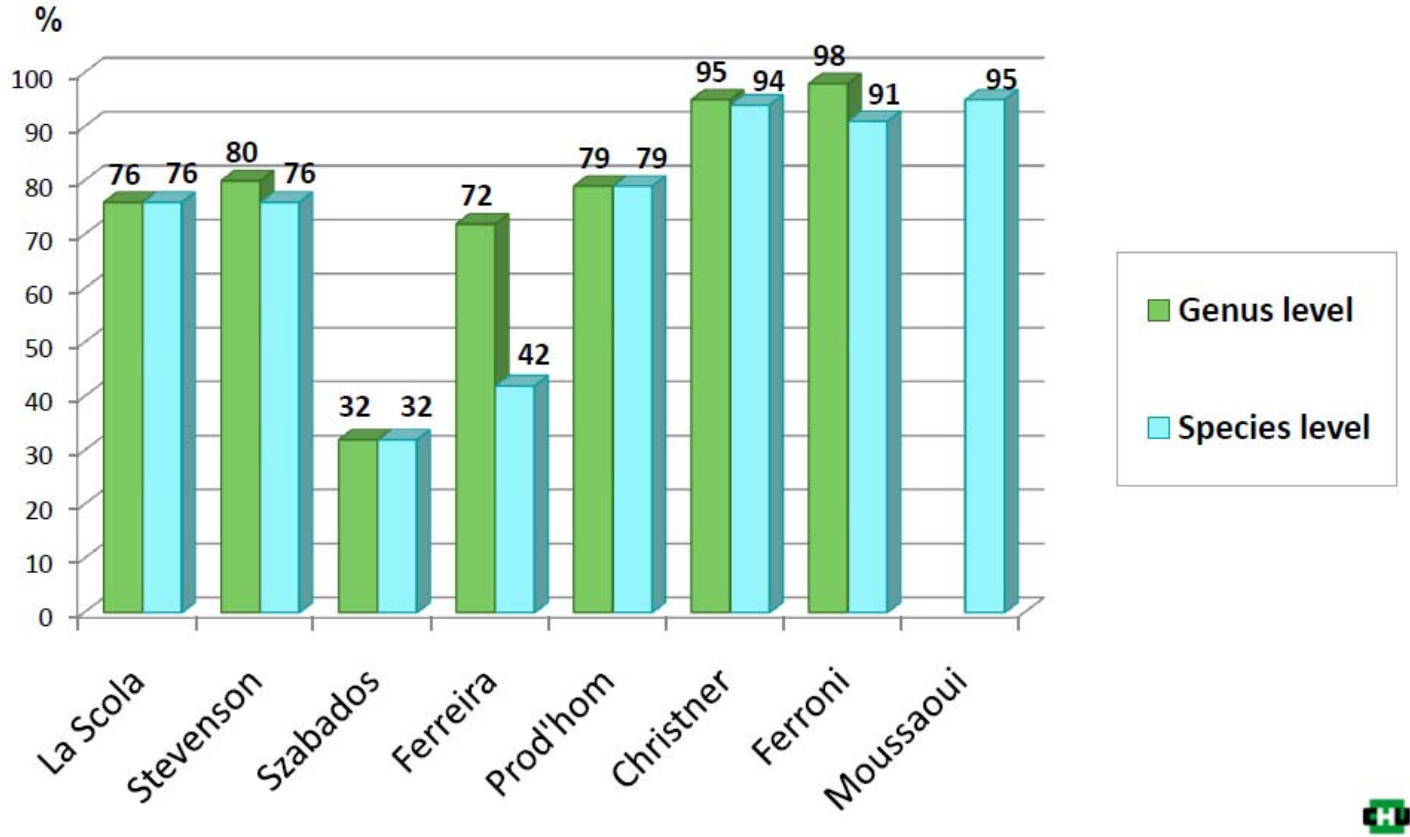


Direct Testing of Positive Blood Cultures by MALDI-TOF

General performance

Percentage of correct identifications

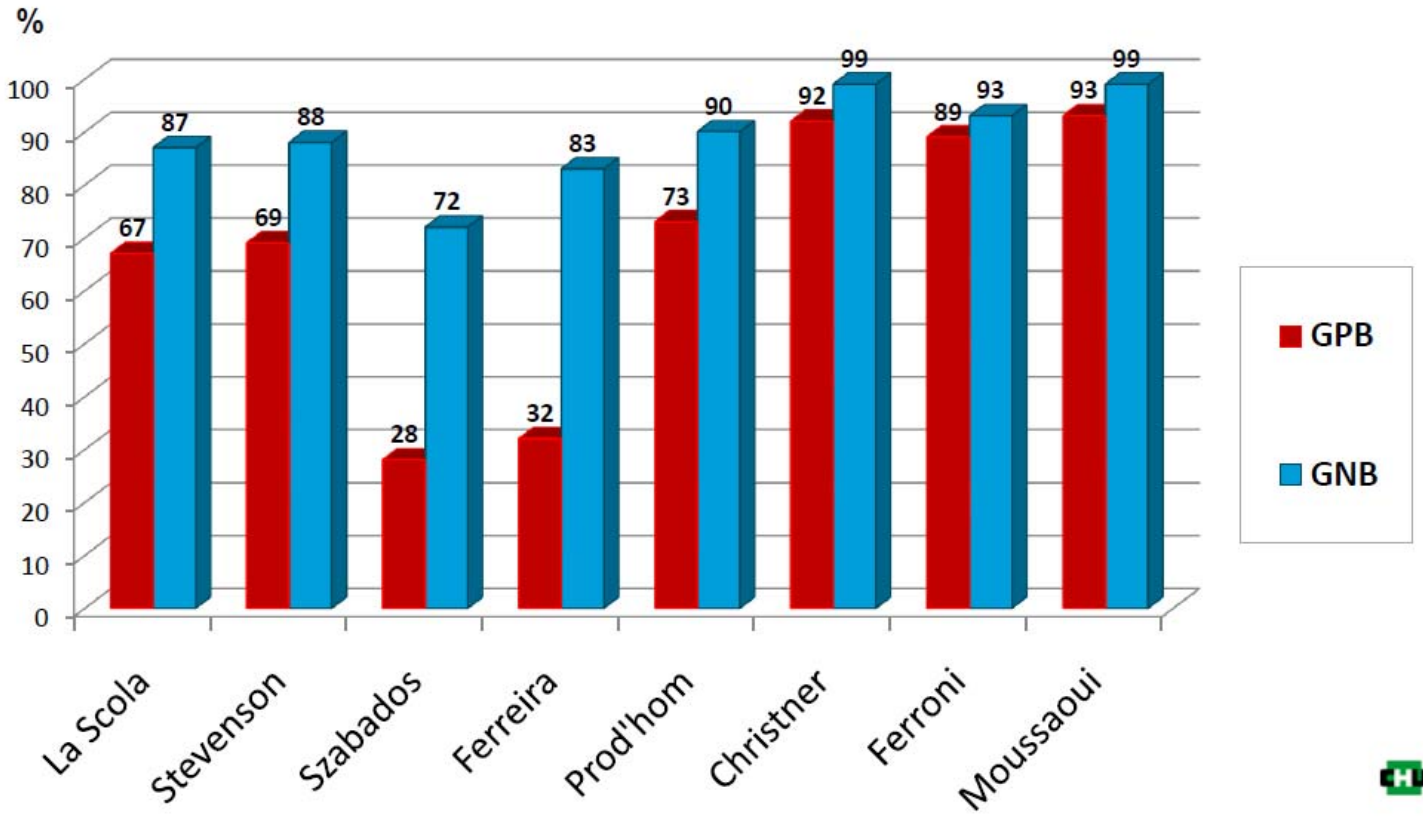
(8 published studies of mono-microbial bacteremia)



Direct Testing of Positive Blood Cultures by MALDI-TOF

Comparative performance for Gram positive and Gram negative organisms

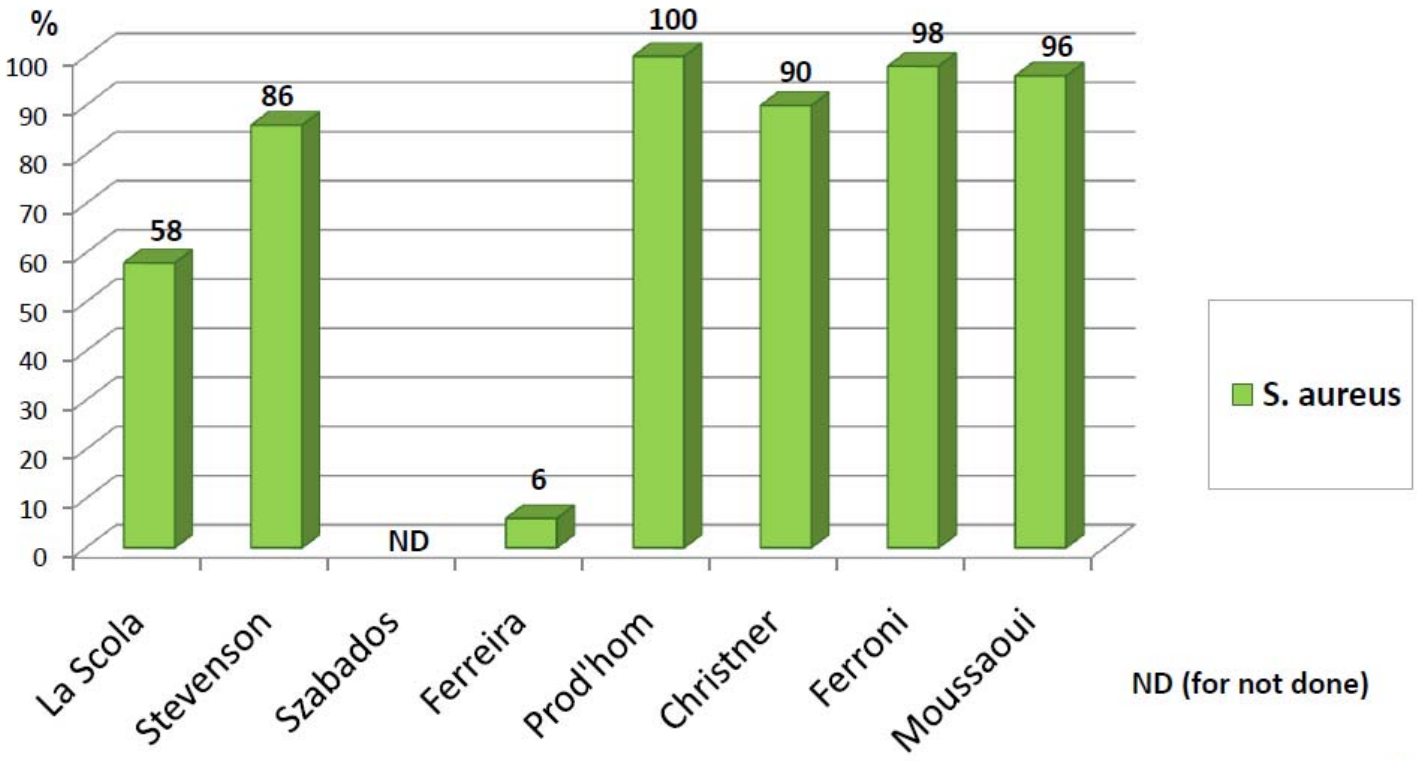
(8 published studies of mono-microbial bacteremia)



Direct Testing of Positive Blood Cultures by MALDI-TOF

Performance for S.aureus identification

(8 published studies of mono-microbial bacteremia)



Strenghts

Almost no misidentification :
(exception: *S.mitis* – *S.pneumoniae*)

Excellent for differentiating :
S.aureus from coag. neg. *staphylococci*
Enterobacteriaceae to the species level

Rapid : ≤ 60 min from positive blood culture signal

Cheap : < 1 US Dol./ test

Applications en routine au laboratoire de microbiologie

- Identification des bactéries y compris mycobactéries
- Identification des levures
- Identification des champignons filamenteux
- Identification des hémocultures positives

Avantages

- Simplicité: formation de l'opérateur en 4 heures
- Coût < 1€ l'identification
- Rapidité < 10 min – plus rapide que le Gram

Method	Delay, minutes	Cost, €[a]	Level of training
Manual			
Gram staining	6	0.6	Medium to high
API system identification (bioMérieux)	1080–2880	4.6–6.0	Medium
Antibiotic susceptibility test	1080–2880	6.6–7.4	Medium
Phoenix system identification and susceptibility test (BD Diagnostics)	300–1200	12.65	Medium
Vitek system (bioMérieux)			
Identification	300–480	5.9–8.23	Medium
Identification and susceptibility test	300–480	10.38–12.71	
MALDI-TOF	6–8.5	1.43	Low to medium

NOTE. MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight.

[a] Costs have been tabulated based on December 2008 price list of the providers in France

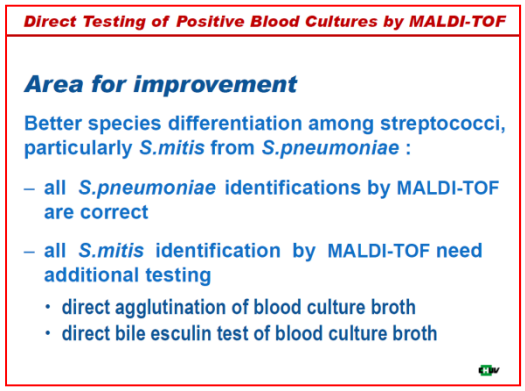
Prospectives médicales

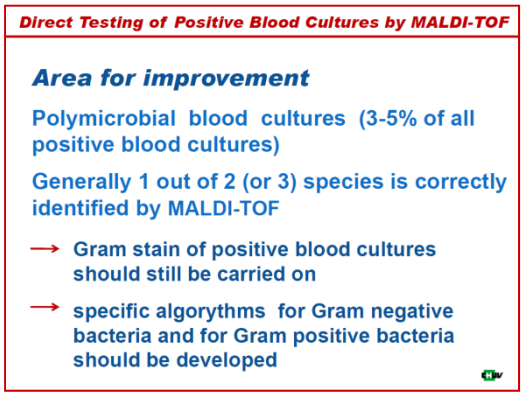
- Mesurer l'impact médical sur la prise en charge des patients
- Coût - efficacité

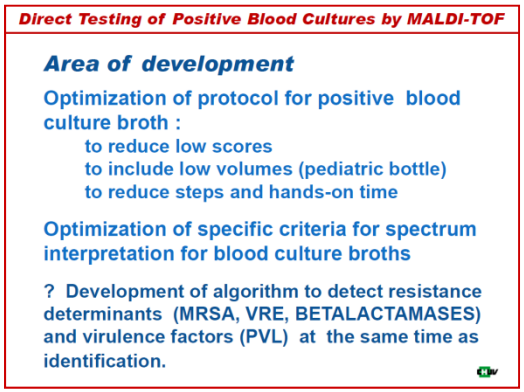
Prospectives techniques

Souches bactériennes

- *S. mitis* ≠ *S. pneumoniae*
- *E. coli* ≠ *Shigella* ?
- Phénotypes de résistance
- Phénotypage
- Déminuer inoculum < 10^6 cfu







Fluides biologiques

- Hemocultures mixtes
- Urocultures

Identification of animals cells

Journal of Virological Methods 164 (2010) 116–121

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Short communication

Rapid characterisation of cell cultures by matrix-assisted laser desorption/ionisation mass spectrometric typing

Axel Karger[a,*], Barbara Bettin[a], Matthias Lenk[b], Thomas C. Mettenleiter[a]

[a] Institute of Molecular Biology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany
[b] Institute of Infectology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany

J Am Soc Mass Spectrom. 2006 Apr;17(4):490-9. Epub 2006 Feb 17.

Identification of mammalian cell lines using MALDI-TOF and LC-ESI-MS/MS mass spectrometry.

Zhang X, Scalf M, Berggren TW, Westphall MS, Smith LM.

Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706-1396, USA.

OPEN ACCESS Freely available online

PLoS one

Global Analysis of Circulating Immune Cells by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

Richard Ouedraogo, Christophe Flaudrops, Amira Ben Amara, Christian Capo, Didier Raoult, Jean-Louis Mege*

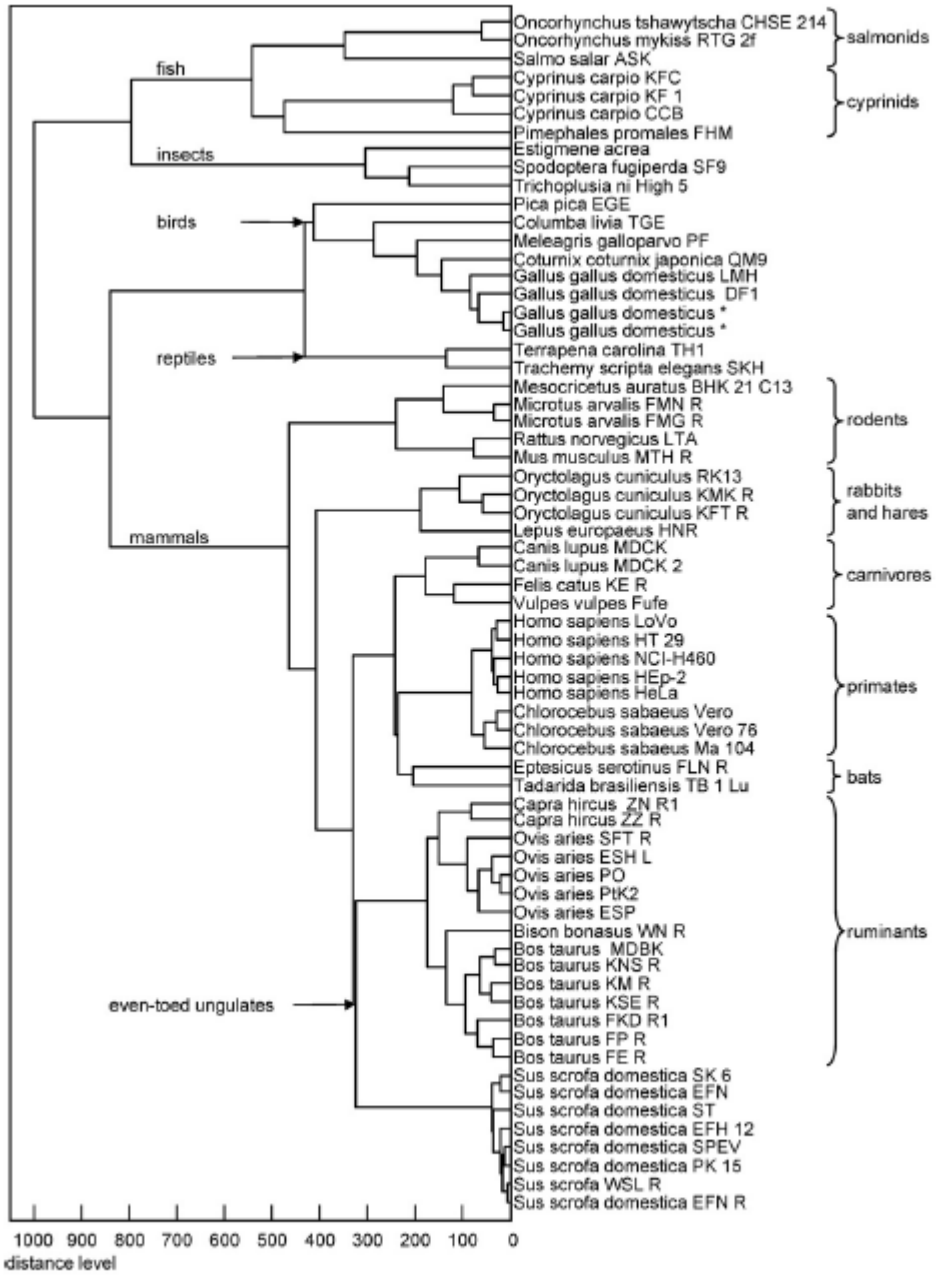


Fig. 3. Phylogenetic tree constructed from reference spectra of cell lines from a wide range of animal species. Systematic species designations and relevant taxonomic groups (in English) are given. Two independent chicken primary cell preparations are indicated with an asterisk. Phylogenetic distances are based on similarity scores calculated from the reference spectra as described in the text.

Identification of human blood cells

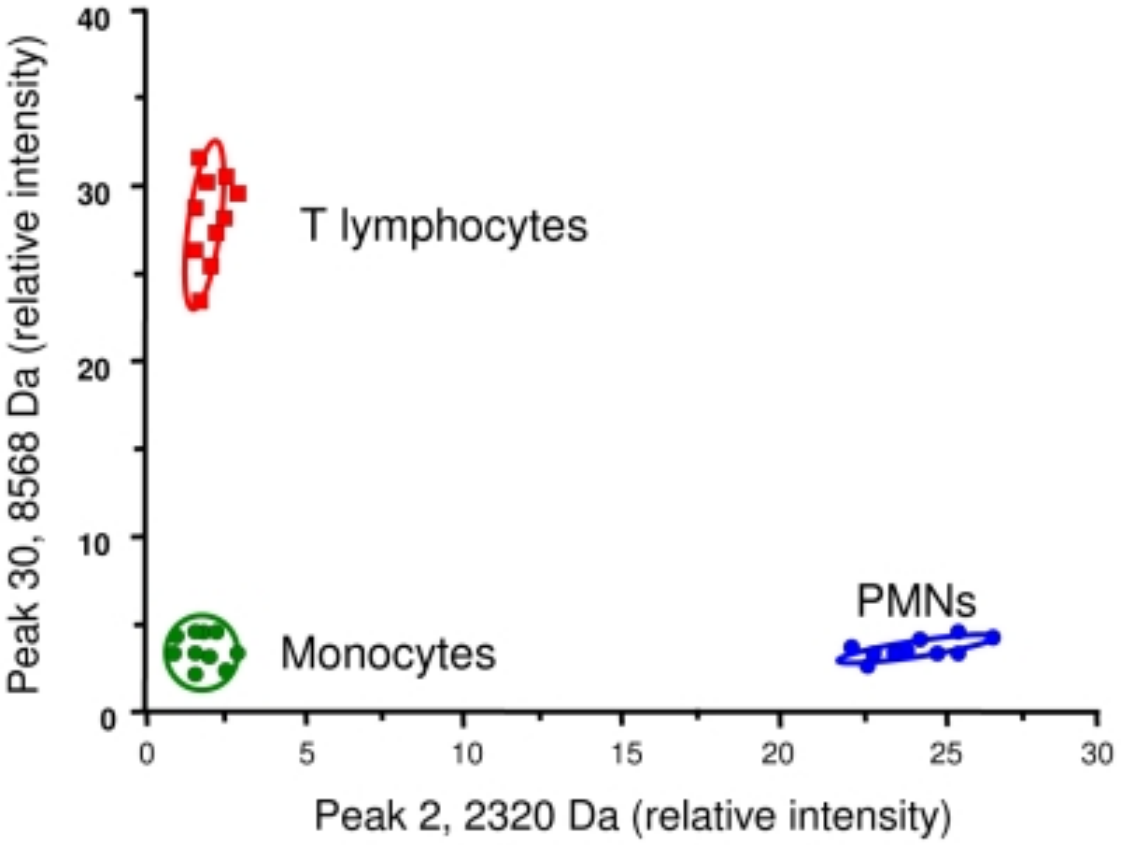


Figure 2. **MALDI-TOF MS spectra of circulating cells.**
T lymphocytes (A), PMNs (B) and RBCs (C) were isolated from a healthy blood donor. Representative MALDI-TOF MS spectra are shown.

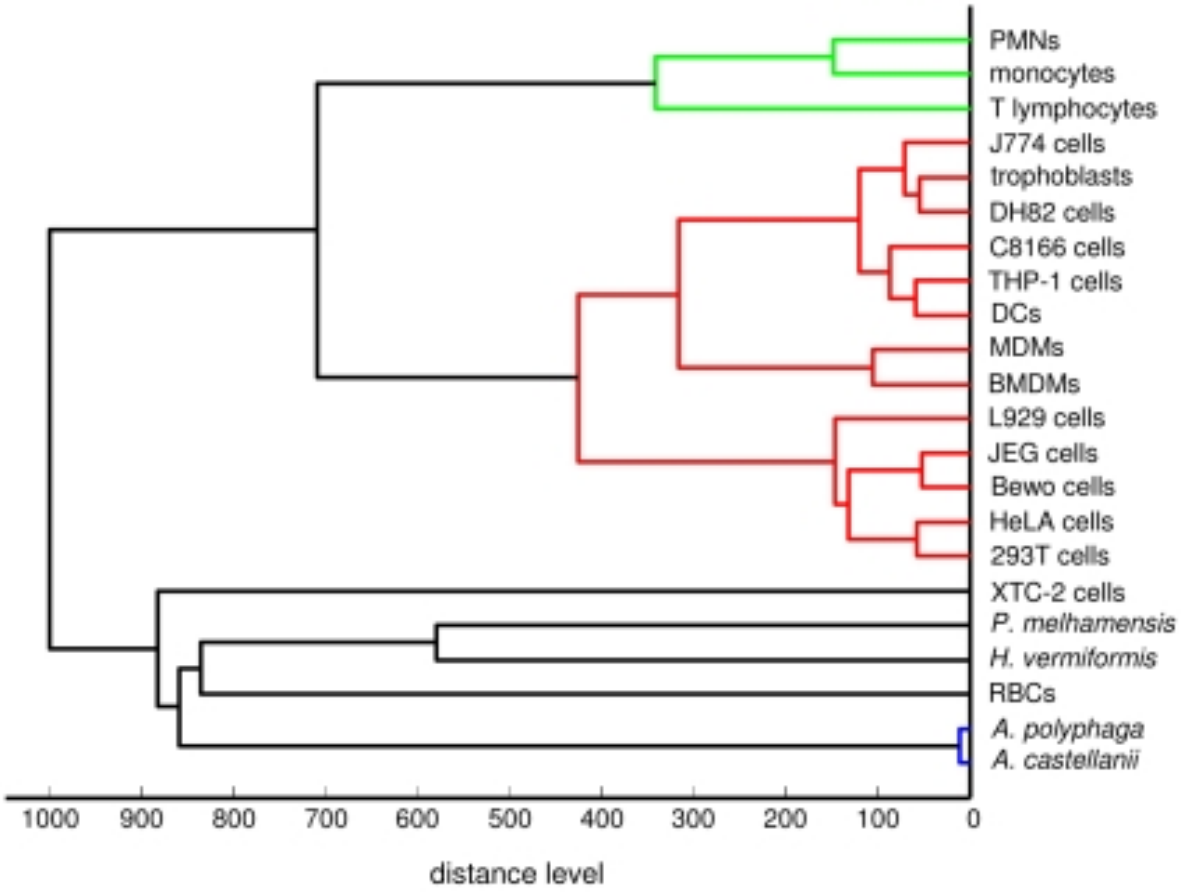
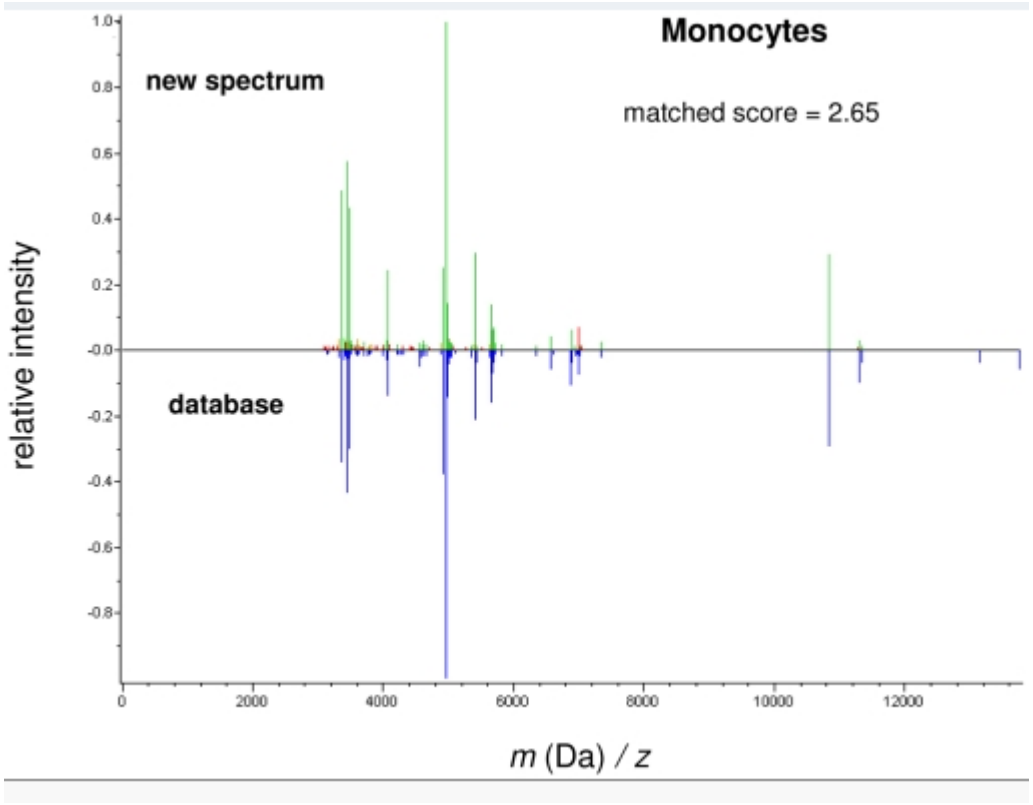


Figure 6**Dendrogram of 22 eukaryotic cell types.**
MALDI-TOF MS was performed on 22 cell types with at least 20 spectra per cell type. An averaged spectrum for each cell type was added to the database using the BioTyper software and the dendrogram creation method.



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Identification of mammals

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PLoS one

Classification of Ancient Mammal Individuals Using Dental Pulp MALDI-TOF MS Peptide Profiling

Thi-Nguyen-Ny Tran[1], Gérard Aboudharam[1], Armelle Gardeisen[2], Bernard Davoust[3], Jean-Pierre Bocquet-Appel[4], Christophe Flaudrops[1], Maya Belghazi[5], Didier Raoult[1], Michel Drancourt[1]*

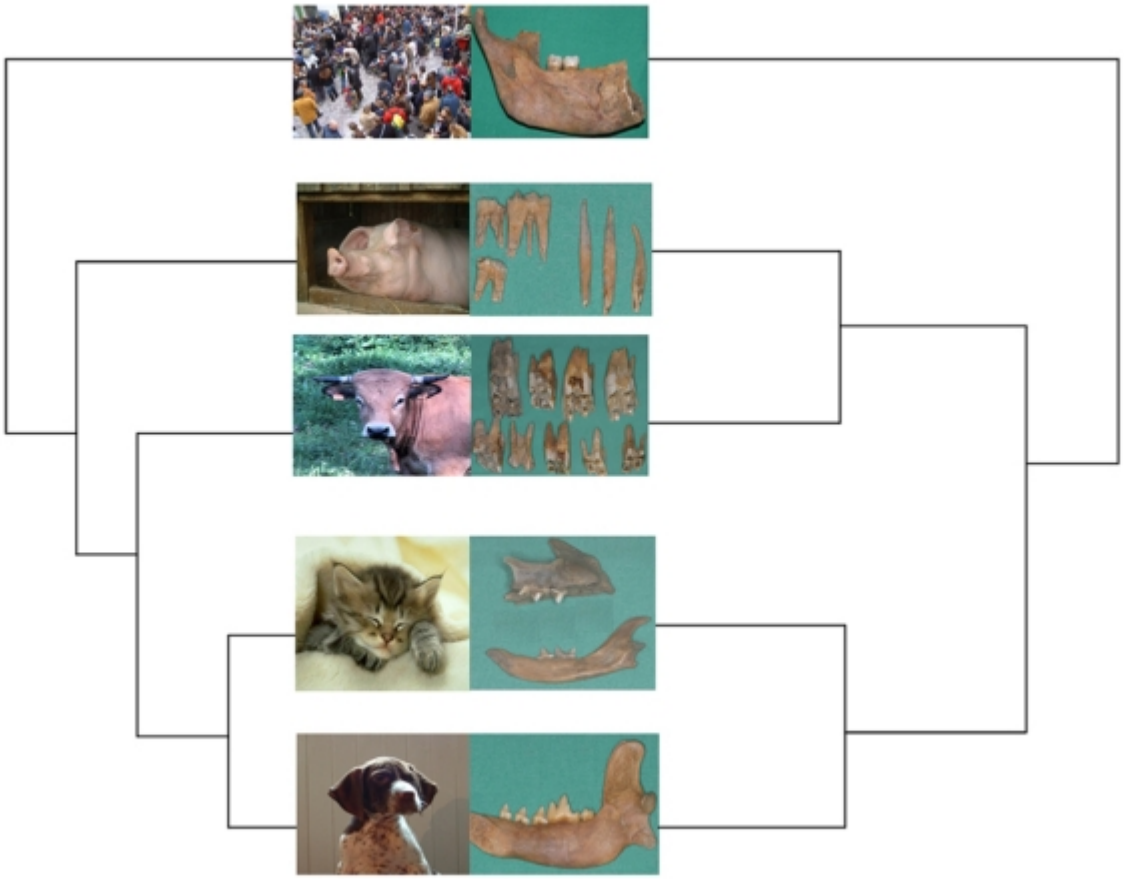
1 Unité de recherche sur les maladies infectieuses et tropicales émergentes (URMITE), UMR CNRS 6236 IRD 198, IFR48, Faculté de médecine, Université de la Méditerranée, Marseille, France, **2** UMR 5140-CNRS, Université Montpellier III, Montpellier, France, **3** Direction régionale du service de santé des armées de Toulon, Toulon, France, **4** Ecole Pratique des Hautes Etudes and CNRS UPR 2147, Paris, France, **5** Centre d'Analyses Protéomiques de Marseille (CAPM), IFR Jean Roche, Faculté de Médecine - Secteur Nord, Université de la Méditerranée, Marseille, France

Abstract

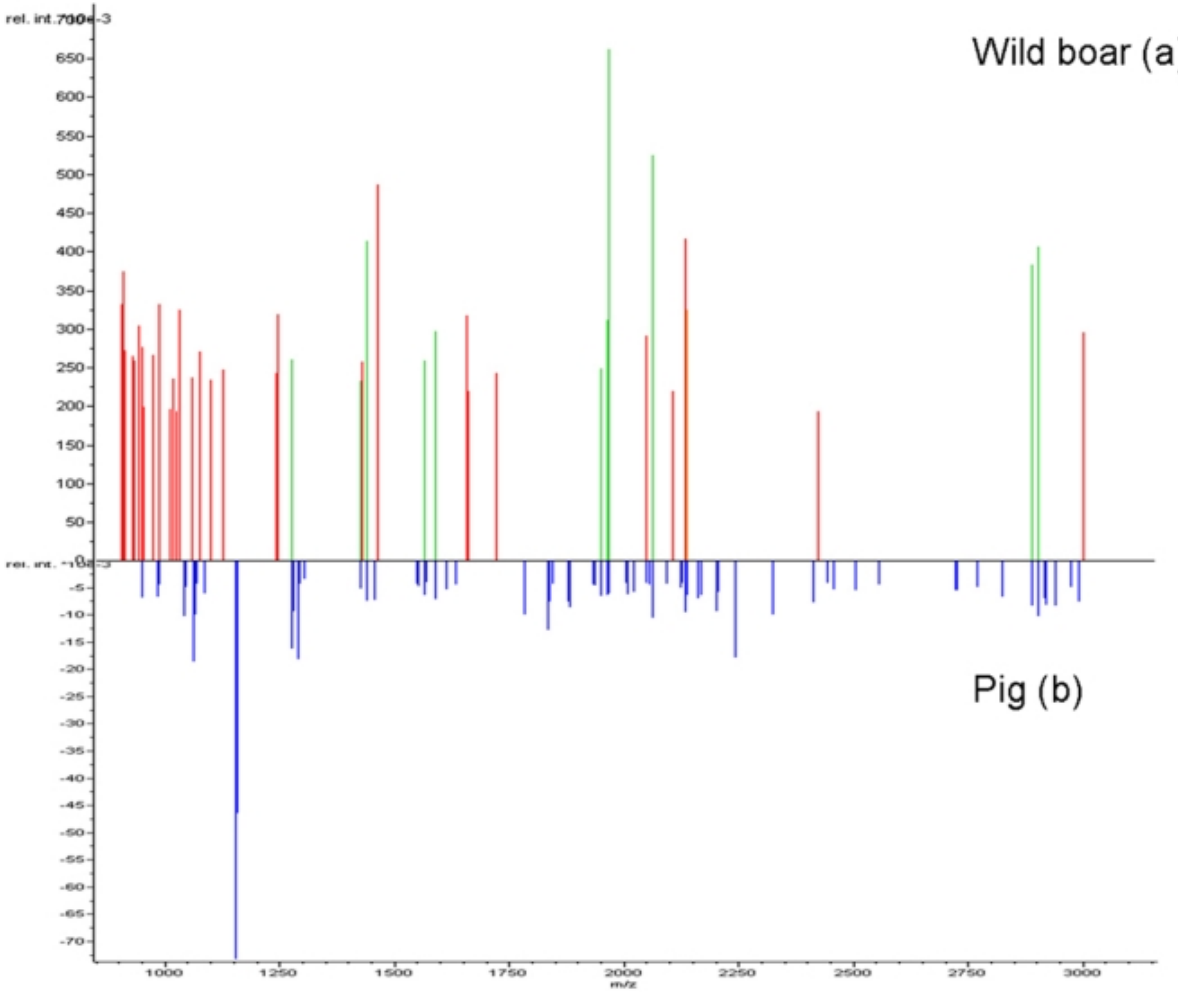
Background: The classification of ancient animal corpses at the species level remains a challenging task for forensic scientists and anthropologists. Severe damage and mixed, tiny pieces originating from several skeletons may render morphological classification virtually impossible. Standard approaches are based on sequencing mitochondrial and nuclear targets.

Methodology/Principal Findings: We present a method that can accurately classify mammalian species using dental pulp and mass spectrometry peptide profiling. Our work was organized into three successive steps. First, after extracting proteins from the dental pulp collected from 37 modern individuals representing 13 mammalian species, trypsin-digested peptides were used for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis. The resulting peptide profiles accurately classified every individual at the species level in agreement with parallel cytochrome *b* gene sequencing gold standard. Second, using a 279–modern spectrum database, we blindly classified 33 of 37 teeth collected in 37 modern individuals (89.1%). Third, we classified 10 of 18 teeth (56%) collected in 15 ancient individuals representing five mammal species including human, from five burial sites dating back 8,500 years. Further comparison with an upgraded database comprising ancient specimen profiles yielded 100% classification in ancient teeth. Peptide sequencing yield 4 and 16 different non-keratin proteins including collagen (alpha-1 type I and alpha-2 type I) in human ancient and modern dental pulp, respectively.

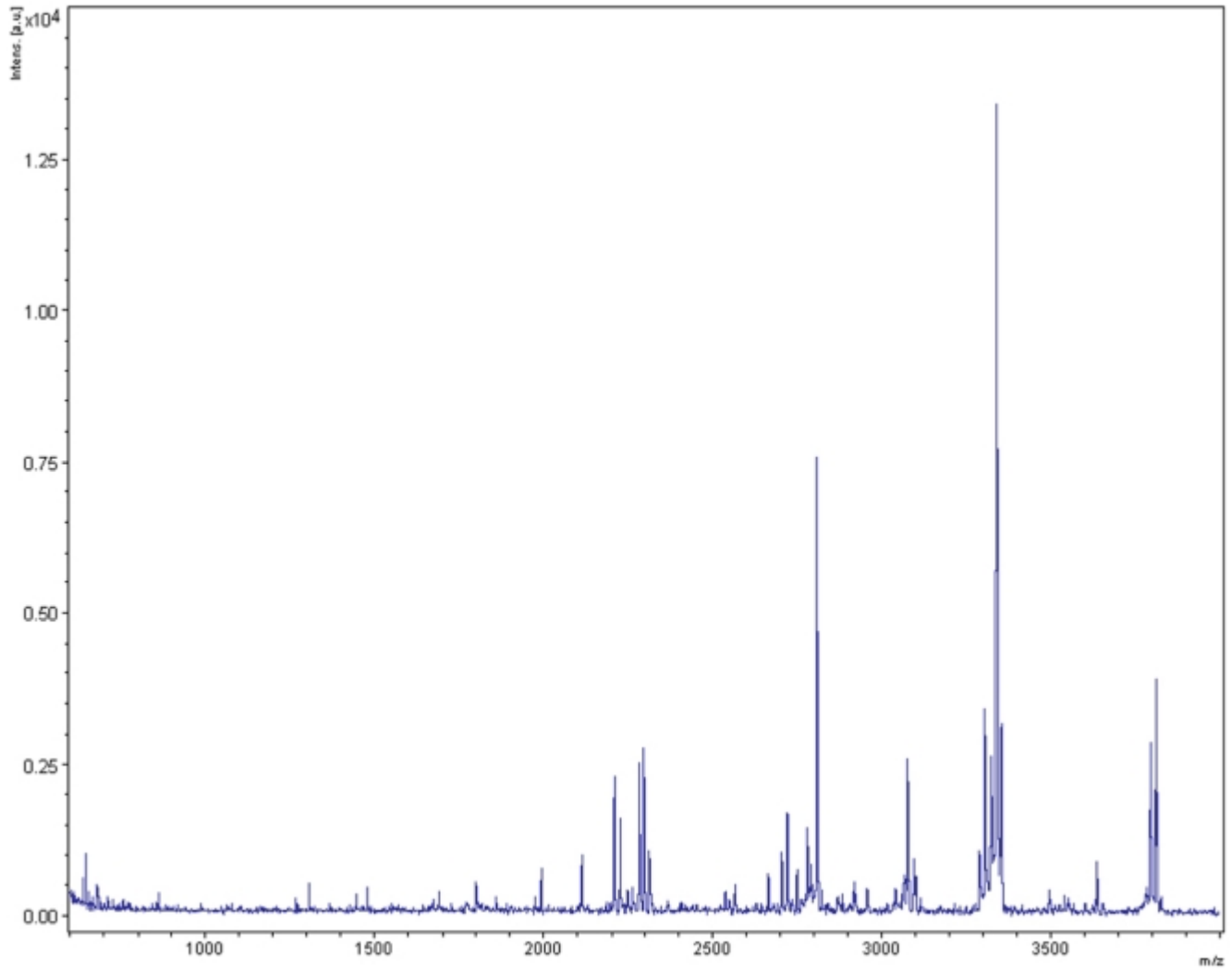
Conclusions/Significance: Mass spectrometry peptide profiling of the dental pulp is a new approach that can be added to the arsenal of species classification tools for forensics and anthropology as a complementary method to DNA sequencing. The dental pulp is a new source for collagen and other proteins for the species classification of modern and ancient mammal individuals.



The dendrogram obtained by the software Maldi Biotyper 2.0 (Bruker Daltonics) after peptide spectral analysis is congruent with the one derived from cytochrome *b* sequencing by the software Tree View (Free down load from the Internet site: http://darwin.zoology.gla.ac.uk/~rpage/treeviewx/download.html**).** The images indicate the mammalian species (left) from which the ancient teeth were identified (right).



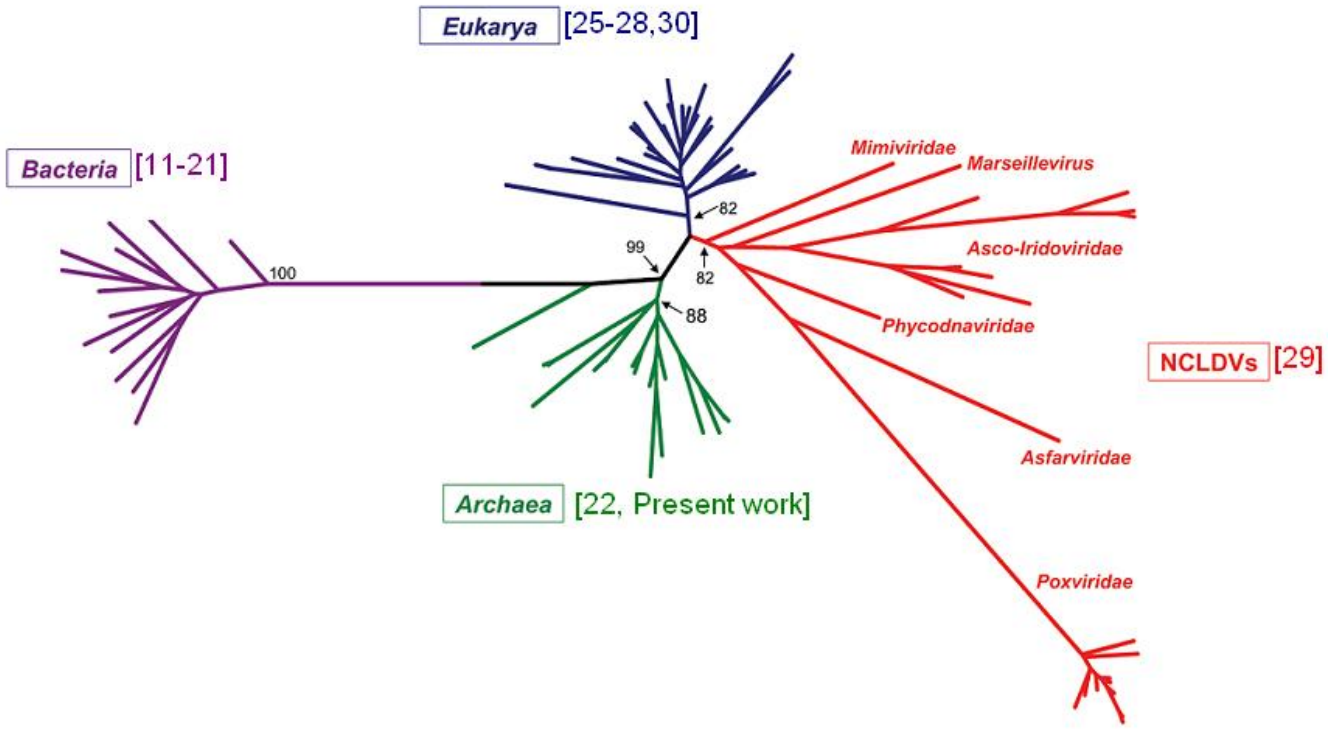
MALDI-TOF MS peptide profiling of proteins extracted from dental pulp. Pseudo-gel displays (Gel View, Bruker Daltonics) of the peptide spectra obtained with wild boar (a) and pig (b) specimens were used as the reference peptide. The mass-to-charge ratio (m/z) of each peptide is indicated on the x-axis, and the relative intensity of each peak is shown on the y-axis. Blue bars correspond to peaks from pig specimens, red bars correspond to peaks from wild boar specimens, and green bars represent peaks shared by both.



MALDI-TOF MS peptide spectrum obtained from 8,500-year-old human dental pulp.

Conclusion: MALDI-TOF-MS

Towards an universal first-line identification approach for living organisms



Schematic illustration of application of MALDI-TOF-MS on identification of organisms belonging to the 4 domains of life with corresponding references indicated in the brackets (adapted from Boyer M. *et al.* 2010 PLoS One)