LA SPECTROMETRIE DE MASSE EN MICROBIOLOGIE CLINIQUE : PROPOSITION D'UNE AUTRE SOLUTION

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La technologie présentée fait l' objet d'une propriété intellectuelle appartenant à l' Assistance Publique-Hôpitaux de Paris et à l'Université Paris Descartes.
Xavier Nassif est l'un des inventeurs

Cette Propriété intellectuelle a été licenciée à ANDROMAS SAS dont Xavier Nassif est l'un des actionnaires fondateur

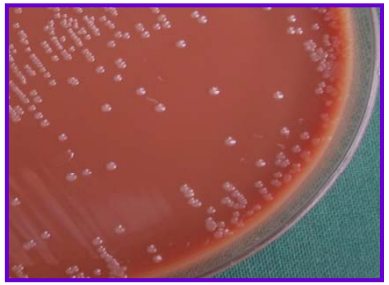
Why accurate identification of bacteria is important?

- *To obtain the proper diagnostic of a bacterial infection*
- *To implement the most appropriate anti-infective therapy before antibiotic susceptibility testing*
- *To identify new bacterial pathogens*
- *To manage nosocomial infections (follow up, epidemiology…)*

Phenotypic identification

Biological sample

Culture



24 hours

Molecular biology

Bacterial identification by MALDI-TOF-MS is a strategy based on the identification of species specific components in the proteome of the pathogen to be identified

Biological sample

Culture

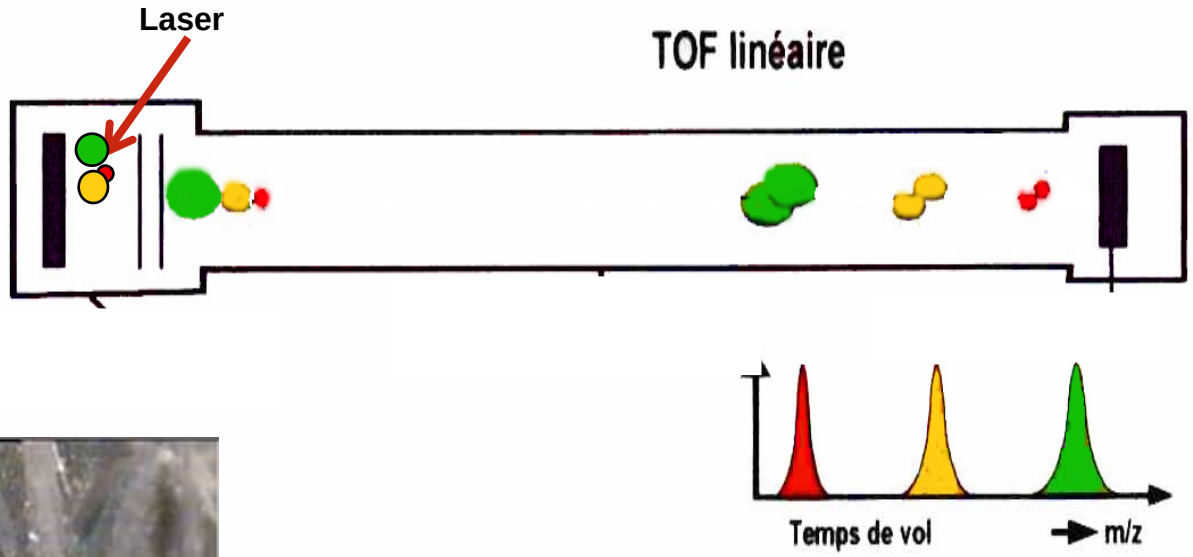
Bacterial identification within minutes

- Speed up the process of bacterial identification **once the pathogen has been cultivated**
- No expertise is required for accurate bacterial identification

MALDI-TOF

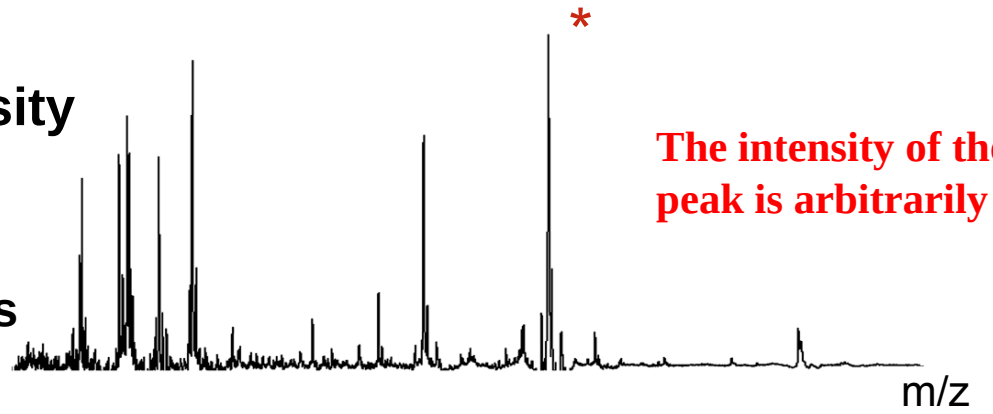
Matrix Assisted Laser Desorption Ionisation-Time Of Flight

Sample= Bacteria embeded in a matrix



DHB, Dihydroxybenzoic acid

HCCA, alpha cyano 4 hydroxy cynic acid



The intensity of the highest peak is arbitrarily set up to 1

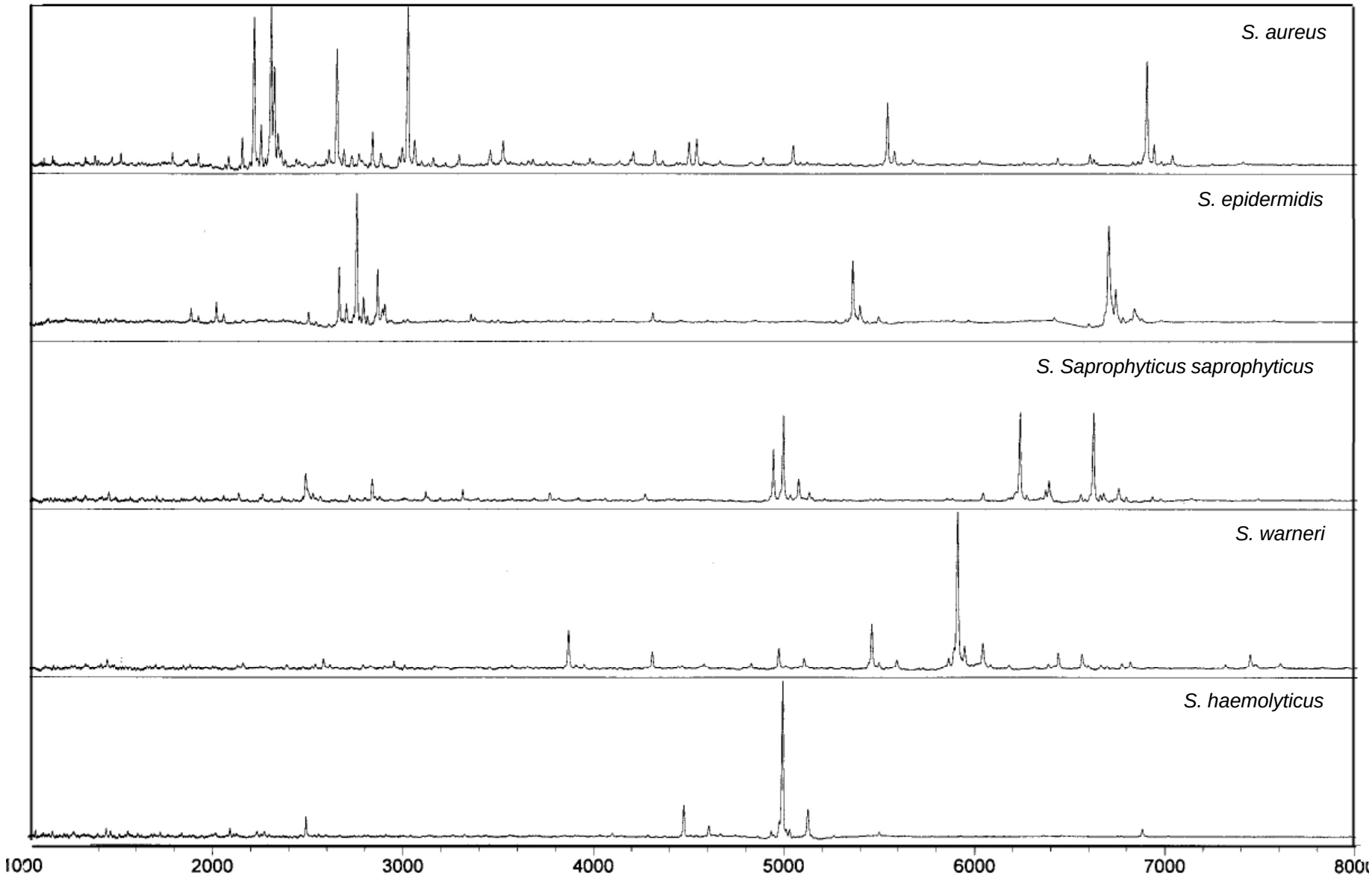
Range between 2 and 20 Kilodaltons

m/z	Rel. Intens.
1012	0,02
1141	0,02
1198	0,03
1253	0,06
1253	0,06
1318	0,02
1323	0,03
1340	0,02
1357	0,06
1375	0,02
1375	0,02
1395	0,02
1412	0,02
1446	0,02
1476	0,04
1477	0,04
1483	0,02
1486	0,02
1494	0,05
1496	0,04
1539	0,03
1624	0,03
1690	0,14
1728	0,04
1750	0,06
1765	0,74
1783	0,67
1804	0,17

m/z	Rel. Intens.
1821	0,12
1845	0,02
1902	0,05
1919	0,03
1927	0,03
2132	0,03
2192	0,72
2215	0,15
2231	0,34
2253	0,06
2280	0,96
2299	0,62
2319	0,29
2337	0,14
2358	0,05
2417	0,02
2539	0,08
2575	0,11
2586	0,03
2601	0,05
2614	0,04
2629	0,73
2650	0,07
2667	0,18
2704	0,03
2765	0,03
2817	0,04
2861	0,03
2959	0,06
2975	0,04

m/z	Rel. Intens.
2986	0,03
3001	0,93
3022	0,06
3039	0,22
3061	0,02
3075	0,04
3137	0,05
3261	0,02
3438	0,10
3504	0,06
3517	0,04
3634	0,04
3762	0,02
3888	0,03
3923	0,02
3978	0,03
4173	0,04
4188	0,03
4304	0,12
4437	0,02
4483	0,23
4505	0,02
4523	0,33
4543	0,03
4561	0,10
4812	0,03
5016	0,04
5032	0,32
5053	0,04
5070	0,10

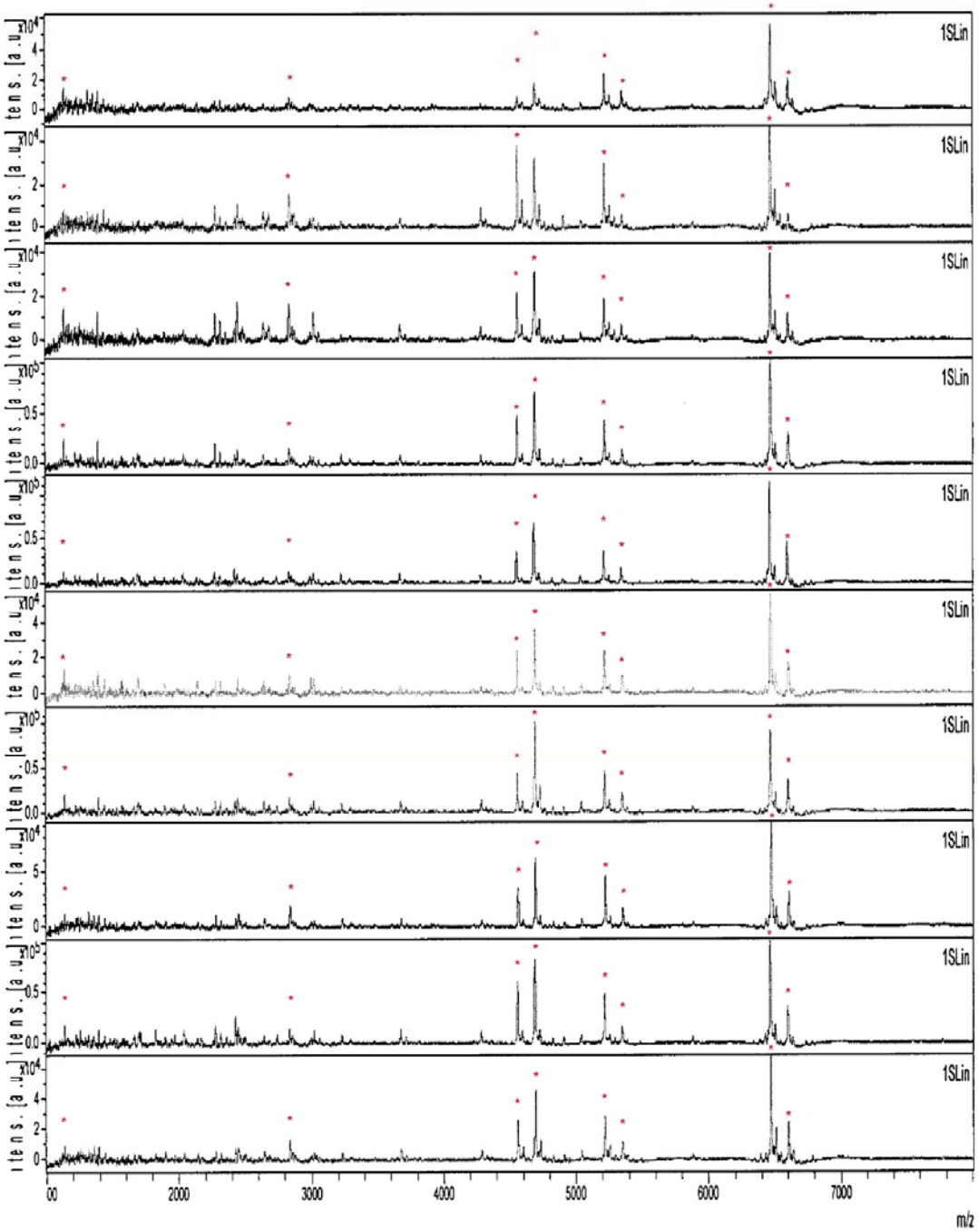
m/z	Rel. Intens.
5107	0,03
5127	0,02
5168	0,03
5438	0,02
5510	0,05
5528	0,55
5547	0,05
5563	0,13
5600	0,02
5662	0,05
5934	0,03
6011	0,03
6032	0,02
6426	0,05
6555	0,13
6575	0,03
6593	0,02
6595	0,02
6616	0,07
6821	0,10
6848	0,05
6876	0,14
6892	1,00
6930	0,19
6953	0,02
6968	0,04
7029	0,10
7402	0,03
8156	0,02
9636	0,07
9665	0,04


S. aureus
S. epidermidis
S. Saprophyticus saprophyticus
S. warneri
S. haemolyticus
1000
2000
3000
4000
5000
6000
7000
8000

S. hominis hominis

As expected different isolates of the same strain have different proteomes. However some species specific components have to be conserved.

Hypothesis : peaks conserved among colonies with an intensity above 0.1 correspond to elements species specific



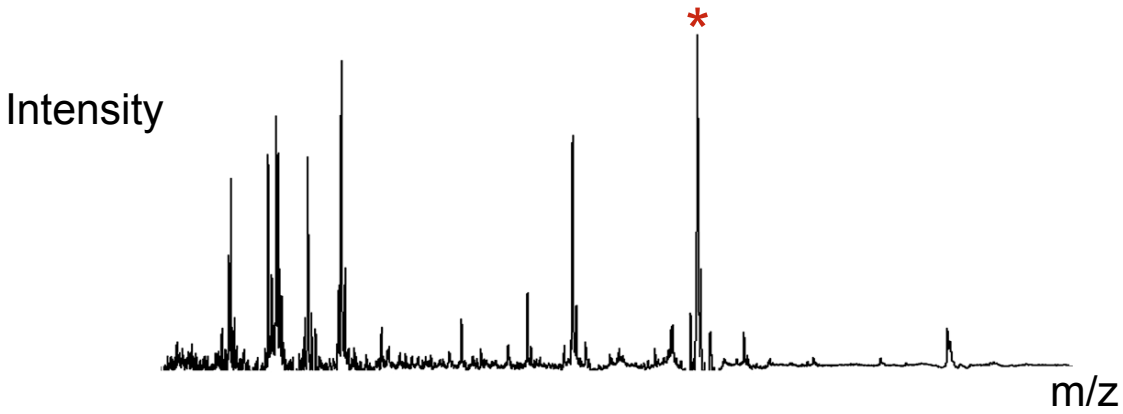
Testing the hypothesis

- To construct a database in a group of bacteria of clinical interest : exemple *Staphylococci* . The peaks with an intensity above 0.1 conserved among 10 isolates of each reference strain were included in the database

-To compare the spectra obtained by MALDI-TOF-MS of a set of clinical isolates with that of the reference isolates in the database

List of bacterial species included in the *Staphylococci* database

species
S. aureus
Micrococcus luteus
Micrococcus lentus
S. epidermidis
S. warneri
S. xylosus
S. intermedius
S. haemolyticus
S. saprophyticus subsp *saprophyticus*
S. saprophyticus subsp *bovis*
S. lugdunensis
S. hominis subsp *hominis*
S. hominis subsp *novobiosepticus*
S. capitis subsp *capitis*
S. capitis subsp *ureolyticus*
S. caprae
S. pasteuri
S. cohni subsp *cohni*
S. cohni subsp *urealyticum*
S. scheiferi subsp *scheiferi*
S. scheiferi subsp *coagulans*
S. sciuri subsp *sciuri*
S. simulans



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1446	0,02
1476	0,04
1477	0,04
1483	0,02
1486	0,02
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8156	0,02
9636	0,07
9665	0,04

Staphylococci database

S.capitis capitis	*S.capitis ureolyticus*	*S.caprae*	*S.cohni cohni*	*S.cohni urealyticum*	*S.epidermidis*	*S.haemolyticus*	*S.hominis hominis*	*S.hominis novobiosepticus*
2611 (+/-2) 2642 (+/-2) 2664 (+/-2) 2681 (+/-2) 2908 (+/-2) 2947 (+/-2) 5099 (+/-3) 6713 (+/-4)	5098 (+/-3) 5137 (+/-3) 5235 (+/-3) 6713 (+/-4) 6751 (+/-4)	2642 (+/-1) 2665 (+/-1) 2681 (+/-1) 2959 (+/-1) 5215 (+/-3)	2074 (+/-1) 2097 (+/-1) 2113 (+/-1) 2690 (+/-1) 4481 (+/-2) 6567 (+/-4)	2075 (+/-1) 2098 (+/-1) 2114 (+/-1) 2136 (+/-2) 6079 (+/-3) 6541 (+/-4)	2836 (+/-4) 5340 (+/-2) 6682 (+/-4) 6720 (+/-4)	4981 (+/-3) 4997 (+/-3) 5036 (+/-3) 5131 (+/-3) 6891 (+/-5)	1138 (+/-2) 2839 (+/-2) 4564 (+/-2) 4695 (+/-2) 5223 (+/-2) 5356 (+/-2) 6476 (+/-2) 6609 (+/-2)	2496 (+/-2)) 2839 (+/-2) 4696 (+/-3) 4916 (+/-3) 5224 (+/-3) 6478 (+/-4)

S.pasteuri	*S.saprophyticus bovis*	*S.saprophyticus saprophyticus*	*S.schleiferi coagulans*	*S.schleiferi schleiferi*	*S.sciuri sciuri*	*S.simulans*	*S. warneri*	*S.xylosus*
2176 (+/-1) 2215 (+/-1) 2352 (+/-2) 2393 (+/-2) 2432 (+/-2) 2784 (+/-2) 2807 (+/-2) 2823 (+/-2) 2866 (+/-2) 2888 (+/-2) 2905 (+/-2)	2478 (+/-2) 2499 (+/-2) 2517 (+/-2) 2828 (+/-3) 4936 (+/-4) 4987 (+/-3) 6288 (+/-5) 6383 (+/-5) 6617 (+/-5)	1004 (+/-1) 2478 (+/-2) 2828 (+/-2) 4935 (+/-4) 4986 (+/-4) 5066 (+/-4) 6228 (+/-5) 6617 (+/-5)	4276 (+/-4) 4851 (+/-4) 4873 (+/-4) 4889 (+/-4) 4984 (+/-4) 6683 (+/-6) 6771 (+/-6)	2577 (+/-2) 2670 (+/-2) 2692 (+/-2) 2708 (+/-2) 2922 (+/-2) 2944 (+/-2) 2961 (+/-2) 2983 (+/-2) 3000 (+/-2)	1342 (+/-1) 1360 (+/-2) 1395 (+/-1) 1407 (+/-1) 1421 (+/-1) 1436 (+/-4) 5520 (+/-2) 5652 (+/-2)	2780 (+/-1) 2893 (+/-1) 2933 (+/-2) 3958 (+/-2) 3996 (+/-1) 4094 (+/-2) 4321 (+/-2) 5694 (+/-3)	2177 (+/-2) 2336 (+/-1) 2375 (+/-2) 2585 (+/-2) 2824 (+/-2) 2936 (+/-2) 5457 (+/-2) 5906 (+/-3) 5945 (+/-3)	2429 (+/-2) 4236 (+/-2) 6065 (+/-2) 6386 (+/-3) 6572 (+/-3)

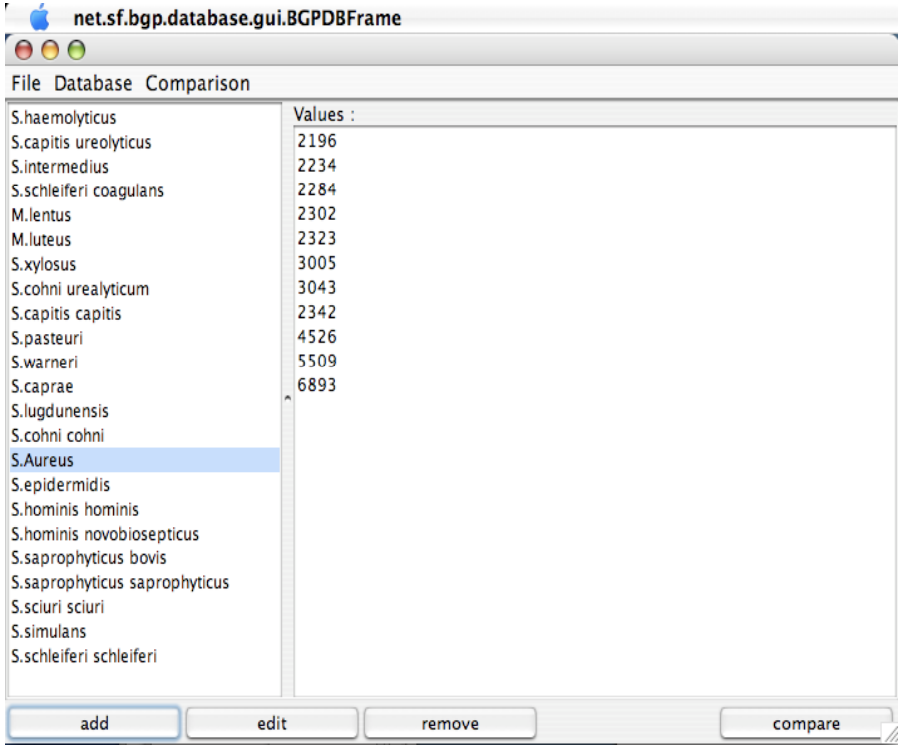
212 isolates were tested. Their identification was confirmed by molecular biology

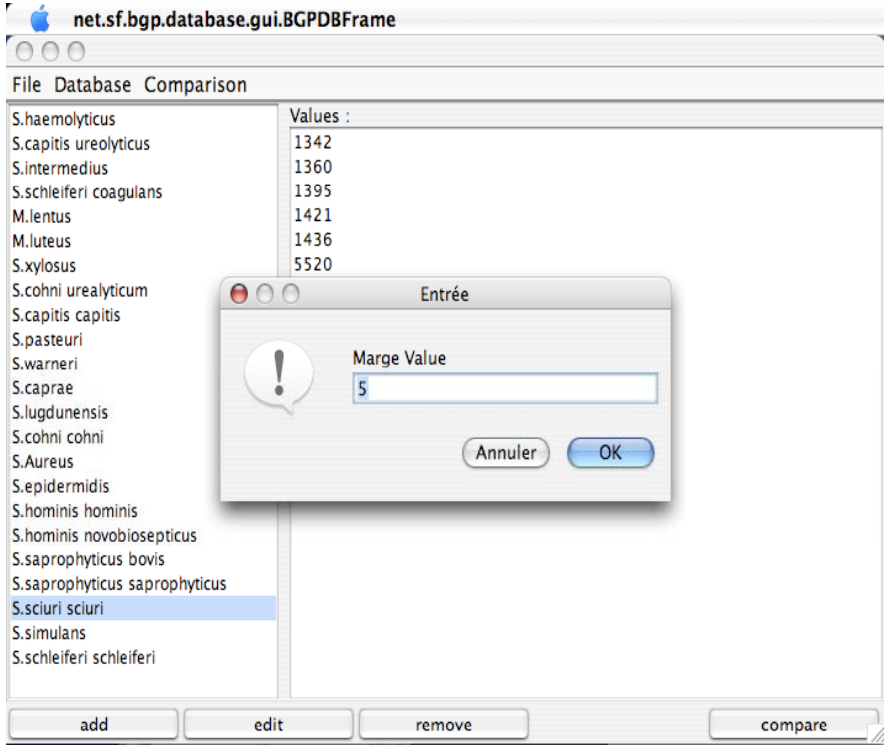
Peaks with an intensity above 0.1 were retained and compared to that of the database.

The species in the database having the best match was retained for the identification of the tested strain

Species	Strain
Micrococcus luteus	CIP A270
S. epidermidis	81 clinical isolates
S. warneri	CIP 106511
	CIP 8165
	6 clinical isolates
S. xylosus	CIP 103720
	CIP 104065
S. intermedius	CIP 81.60
S. haemolyticus	CIP 104114
	13 clinical isolates
S. saprophyticus subsp *saprophyticus*	CIP 76.125T
	CIP 103545
S. saprophyticus subsp *bovis*	CIP 105262
	CIP 105261
S. saprophyticus spp *	6 clinical isolates
S. lugdunensis	CIP 103584
S. hominis subsp *hominis*	CIP 81.57
	CIP 104689
S. hominis subsp *novobiosepticus*	CIP 105719T
S. hominis spp *	10 clinical isolates
S. capitis subsp *capitis*	CIP 103688
S. capitis subsp *ureolyticus*	CIP 104192T
S. caprae	CIP 104000T
	CIP 104520
S. pasteuri	CIP 105540T
	CIP 103830
	CIP 103832
S. cohni subsp *urealyticum*	CIP 104024T
	CIP 104025
S. scheiferi subsp *coagulans*	CIP 104366
S. sciuri subsp *sciuri*	CIP 8162T
	CIP 103583
	CIP 103825
S. aureus	68 clinical isolates

Bacteria Graphic Profile Data Base





Results

	Number of strains	Number of peaks shared with the reference strain in the database
S.aureus	68	9,6/11
S.capitis capitis	1	8/8
S.capitis urealyticus	1	7/7
S.caprae	2	5/5
S.cohni urealyticus	2	6/6
S.epidermidis	81	3,2/4
S.haemolyticus	14	3,4/5
S.hominis hominis	6	5,6/8
S.hominis novobio	7	4/6
S.intermedius	1	10/10
S.lugdunensis	1	8/8
S.saprophyticus bovis	3	7,6/9
S.saprophyticus saprophyticus	7	6/8
S.schleiferi coagulans	1	7/7
S.pasteuri	3	11/11
S.sciuri sciuri	3	7/7
S.xylosus	2	5/5
S.warneri	8	6,7/9
M.luteus	1	7/7

MALDI-TOF versus automate for the identification of coagulase negative staphylococci

	Species	(Nb)	MALDI							Phoenix						
			DB	Id	(%)	Mis Id	(%)	No Id	(%)	DB	Id	(%)	Mis Id	(%)	No Id	(%)
1	*S. auricularis*	(1)	y	1	(100)					y			1	(100)		
2	*S. capitis*	(20)	y	20	(100)					y	20	(100)				
3	*S. caprae*	(14)	y	13	(92.9)			1	(7.1)	y	9	(64.3)	5	(35.7)		
4	*S. cohnii*	(15)	y	14	(93.3)			1	(6.7)	y	10	(66.7)	5	(33.3)		
5	*S. epidermidis*	(56)	y	56	(100)					y	50	(89.3)	6	(10.7)		
6	*S. haemolyticus*	(14)	y	12	(85.8)	1	(7.1)	1	(7.1)	y	14	(100)				
7	*S. hominis*	(29)	y	29	(100)					y	20	(69)	9	(31)		
8	*S. intermedius*	(1)	y	1	(100)					y			1	(100)		
9	*S. lugdunensis*	(14)	y	14	(100)					y	13	(92.9)	1	(7.1)		
10	*S. pasteuri*	(12)	y	10	(83.3)	2	(16.7)			y	5	(41.7)	7	(58.3)		
11	*S. saprophyticus*	(12)	y	12	(100)					y	11	(91.7)	1	(8.3)		
12	*S. schleiferi*	(2)	y	2	(100)					y	2	(100)				
13	*S. sciuri sciuri*	(1)	y	1	(100)					y	1	(100)				
14	*S. simulans*	(14)	y	14	(100)					y	11	(78.6)	3	(21.4)		
15	*S. warneri*	(16)	y	16	(100)					y	8	(50)	8	(50)		
16	*S. xylosus*	(3)	y	3	(100)					y	3	(100)				
Total database species only		**(224)**		**218**	**(97.4)**	**3**	**(1.3)**	**3**	**(1.3)**		**177**	**(79)**	**47**	**(21)**	**0**	**(0)**

	Species	(Nb)	VITEK-2								
			DB	Id	(%)	Mis Id	(%)	No Id	(%)	Low Id	(%)
1	*S. auricularis*	(1)	y	1	(100)						
2	*S. capitis*	(20)	y	12	(60)	3	(15)	1	(5)	4	(20)
3	*S. caprae*	(14)	y	14	(100)						
4	*S. cohnii*	(15)	y	13	(86.7)	2	(13.3)				
5	*S. epidermidis*	(56)	y	52	(92.9)	4	(7.1)				
6	*S. haemolyticus*	(14)	y	11	(78.6)	2	(14.3)			1	(7.1)
7	*S. hominis*	(29)	y	25	(86.2)	3	(10.4)			1	(3.4)
8	*S. intermedius*	(1)	y	1	(100)						
9	*S. lugdunensis*	(14)	y	12	(85.8)	1	(7.1)			1	(7.1)
10	*S. pasteuri*	(12)	y			2	(16.7)			10	(83.3)
11	*S. saprophyticus*	(12)	y	10	(83.4)	1	(8.3)			1	(8.3)
12	*S. schleiferi*	(2)	y	1	(50)			1	(50)		
13	*S. sciuri sciuri*	(1)	y	1	(100)						
14	*S. simulans*	(14)	y	11	(78.6)	2	(14.3)			1	(7.1)
15	*S. warneri*	(16)	y	9	(56.3)	3	(18.7)			4	(25)
16	*S. xylosus*	(3)	y	3	(100)						
Total database species only		**(224)**		**176**	**(78.6)**	**23**	**(10.3)**	**2**	**(0.9)**	**23**	**(10.3)**

Construction of databases for the main group of bacteria isolated in clinical microbiology laboratories

- **Gram + cocci catalase + (*Staphylococci*)**
- **Gram + Cocci catalase - (*Streptococci*, *Enterococci*)**
- **Gram - cocci (*Neisseria*)**
- **Gram + Bacilli**
- **Gram - oxydase - bacilli (*Enterobacteriaceae*)**
- **Gram - oxydase + bacilli (*Pseudomonas*)**
- **Fastidious Gram - bacilli (HACECK, *Haemophilus*..)**
- **Anaerobes**
- ***Campylobacter/Helicobacter***
- **Mycobacteria**
- ***Aspergillus***
- **Yeast**
- **Dermatophytes**

3 Steps

- Engineering the database (choice of the species to be included in each group)
- Testing the database with a set of hundreds of clinical strains identified by molecular biology
- Routine use

MALDI Biotyper 2.0

BRUKER

-Each spectra in the database correspond to the common peaks (up to 100) per spectra obtained in various growth conditions

-Results = log of a value obtained by measuring identities and differences between the spectra of the tested strain and that of database

SARAMIS

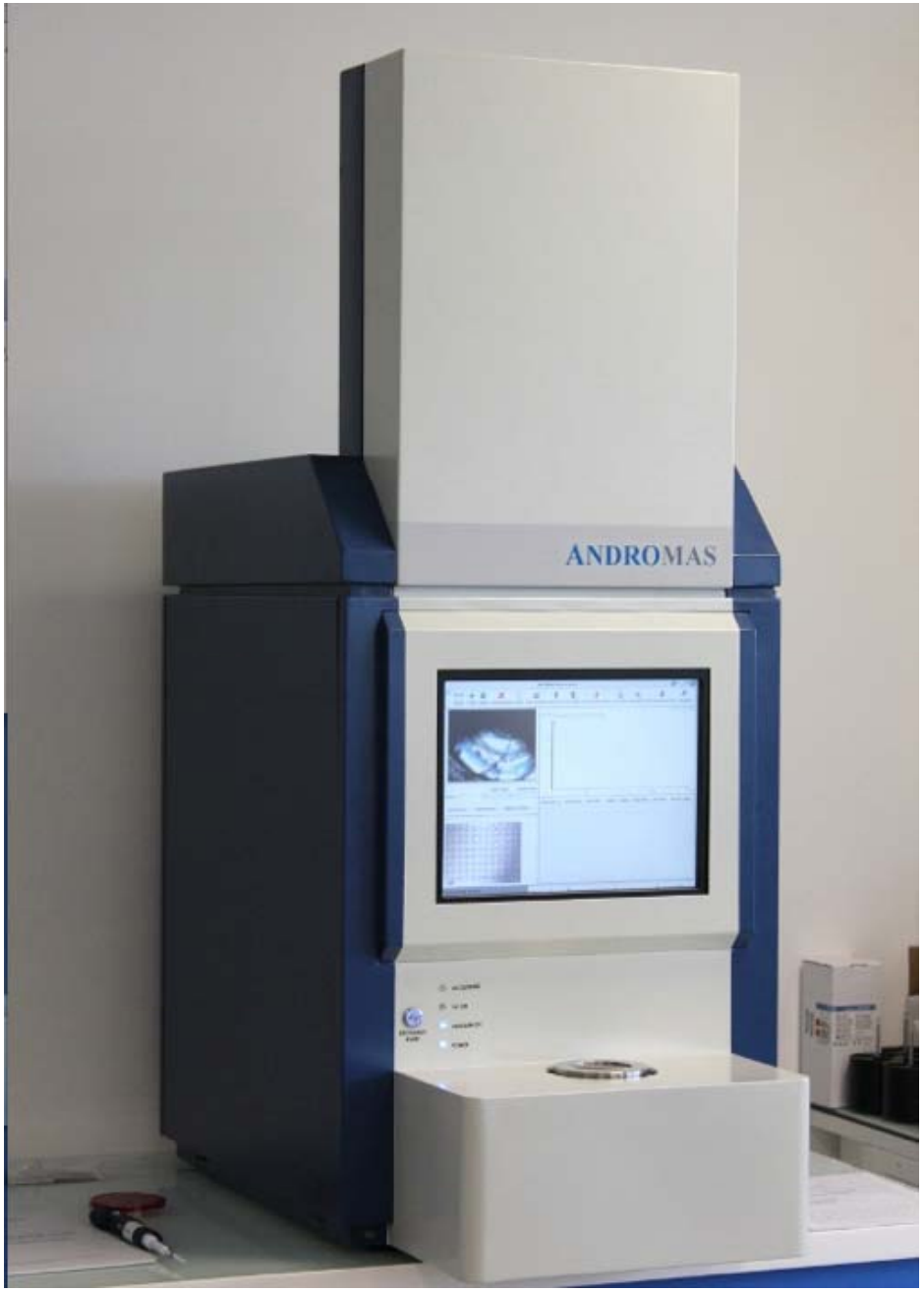
Spectral ARchive And Microbial Identification System

AnagnosTec

BIOMÉRIEUX

•Database is made of superspectra and spectra. Superspectra : peaks found in 90% of the strains of a species (20-40 peaks). There may be several superspectra for each species.

•Results : Comparison between the spectrum of the tested strain with that of the database
if ≥70% : identification is good (>80% for yeast)
if <70% : identification not certain and there is a possibility to search the spectrum database and if 10 to 20 spectra of the same species have a score above 45%, the identification is good.



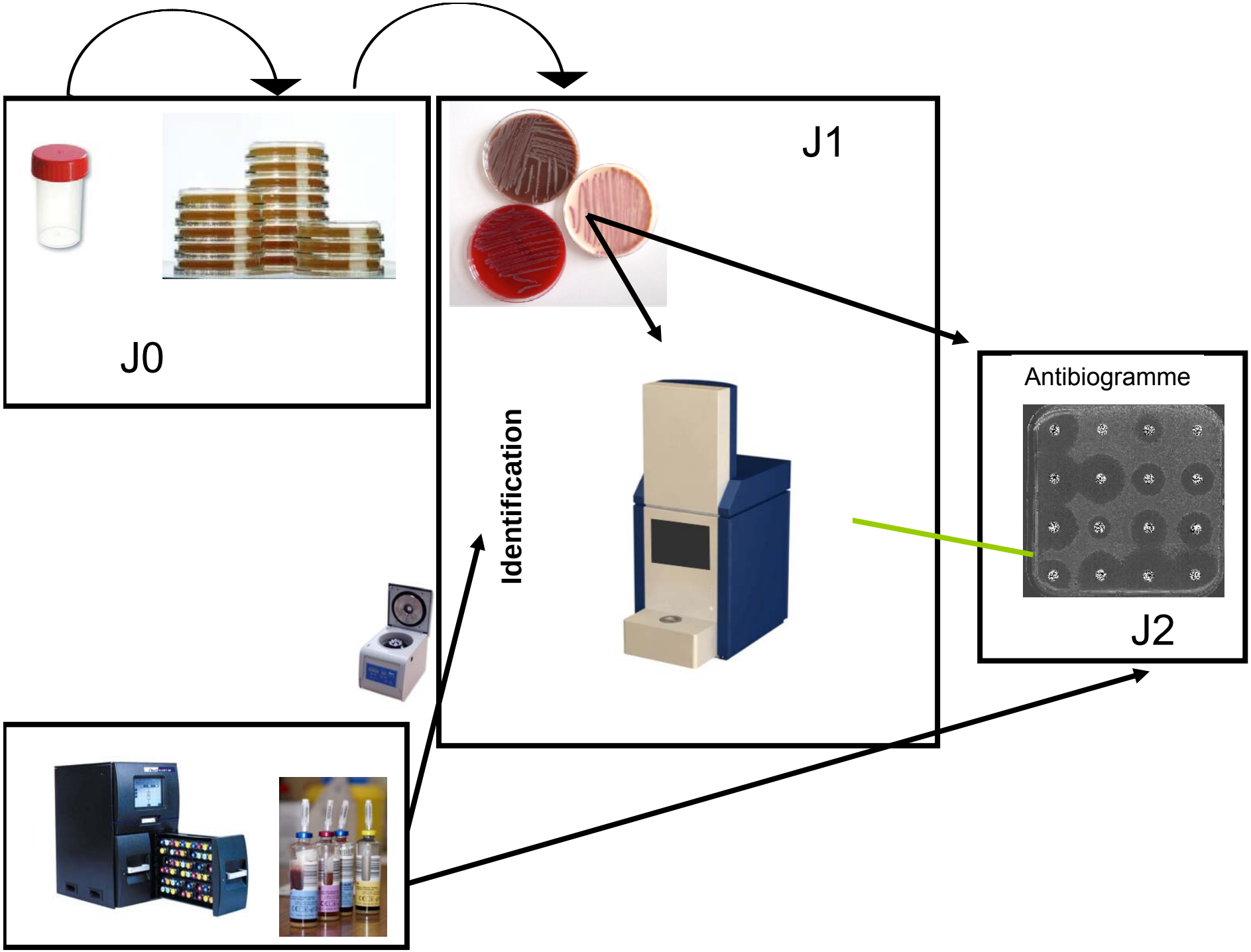
ANDROMAS

andromas.com

Pépinière Paris Santé Cochin 29 rue du Faubourg Saint Jacques 75014 Paris

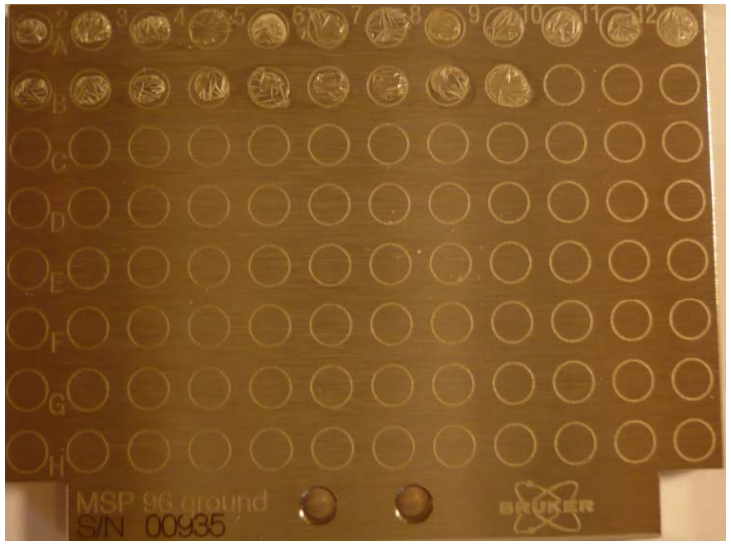
The Necker-Enfants malades Hospital

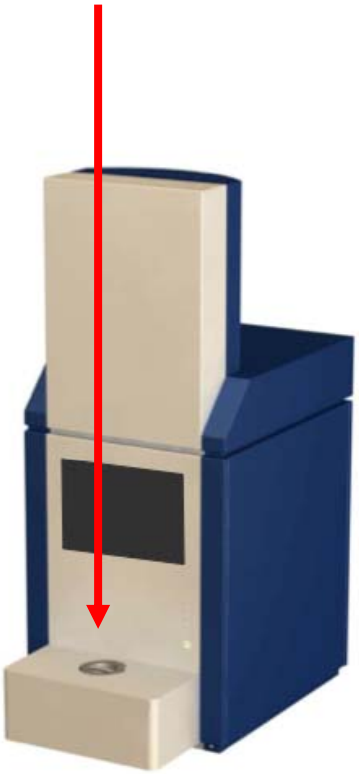
- Primary and tertiary referral center
- 489 beds
  - **130 surgical beds**
    - » **30 ICU beds**
  - **304 medical beds**
    - » **29 ICU beds**
  - **40 Obstetrical beds**
  - **4 Emergency beds**
- 55 000 hospitalizations/year


J0
J1
Identification
Antibiogramme
J2

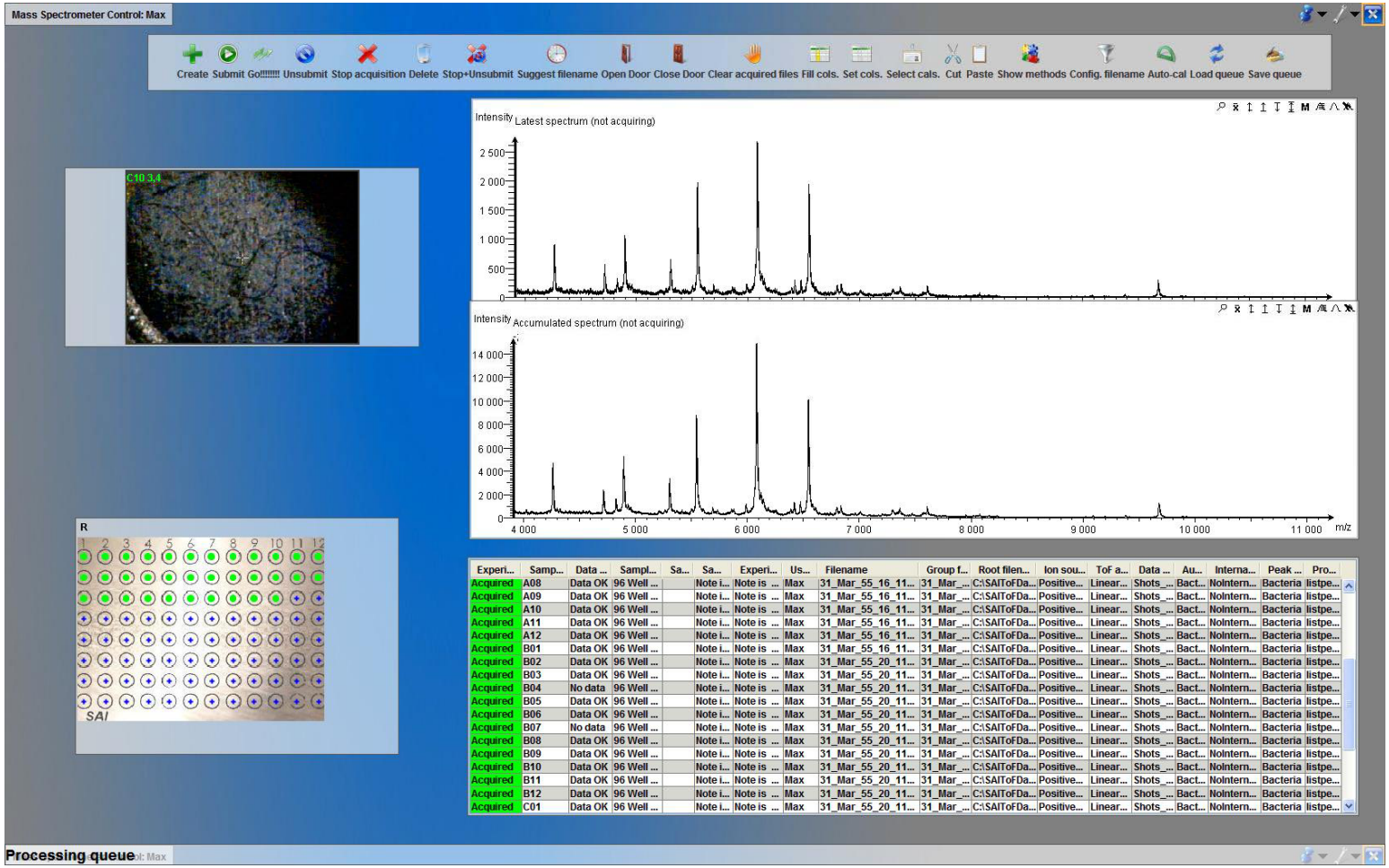
In each well

-Bacteria are deposited with a swab
-1 µl of ethanol is added
- 1 µl of matrix is added
- left at room T





10 series of 50 shots each
Peaks between 3500 et 20000 m/z were retained



- The spectra of the strain to be identified was compared with that of the reference spectra in the databases.
- For bacteria grown on plates
  - Good identification = the best match has at least 65% identity
    - with at least 10% difference with the second best match the identification was considered as being good at the species level
    - with less than 10 % with the second best match, but the first two best matches correspond to species of the same genus, the identification was considered as being good at the genus level
  - Identification likely = the best match has between 60 and 65% identity with at least 10% difference with the second best match the identification was considered as being likely
  - In all other cases the answer was no identification

RESULTS

Between 01/06/2010 and 31/07/2010 MALDI-TOF-MS was used to identify 2630 bacteria grown on plate

After the first acquisition on one colony

Good identification	= 2481	(94,3%)
Likely identification	= 43	(1,6%)
No identification	= 106	(4,1%)

After a second acquisition (106+43 strains)

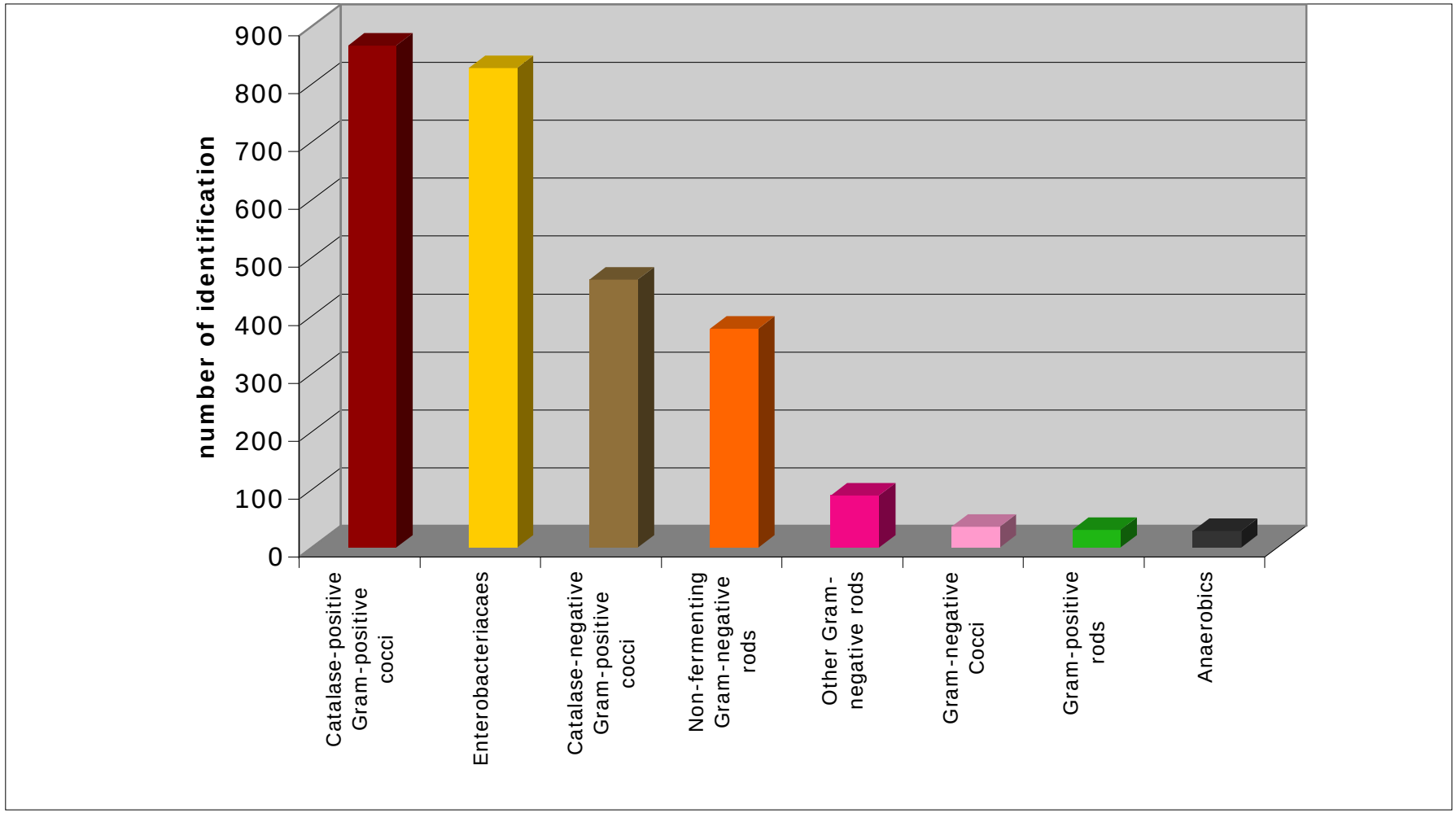
Good identification	= 123	(99%)
Likely identification	= 5	(0,2%)
No identification	= 21	(0,8%)

Staphylococcus auricularis,
Staphylococcus lugdunensis
Staphylococcus pettenkoferi

Hafnia alvei
Raoultella terrigena
Pantoea ananatis

Enterococcus avium
Granulicatella adiacens
Streptococcus vestibularis
Pediococcus acidilactici

Chryseomonas luteola
Ochrobactrum anthropi
Comamonas kerstersii
Acinetobacter haemolyticus



Capnocytophaga sputigena
Haemophilus paraphrohaemolyticus
Eikenella corrodens
Kingella kingae

Corynebacterium amycolatum
Gardnerella vaginalis
Lactobacillus rhamnosus
Turicella otitidis

Parvimonas micra
Propionibacterium acnes
Bacteroides thetaiotaomicron
Propionibacterium avidum

21 strains not identified by MALDI-TOF

MB: Atopobium vaginae
MB: Kocuria kristinae
MB: Dialister pneumosintes
MB: Staphylococcus croceolyticus
Enterococcus faecium
Streptococcus agalactiae
MB: Actinomyces graevenitzii
BGP Molecular biology in process
MB: Enterobacter pulveris
Enterobacter aerogenes
Escherichia coli
MB: Streptococcus mitis
MB: Aeromonas caviae
MB: Aeromonas caviae
MB: Pseudomonas putida
BGP Molecular biology in process
CGP Molecular biology in process
Clostridium difficile
Enterococcus faecalis
Streptococcus agalactiae
MB: Acinetobacter radioresistens

Identification of bacteria grown directly out of blood culture bottles

- Speed up the establishement of an adequate antibiotic treatment by 24 hrs

METHOD

- Bacteria are released from the cells in one step after solubilization of blood cells
- Removal of the cellular membranes by centrifugation

- For bacteria grown in blood culture liquid
  - Good identification = the best match has at least 60% identity
    - with at least 10% difference with the second best match the identification was considered as being good at the species level
    - with less than 10 % with the second best match, but the first two best matches correspond to species of the same genus, the identification was considered as being good at the genus level
  - Identification likely = the best match has betwwen 50 and 60% identity with at least 10% difference with the second best match the identification was considered as being likely
  - In all other cases the answer was no identification

RESULTS

Between 01/06/2010 and 31/07/2010 MALDI-TOF-MS was used to identify bacteria grown in 162 blood culture bottles. The acquisiton was performed in duplicate

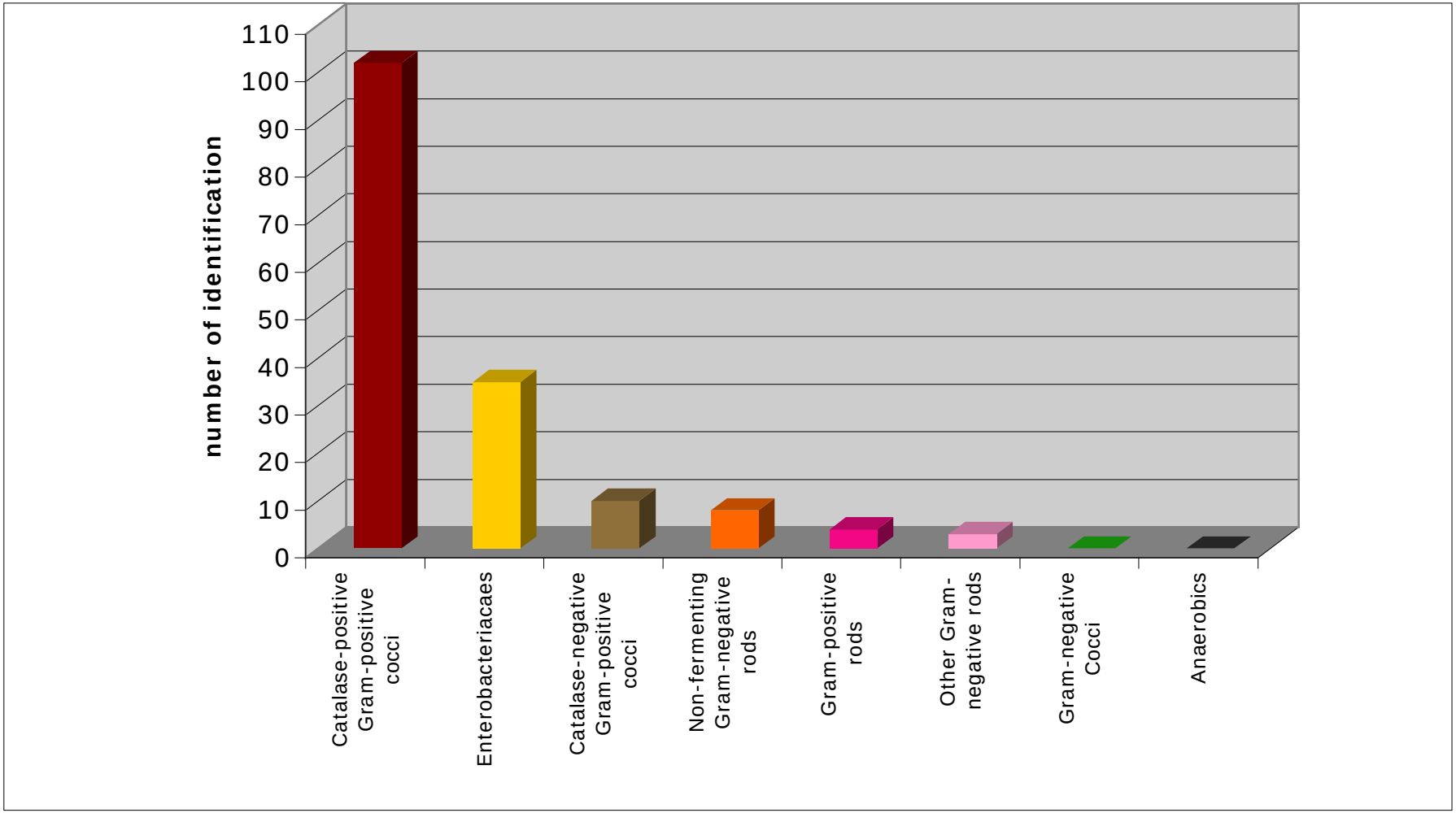
Good identification	= 142	(87,6%)
Likely identification	= 3	(1,8%)
Identification at the genus level	= 2	(1,2%)
No identification (2 mixed culture)	= 15	(9,2%)

Gram staining on positive blood culture +++ to eliminate positive blood culture with mixed bacteria

Staphylococcus aureus
Staphylococcus lugdunensis
Micrococcus luteus

Granulicatella adiacens

Pseudomonas alcaligenes
Pseudomonas aeruginosa



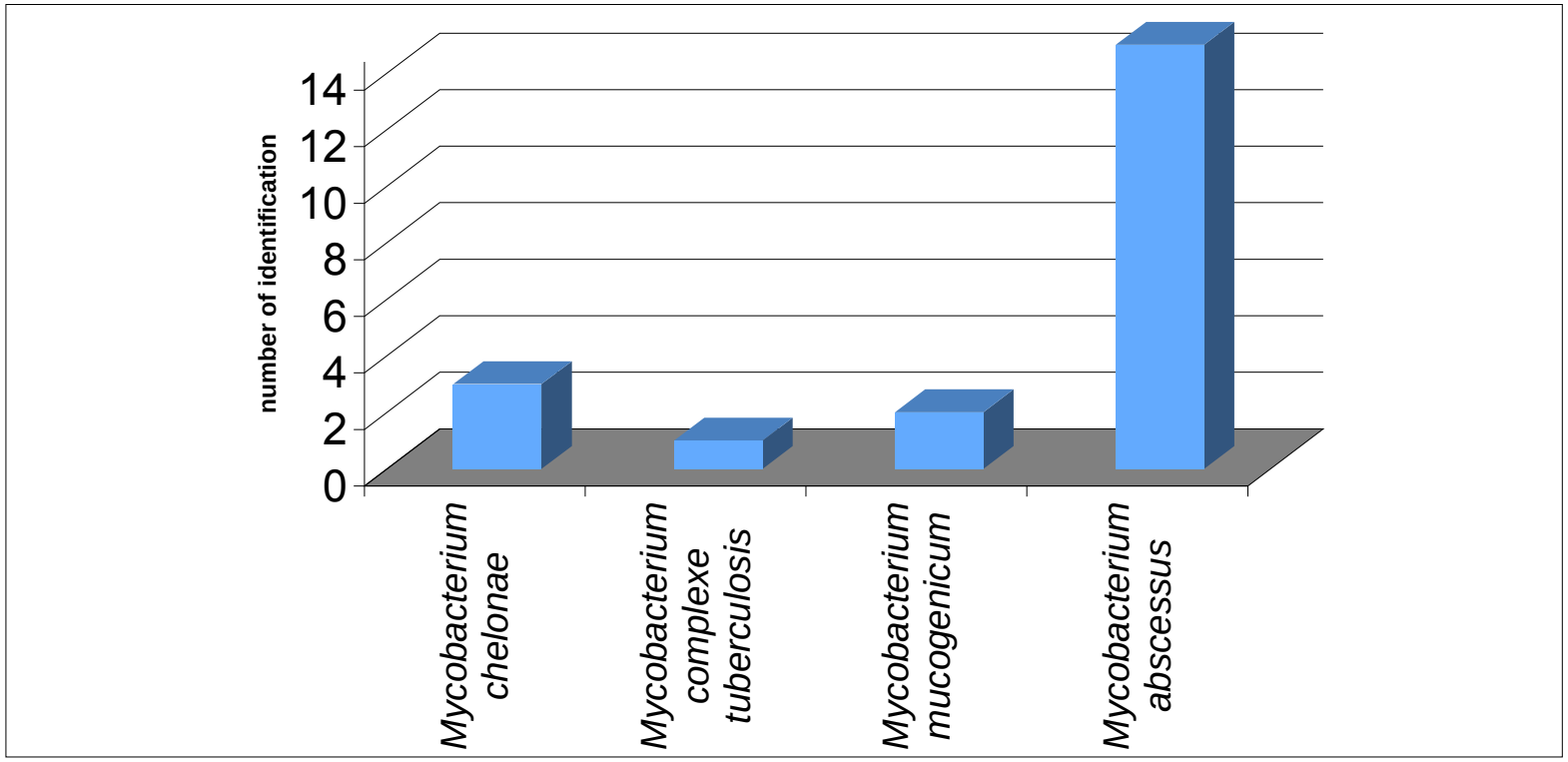
Capnocytophaga sputigena
Haemophilus influenzae

15 strains non identified

	MB: Virgibacillus proomii
	Capnocytophaga sputigena
	Corynebacterium bovis
Two strains	*Escherichia coli + Enterobacter cloacae*
	Pseudomonas fluorescens
	Rhizobium radiobacter
	Staphylococcus aureus
	Staphylococcus aureus
	Staphylococcus epidermidis
	Staphylococcus haemolyticus
	Staphylococcus haemolyticus
	Staphylococcus hominis
	Streptococcus constellatus
	Streptococcus mitis
Two strains	*Streptococcus pyogenes+ Staphylococcus aureus*

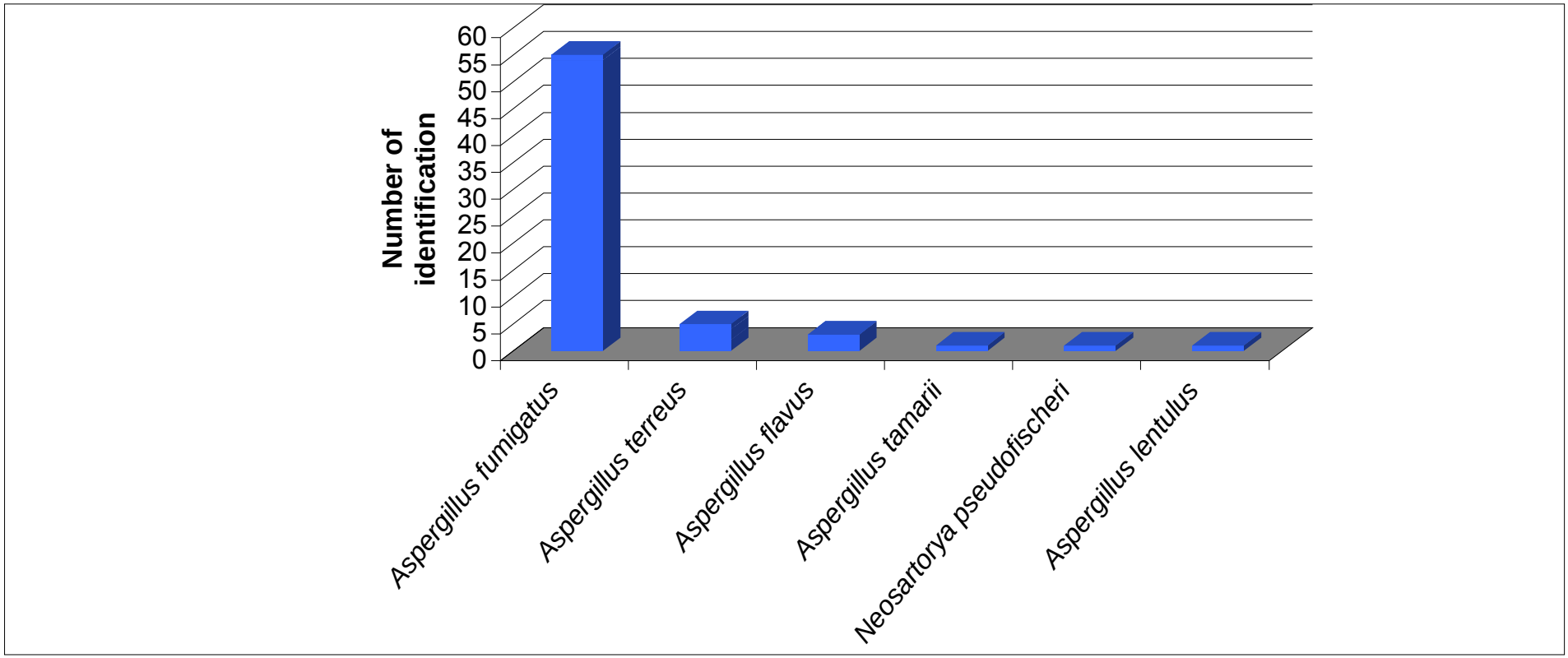
Identification of Mycobacteria

21 Mycobacteria identified



Aspergillus Identification

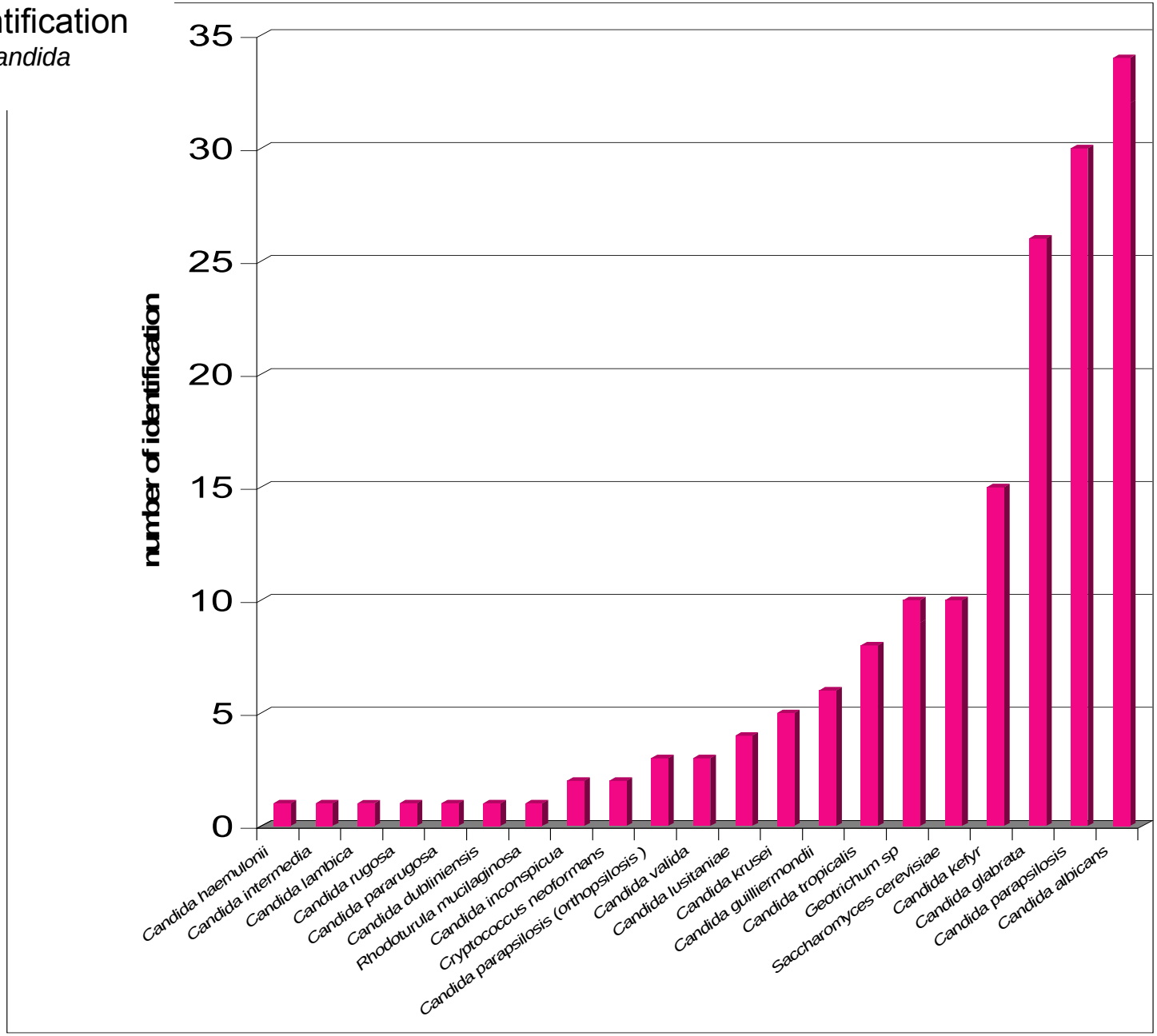
63/64 (98.4%) of identification
1/64 (1.6%) likely identification (*A. terreus*)



Identification of yeasts

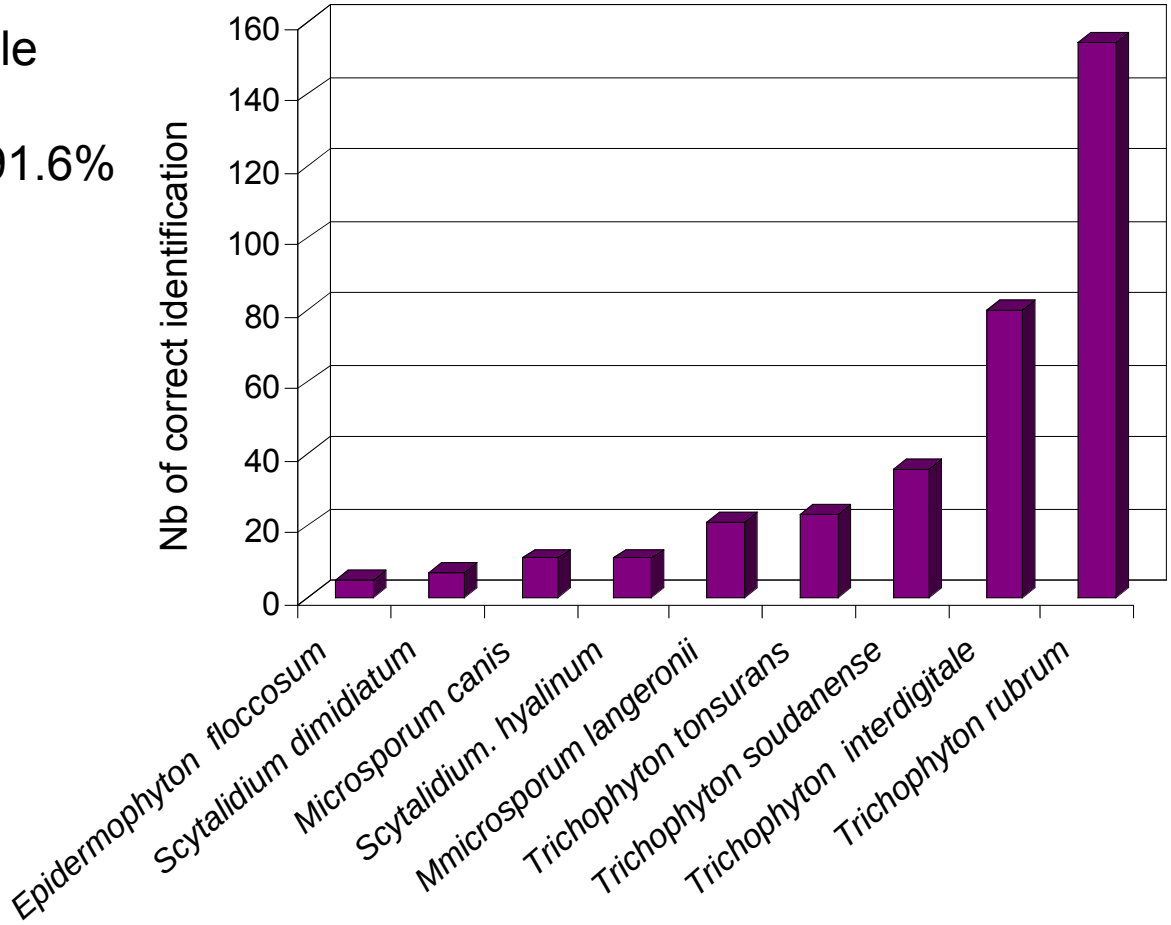
161/162 (99.4%) of identification

Only one yeast not identified: *Candida lambica*



Successful identification of clinical dermatophytes and *Scytalidium* species

- 9 species responsible for dermatomycoses
- 348/380 isolates = 91.6%
- No misidentification



Comments

- Obviously molecular biology was not performed on 3000 isolates
- Validity of the results were supported by the morphology of the colony, the antibiotic susceptibility testing, the clinical presentation of the patient, and the outcome of the antibiotic treatment.

The problems

- Acquisition

- Bacteria belonging to the same genospecies (S.pneumoniae/S.mitis)

The plus of the bacterial identification by MALDI-TOF-MS

- The speed
- The easiness
- The ability to identify bacteria grown on blood culture bottles in less than 30 min.

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