



What is new in
diagnosing bacterial
infections ?

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Hosts***

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What is new in diagnosing bacterial infections ?

Menu

Need for new tests

Steps and timing

Direct tests on blood

Rapid tests on positive culture

Rapid tests for antibiotic susceptibility

Conclusions

What is new in diagnosing bacterial infections ?

Need for new tests

- **Conventional tests (culture, antibiotic susceptibility testing) are slow and/or insensitive, particularly in patients receiving prophylactic or empirical antibiotics**
 - **The prognosis of severe infection improves with a rapid appropriate antibiotherapy**
- new tests, particularly more rapid tests are needed**

What is new in diagnosing bacterial infections ?

Steps in diagnosing bacterial infection

	Detection (day 0)	Identification (day 1-2)	Antibiotic susceptibility testing (day 2-3)
Conventional tests	direct examination	biochemical or molecular from cultures	phenotypic from cultures
New test	NAT (PCR)	Maldi-Tof PNA FISH microarray	Maldi-Tof ? NAT microarray

NAT : nucleic acid test

What is new in diagnosing bacterial infections ?

Detection step (time 0)

Direct detection of bacteria in blood or other body fluids by NAT

- **genus/species specific PCR**
(ex: MRSA, *N.meningitidis*, *M.tuberculosis*)
- **multiplex PCR** (ex: Septifast)
- **eubacterial PCR** followed by sequencing

What is new in diagnosing bacterial infections ?

Molecular-based tests for bloodstream infections

<u>Product</u>	<u>Volume (ml) of blood</u>	<u>DNA enrichment</u>	<u>DNA detection</u>	<u>Bacterial species</u>	<u>Fungi</u>
Septifast Roche	1.5	-	fluorescent probes	19	<i>Candida</i> spp (5) <i>Aspergillus fumigatus</i>
LOOXTER/VYOO SIRS-Lab	5	via affinity chromatography	gels	40	<i>Candida</i> <i>Aspergillus panfungal</i>
Sepsi Test Molzym	5	selected lysis	gels, sequencing	>300	<i>Candida</i> <i>Cryptococcus</i>
PLEX-ID BAL Abbott-Ibis	1.5	-	Mass spectro	>300	<i>Candida</i> spp (9)

What is new in diagnosing bacterial infections ?

Detection step – **Light Cycler Septifast Test**

To date, the only multiplex real-time commercial PCR assay for the diagnosis of sepsis

Detects 25 bacterial and fungal pathogens
(target : ITS regions)

Mechanical DNA extraction

3 parallel multiplex real-time PCR reactions

Detection limit : 3-30 CFUs/ml

TAT 6h

> 20 studies in neutropenic, pediatric, ICU, ER patients

What is new in diagnosing bacterial infections ?

Detection step – Performance of Septifast in immunocompromised hosts

4 studies, 2 with more than 100 episodes of febrile neutropenia (FN)

	FN episodes without antibiotics			FN episodes with antibiotics		
	n=	+BC	+SF	n=	+BC	+SF
Von Lilienfeld M	119	36%	24%	?	3%	15%
Lamoth F	141	31%	33%	52	8%	37%

1) von Lilienfeld M et al, *J Clin Microbiol* 2009; 47:2405

2) Lamoth F et al, *J Clin Microbiol* 2010; 48:3510

What is new in diagnosing bacterial infections ?

Detection step – Performance of Septifast in immunocompromised hosts

Microorganisms identified by the LightCycler SeptiFast test (SF) only in samples from patients with blood culture-negative neutropenic episodes and with persistent fever on day 3 or beyond 3 days after onset of fever according to the clinically and/or radiologically documented site of infection

Site of infection	Species identified on indicated day of fever (no. of episodes)	
	3	> 3
Upper gastrointestinal tract	<i>P. aeruginosa</i> , <i>S. aureus</i>	
Lower gastrointestinal tract	<i>P. aeruginosa</i> (4), <i>S. aureus</i> , <i>E. faecium</i> , <i>A. fumigatus</i> , <i>C. parapsilosis</i>	<i>E. coli</i> (3), <i>Klebsiella</i> spp., [‡] <i>Enterobacter</i> spp., [‡] <i>P. aeruginosa</i> , <i>E. faecium</i> (2), <i>C. albicans</i> (2), <i>C. tropicalis</i>
Lung	<i>P. aeruginosa</i> , <i>S. pneumoniae</i>	<i>C. albicans</i>
Catheter/skin	<i>S. aureus</i>	
No site	<i>P. aeruginosa</i> , <i>A. baumannii</i>	<i>P. aeruginosa</i>

[‡]*Klebsiella* spp. and *Enterobacter* spp. cannot be distinguished at the species level by SF.

Lamoth F et al, *J Clin Microbiol* 2010; 48:3513.

What is new in diagnosing bacterial infections ?

Light Cyler Septifast Test

10 studies with > 100 febrile episodes or samples in NF, ICU, or ER patients.

Performance for the detection of fungi compared to blood cultures

	<u>BC + SF</u>	<u>BC only</u>	<u>SF only</u>
<i>Candida albicans</i>	6	2	10
<i>glabrata</i>	0	5	2
<i>tropicalis</i>	1	0	2
<i>parapsilosis</i>	0	1	2
<i>krusei</i>	0	0	3
Total	7	8	19
<i>Aspergillus fumigatus</i>	0	0	14

What is new in diagnosing bacterial infections ?

Septifast – Pros and Cons

⊕

Proof of concept

Additional value to blood cultures

- in patients on antibiotics
- in patients with suspected invasive fungal infection

moderately rapid

Low sensitivity

**low volume of blood
low DNAemia**

False negative

not included in the spectrum

False positive?

**no gold standard
contamination**

work-intensive

high cost

lack of automation

cannot replace blood cultures

What is new in diagnosing bacterial infections ?

Identification step

NAT-based commercial assays for identification of pathogens from positive blood cultures

<u>Assays</u>	<u>Type</u>	<u>Pathogens</u>	<u>TAT (h)</u>
PNA-FISH (advanDx)	fluorescent based hybridization	different kits	3
Hyplex blood screen (BAG)	multiplex PCR + hybridization or ELISA	10	3
prove-it Sepsis (Mobidiag)	multiplex PCR + hybridization on a microarray	50	3

Adapted from Mancini N et al, *Clin Microbiol Rev* 2010: 23:235-251.

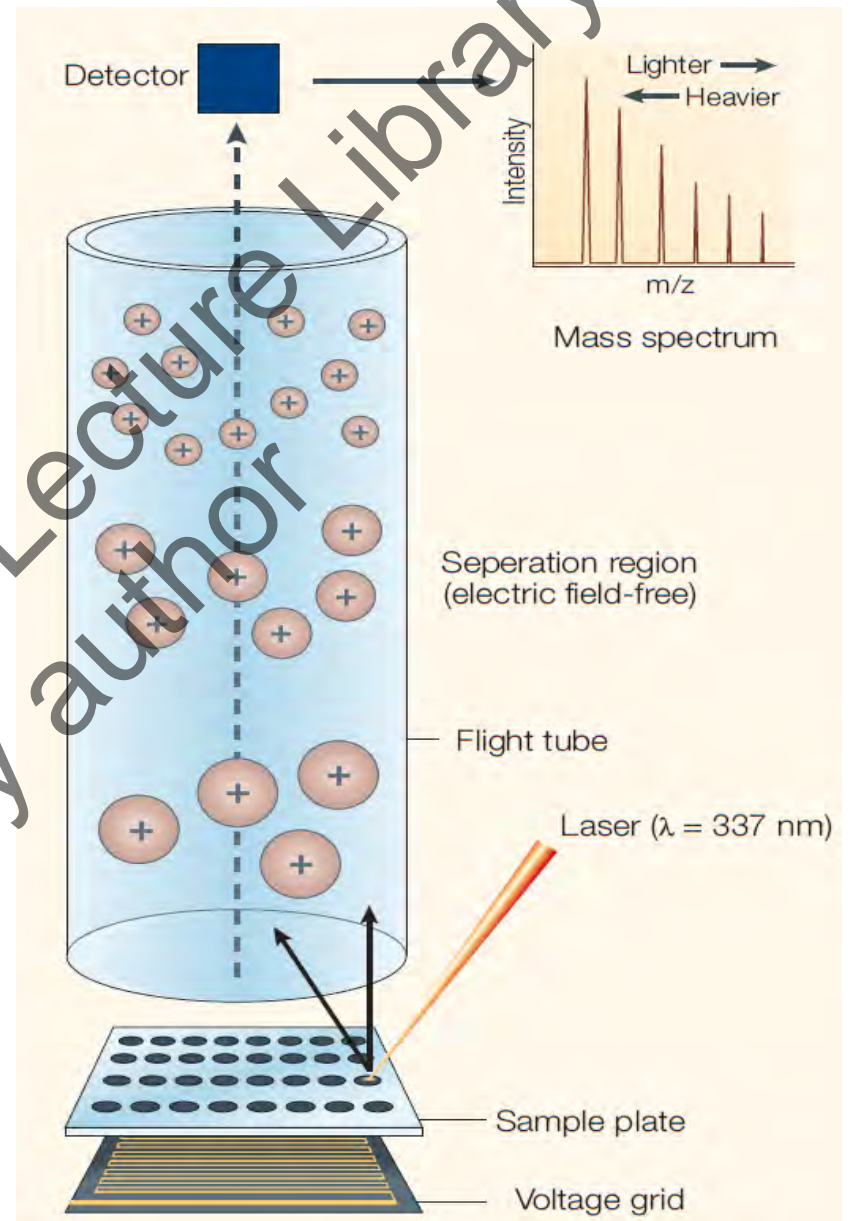
What is new in diagnosing bacterial infections ?

Identification step – **Maldi-Tof**

- **Identification from pure colonies**
 - bacteria**
 - yeast**
 - filamentous fungi**
- **Identification from positive broth**
 - blood cultures**
 - urines**
 - other samples**
- **Direct identification from native clinical samples**

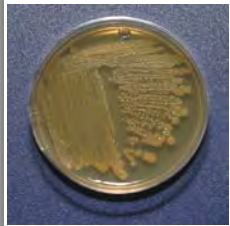
What is MALDI-TOF ?

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

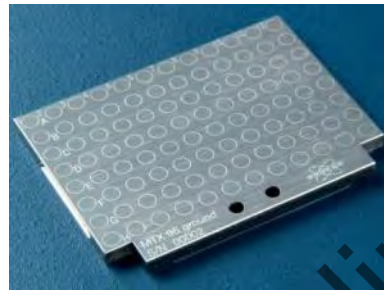


Digital genotyping using molecular affinity and mass spectrometry, Sobin Kim, Hameer D. Ruparel, T. Conrad Gilliam and Jingyue Ju, Nature Reviews Genetics - VOLUME 4 | DECEMBER 2003

Identification procedure



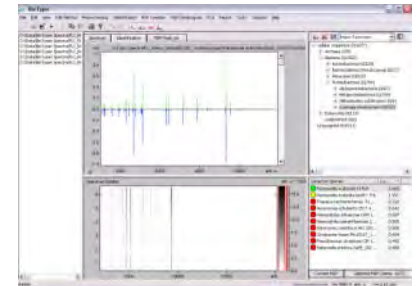
Select bacterial colonies



Deposition of bacteria on MALDI microplate



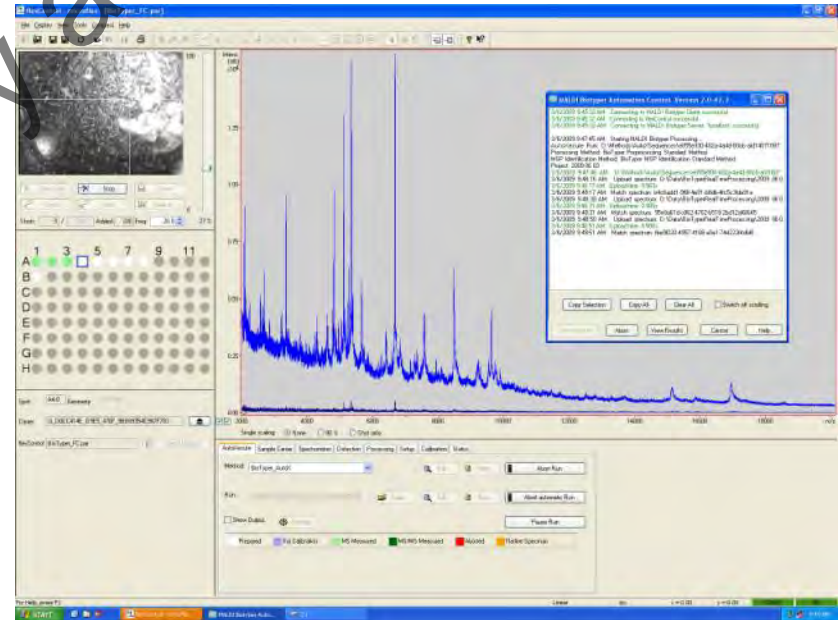
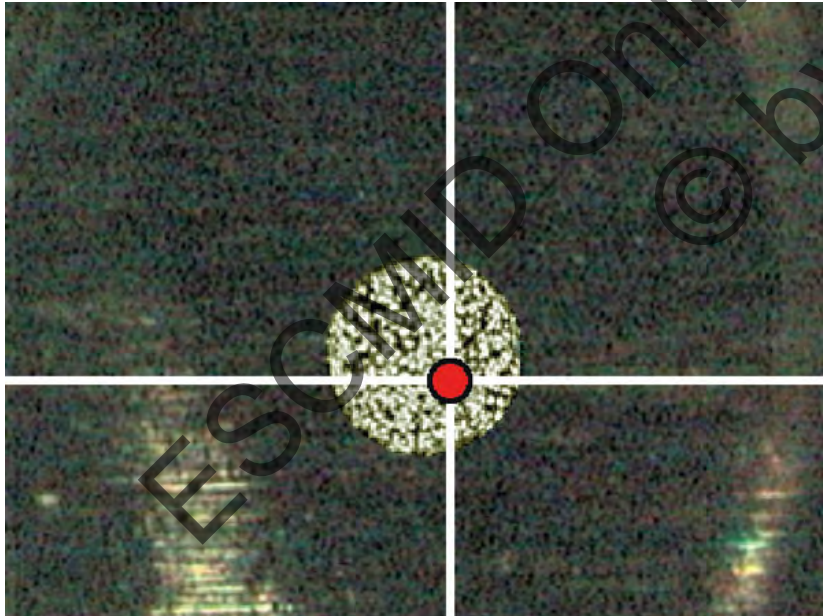
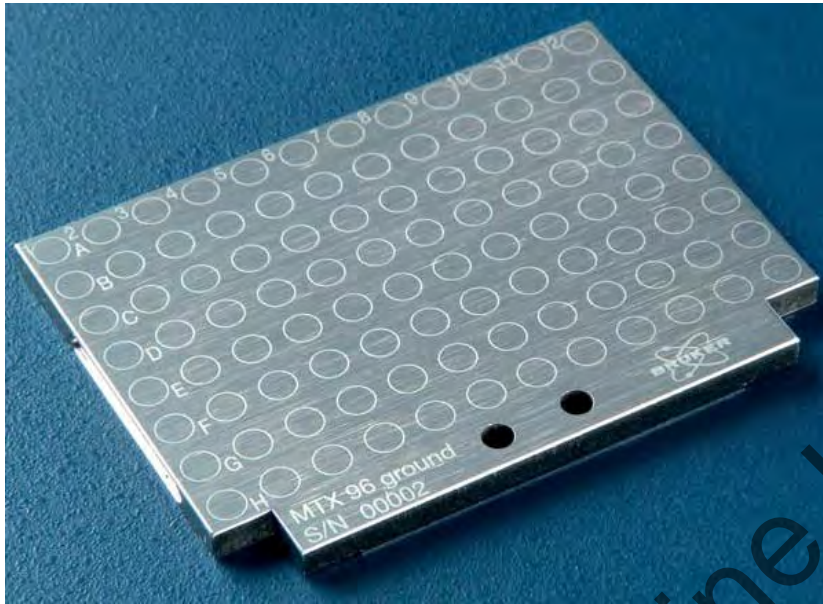
Acquisition of the proteic profile



Comparison with a database

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What is new in diagnosing bacterial infections ?



What is MALDI-TOF ?

Bruker Daltonics MALDI Biotyper Classification Results

Project Info

Project Name: 2009 06 03
 Project Description:
 Project Owner: TOF-USER
 Project Creation Date/Time: 2009-03-06 09:45:47.578
 Project Analyte Count: 9
 Project Type: RUO (Research Use Only)

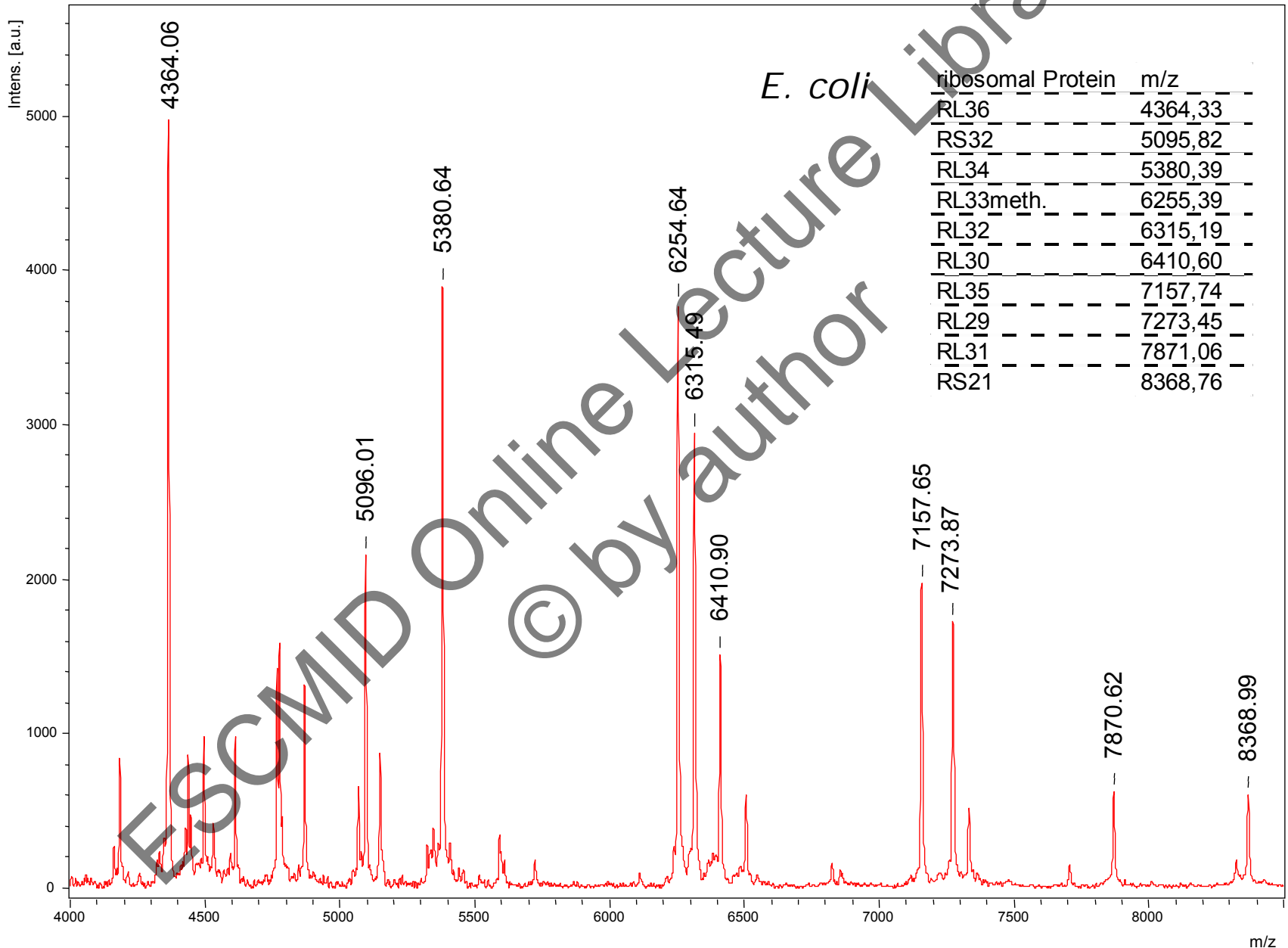
Score > 2
 Score 1.7-2
 Score < 1.7

Result Overview

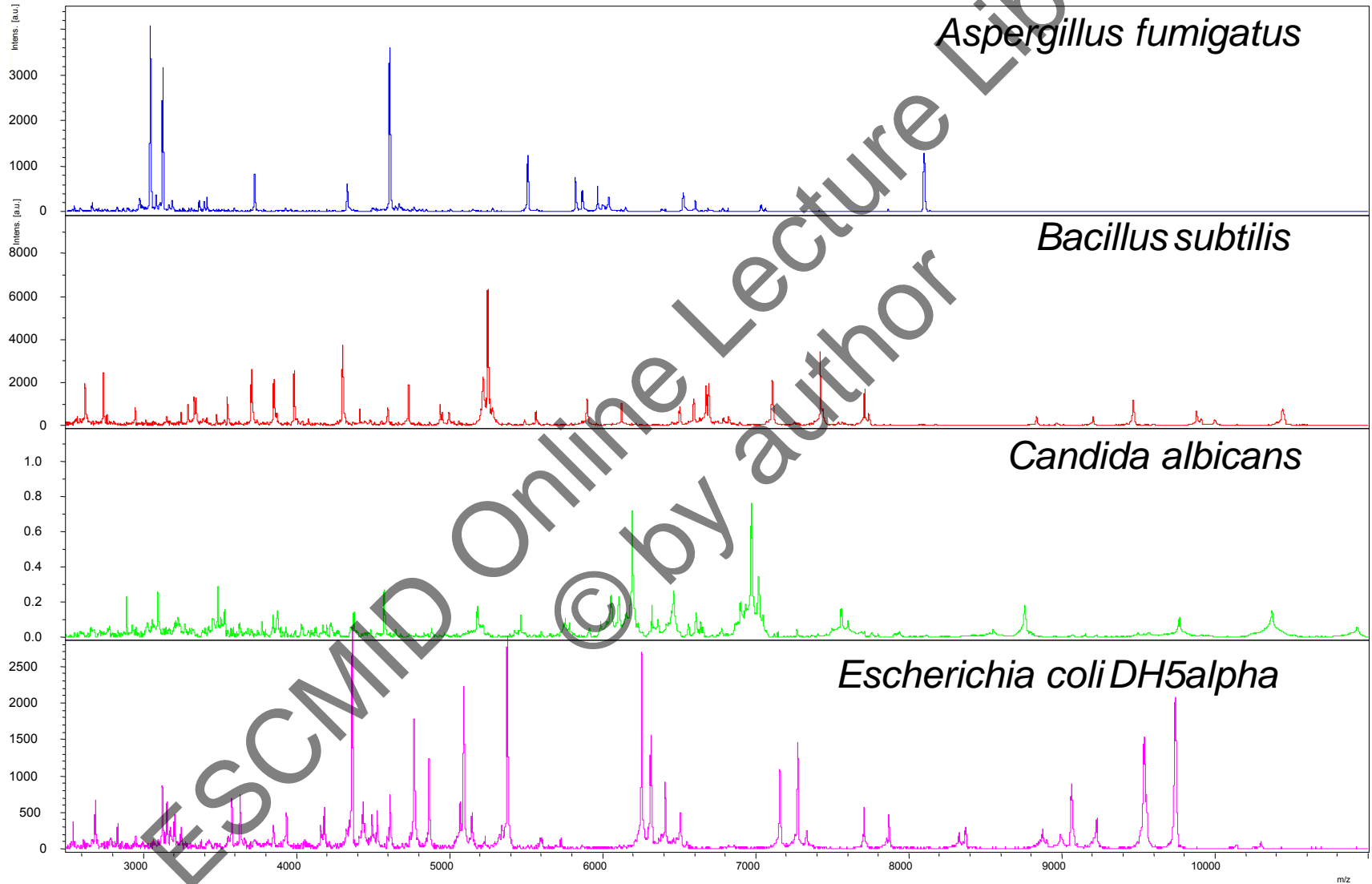
Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
MI09104293 (+++)	A1	Pseudomonas aeruginosa	2.362	Pseudomonas aeruginosa	2.362
MI09103308 (+)	A2	Staphylococcus similans	1.853	not reliable identification	0.000
MI09103259 (+)	A3	Escherichia coli	1.844	Escherichia coli	1.728
MI09103148 (++)	A4	Staphylococcus epidermidis	2.056	Staphylococcus epidermidis	1.975
MI09103021 (+++)	A5	Enterococcus faecium	2.412	Enterococcus faecium	2.394
MI09102185 (++)	A6	Staphylococcus epidermidis	2.138	Staphylococcus epidermidis	2.102
MI09094359.1 (-)	A7	not reliable identification	0.000	not reliable identification	0.000
MI09094359.2 (++)	A8	Corynebacterium amycolatum	2.148	Corynebacterium amycolatum	2.002
MI09104097 (++)	B1	Staphylococcus aureus	2.228	Staphylococcus aureus	2.215

Score = f (database; spectra; ...)

What is new in diagnosing bacterial infections ?



What is new in diagnosing bacterial infections ?



What is new in diagnosing bacterial infections ?

Performance of Maldi-Tof MS identification with routine samples

Studies	MT	n=	Correct identification (%)		GNB	GPB
			at species level	at genus level		
Eigner ¹⁾	a)	1116	95.2	4.8	93.8	97.7
Seng ²⁾	a)	1660	84.1	11.3	-	-
Cherkaoui ³⁾ id.	a)	720	93.6	-	98.2	83.9
	b)	720	88.3	-	94.8	75.6
Bizzini ⁴⁾	b)	1371	95.1	2.8	92.2	98.8

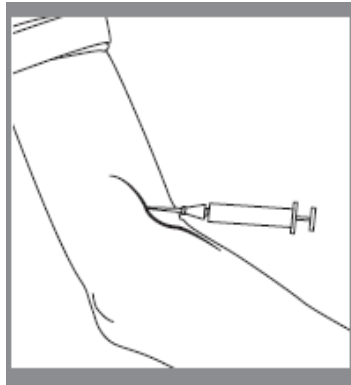
1) Eigner U et al, *Clin Lab* 2009; 55:289

2) Seng P et al, *Clin Infect Dis* 2009; 49:543

3) Cherkaoui A et al, *J Clin Microbiol* 2010; 48:1169

4) Bizzini A et al, *J Clin Microbiol* 2010; 48:1549.

Direct Testing of Positive Blood Cultures by MALDI-TOF



Sampling



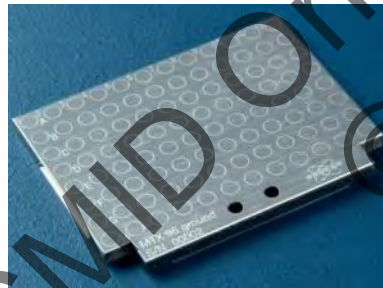
Incubation of blood culture bottles



WHEN POSITIVE



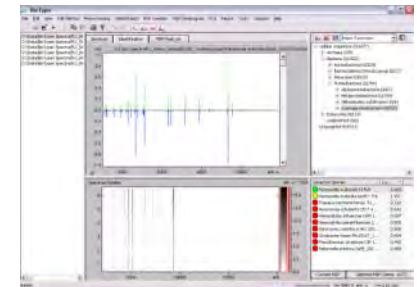
Preparation of a bacterial pellet



Deposition of bacterial pellet on MALDI microplate



Acquisition of the proteic profile

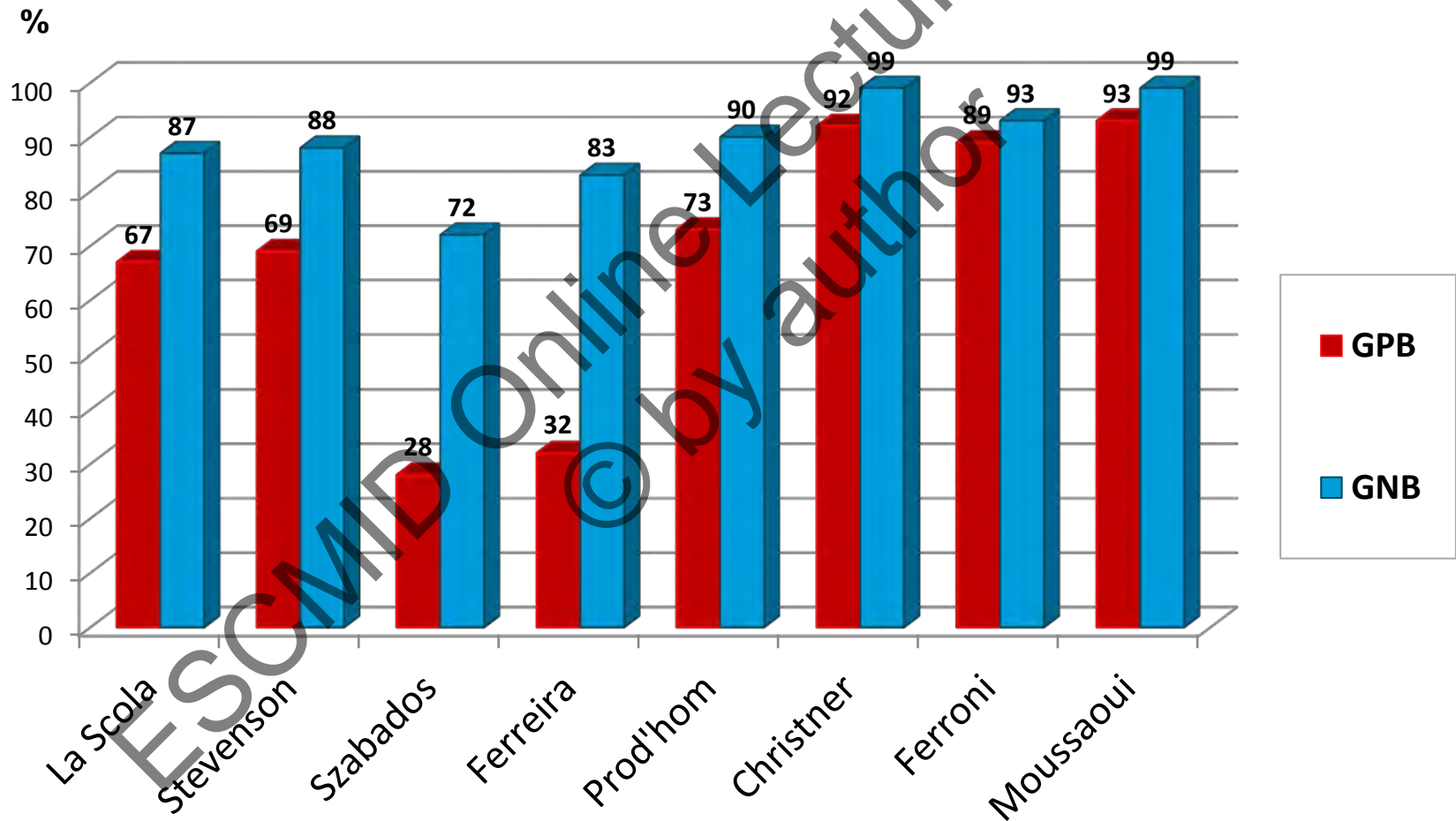


Comparison with a database

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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)



What is new in diagnosing bacterial infections ?

Maldi-Tof MS in your clinical microbiology laboratory – **Pros and Cons**



Rapidity

Ease to use

Cost per test

Accuracy of identification

Reproducibility

Access to database

Prospect for typing



- **cost of the instrument**

- **misidentifications (rare)**

S.mitis – S.pneumoniae

Shigella – E.coli

- **not applicable to native specimens**

- **cross-contamination**

- **mixed culture**

- **lack in the commercial database (filamentous fungi)**

- **limited application to antibiotic susceptibility testing**

**You must buy a Maldi-Tof MS instrument
But you still need to do systematic cultures
and conventional antibiotic susceptibility testing**

What is new in diagnosing bacterial infections ?

Antibiotic susceptibility testing step

- **Maldi-Tof MS**
- **Commercial POCT PCR (MRSA, VRE)**
- **Microarray (betalactamases)**

What is new in diagnosing bacterial infections ?

Maldi-Tof MS for detection of ANTIBIOTIC RESISTANCE

MRSA ¹⁾

proteome related
alterations

limited data
controversial

GNB betalactamases

cephalosporinases ²⁾

enzymes
degradation of drug

very recent studies
look excellent

Yeast-azoles ³⁾

proteome shift

preliminary work

1) Edwards-Jones V et al, *J Med Microbiol* 2000; 49:295

2) Burckhardt I et al, *J Clin Microbiol* 2011; 49:3321

3) Marinach C et al, *Proteomics* 2009; 9:4627.

What is new in diagnosing bacterial infections ?

POCT PCR MRSA Impact on antimicrobial program ¹⁾

Single center sequential cohort analysis of pre-PCR cases (n= 74) of *S.aureus* bacteremia with post-PCR cases (n= 82)

	<u>pre-PCR</u>	<u>post-PCR</u>
Mean-time to switch from vanco to cefazolin/nafcillin (d)	3.6	2.0
Mean lengths of stay (d)	21	15
Mean hospital cost (K USD)	70	48

1) Bauer KA et al, *Clin Infect Dis* 2010; 51:1074.

What is new in diagnosing bacterial infections ?

2 major developments

- **Commercial PCR systems**

- expected for > 15 years
 - not optimal
 - too cumbersome (blood)
 - and/or too expensive
 - not automated enough

- **Maldi-Tof MS instruments**

- unexpected
 - complete change in the work load of a bacteriology laboratory
 - outstanding for a rapid identification of bacteria, yeast and fungi
 - promising for the detection of resistance determinant
 - your next Christmas present

- **Combination of both**