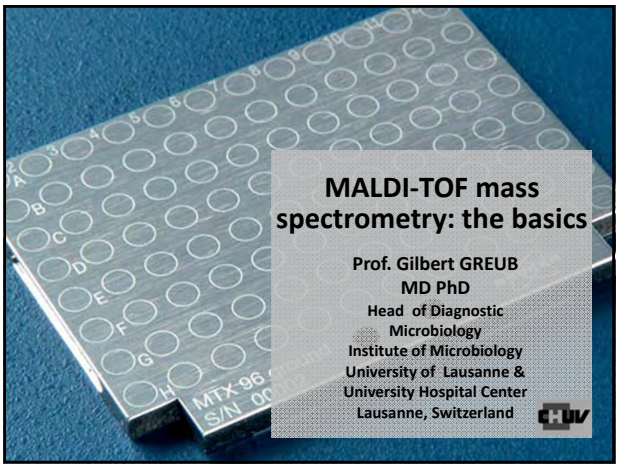

Conflict of interests ?

- In Lausanne, we use a Bruker mass spectrometer but we have no conflict of interest with Bruker

Relationship with industry

- Research grant with **SUEZ-ONDEO** (France)

1. What is MALDI-TOF ?

Mass spectrometers

1. **Ion source**
   transfer molecules into a gas phase
2. **Mass analyzer**
   separate ions according to their mass to charge ratio (m/z)
3. **Detection device**
   monitor amount separated ions

Croxatto, Prod'hom & Greub, FEMS Microbiol Reviews 2011

1. What is MALDI-TOF ?

Ionization methods

1. Plasma desorption (PD)
2. Fast atom bombardment (FAB)

Bacterial lipids

3. Chemical ionization (CI)
4. Atmospheric pressure CI
5. Laser desorption
6. Electrospray ionization (ESI)
7. MALDI

Intact proteins

Croxatto, Prod'hom & Greub, FEMS Microbiol Reviews 2011

Table

1. What is Maldi-tof ?
2. Routine identification of bacterial isolate
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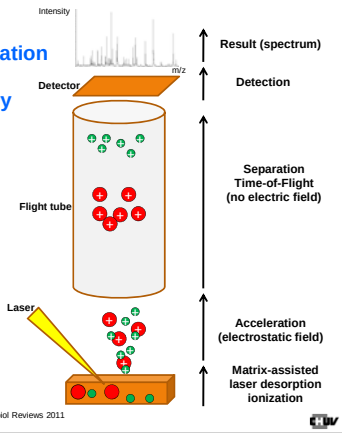
6. Clinical impact of MALDI-TOF
7. Conclusions

1. What is MALDI-TOF ?

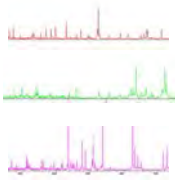
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)



Adapted from: Croxatto, Prod'hom & Greub, FEMS Microbiol Reviews 2011

1. What is MALDI-TOF ?

Different spectra will be obtained for different microbial species



S. aureus

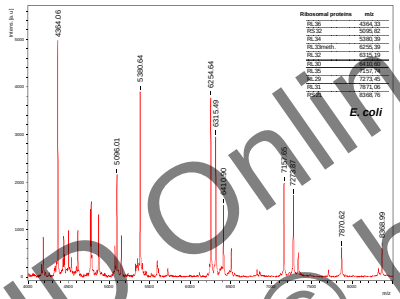
E. faecium

E. coli

with spikes ranging from 1'000 to 20'000 m/z

1. What is MALDI-TOF ?

About 50% of detected proteins are ribosomal proteins



organic solvants and acidic conditions used for lysis are favouring extraction of ribosomal proteins, which are abundant and basic

Stahl et al 2004

1. What is MALDI-TOF ?

About 50% of detected proteins are ribosomal proteins

Others proteins (*Salmonella*) include:

- Cold shock-like protein (Csph)
- Translation initiation factor IF-1
- Ribosome modulation factor
- Integration host factors A & B

pI > 9

- Nucleoid-associated proteins H-NS
- RNA chaperone CspeE
- Glutaredoxin-1
- Phosphocarrier protein HPr

Abundant

= abundant and basic

Dieckmann et al. Appl Env Microbiol 2008

1. What is MALDI-TOF ?

Commonly used matrices

2,5 dihydroxybenzoic acid (DHB) — Glycoproteins glycopeptides

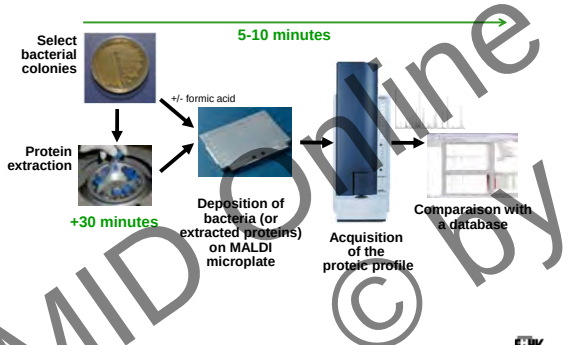
Alpha-cyano-4-hydroxycinnamic acid (CHCA)
Sinapinic acid (SA)
Ferulic acid (FA)
— Proteins

2,4 hydroxyphenylbezoic acid

1.5 microl CHCA
in 50% acetonitrile/2.5% trifluoroactetate

1. What is MALDI-TOF ?

Identification procedure



1. What is MALDI-TOF ?

Identification procedure

1. Mass spectra comparaison with fingerprints database
   - rapid, simple
   - applicable in diagnostic lab

2. Matching of biomarkers masses to a proteome database
   - most moelcules > 4000 Da are proteins
   - in silico prediction of mass from genomes data
   - tolerates variation in culture conditions/samples prep.

1. What is MALDI-TOF ?

MALDI Biotyper User Manual, Version 2.0 (Bruker)

Comparaison with references spectra

Score > 2
Score 1.7-2
Score < 1.7

Score = function (database; spectra; …)

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2. Routine identification of bacterial isolate

Identification of bacterial isolate
(n=1371)

- **Scores**
  - Valid (≥2) 1278 93.2%
  - Intermediate (1.7-2) 73 5.3%
  - No identification (<1.7) 20 1.5%

- **Identification (among scores >2)**
  - Correct at species level 1215/1278 95.1%
  - Correct at genus level 39/1278 3%
  - Discordant results 24/1278 1.9%

Bizzini *et al.* J Clin Microbiol (2010)

2. Routine identification of bacterial isolate

Quality of the database

Errors in the reference spectra
Propionibacterium acnes wrongly identified as Eubacterium brachy

Similarities of spectra present in the database
Klebsiella oxytoca and *Raoultella planticola* …

Absence of reference spectra in the database
No spectra of non-*Clostridium anaerobes*

Insufficient number of reference spectra in the database
Only 1 spectrum of *Propionibacterium acnes* or *Bacillus cereus*
Not enough to be representative of the true diversity of their profiles

Taxonomical "errors" in the database
Stenotrophomonas maltophila misidentified as *Pseudomonas hibiscicola*

Taxonomical "discordances" with common use
Agrobacterium tumefaciens is a synonym of *Rhizobium rhizogenes*
Enterobacter cloacae (group) versus *Enterobacter hormaechei*

Croxatto, Prod'hom & Greub, FEMS Microbiology Reviews 2011

2. Routine identification of bacterial isolate

Discriminotary power of MALDI-TOF insufficient to differentiate closely related species

Even with good spectra (definition of species)
E. coli and *Shigella* spp.

Especially with spectra of slightly lowered quality,
(partially also due to limited number of spectra)

Streptococcus pneumoniae and *Streptococcus mitis*
Streptococcus pneumoniae and *Streptococcus parasanguinis*
Streptococcus constellatus versus *Streptococcus anginosus*
Streptococcus pyogenes versus *Streptococcus dysgalactiae*
…
Klebsiella oxytoca and *Raoultella planticola*, …

Croxatto, Prod'hom & Greub, FEMS Microbiology Reviews 2011

2. Routine identification: benefits from database extension

Benefits of database extension (1)

Gemella, Granulicatella, Abiotrophia

Christensen JJ
J Clin Microbiol. 2012 May;50(5):1787-91

Anaerobes:
- 179/253 (70.8%) correct identific. at species level
- 232/253 (91.7%) correct identific. at genus level

Schmitt BH.
J Clin Microbiol 2013; 51:782-6.

Spirochetes:
Identification of human & animal *Brachyspira* species (anaerobes of the intestinal tract)

Calderaro A et al.
J Proteomics. 2013 Jan 14;78:273-80.

2. Routine identification: benefits from database extension

Benefits of database extension (2)

Prevotella spp.

Extension of the commercial database improved identification rate at species level from 62.7% (of 102 isolates) to 83.3%

Wybo I. et al.
J Clin Microbiol. 2012 Apr;50(4):1415-8.

Useful to have an international shared extended curated non-commercial database

2. Routine identification: benefits from database extension

Importance of having a complete database

Anthrax infection in an injecting drug user in Germany
Anthrax was not suspected initially: the patient died

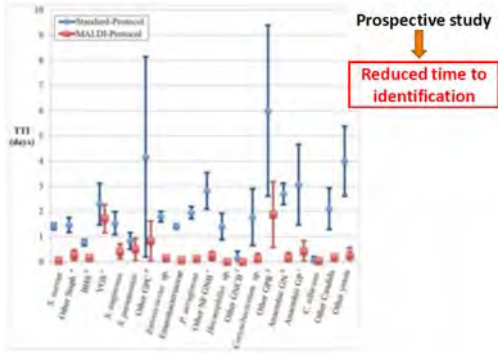
Day 1
- Patient admitted to the hospital
- Blood cultures sent to the laboratory
- Patient dies due to septic shock
- Blood cultures positive with Gram-positive bacilli (late afternoon)

Day 2
- Growth of *Bacillus* spp. on subcultures
- MALDI-TOF: *Bacillus cereus* → Retrospectively identified as *B. anthracis*
- Discussions on anthrax suspicion
- Different PCRs and 16S sequencing over night

Day 3
- *B. anthracis* confirmed by PCR

Fatal anthrax infection in a heroin user from southern Germany, June 2012.
Holzmann T et al.
Euro Surveill. 2012 Jun 28;17(26).

2. Routine identification: reduced time to identification (TTI)



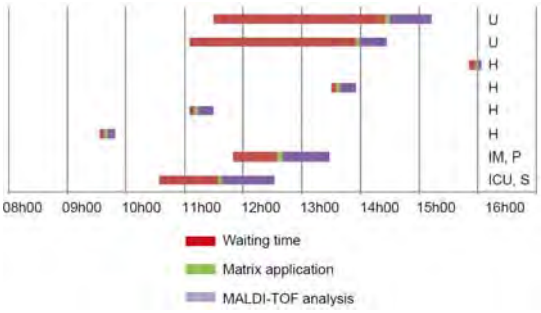
Prospective study

Reduced time to identification

Tan KE et al. J Clin Microbiol. 2012 Oct;50(10):3301-8

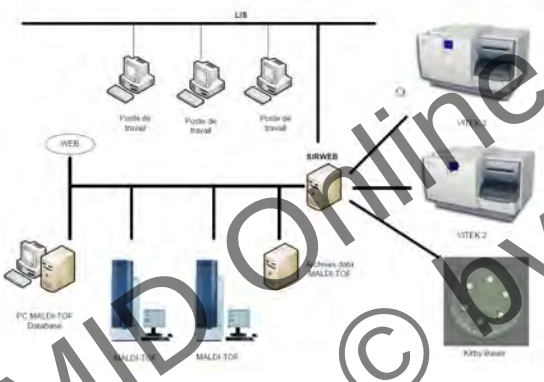
2. Routine identification: reduced time to identification (TTI)

Real life with a single MALDI-TOF



➡ Need 2nd MALDI-TOF

2. Routine identification: importance of connection to LIS



3. Quality control: importance of negative controls

MALDI microplates

Problems:

Residues of proteins ? Inadequate cleaning?

Quality control

Visual inspection

Negative controls at each run

Occasionnaly check microplates by applying the matrix on all 96 wells after routine wash

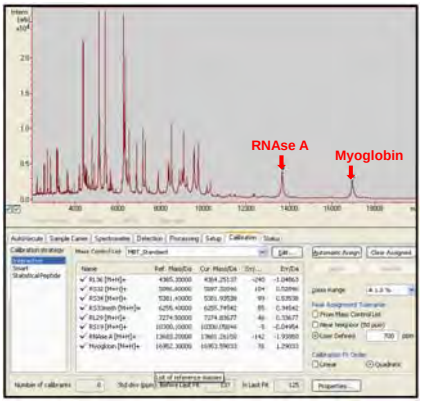
➡ **Disposable MALDI microplates**

2. Routine identification: quality controls & maintenance

Calibration
«BTS» control

E. coli
(RL36, RS32, RS34, RS33, RL29, RS19)

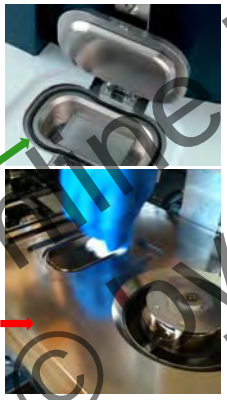
RNAse A
Myoglobin



2. Routine identification: quality controls & maintenance

Problems:

1. vacuum failure
   - dust on plastic joints
   - loss of etancheity of the plastic joints
2. carbonisation of bacteria (laser may be soiled)



Croxatto, Prod'hom & Greub, FEMS Microbiology Reviews 2011

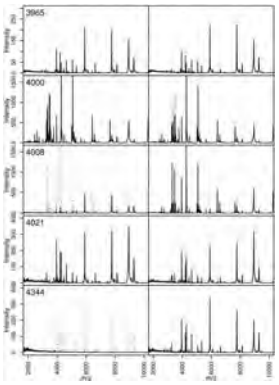
2. Routine identification: quality controls & maintenance

Maintenance
1x/3 months in Lausanne
(~ 5 run/days)

2. Routine identification: reproductibility

Reproductibility

Most data available from clinical chemistry



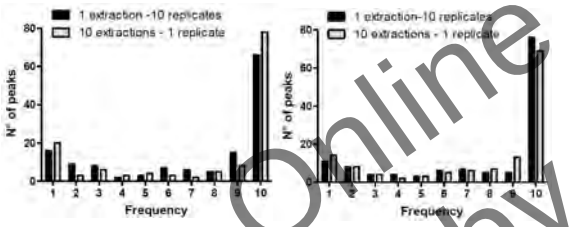
Duplicate of 5 samples
In the left hand panel, the first technical replicate is shown with the black solid line and the second technical replicate with the red dotted line

Cairns D.A. et al. BMC bioinformatics 2008; 9: 519

2. Routine identification: reproductibility

Reproductibility

***E. coli* 10^6** ***S. aureus* 10^6**



→ Variability is mainly due to the deposit (high reproductibility of extraction)

Croxatto, Prod'hom & Greub, FEMS Microbiology Reviews 2011

2. Routine identification: reproductibility

Temporal reproductibility

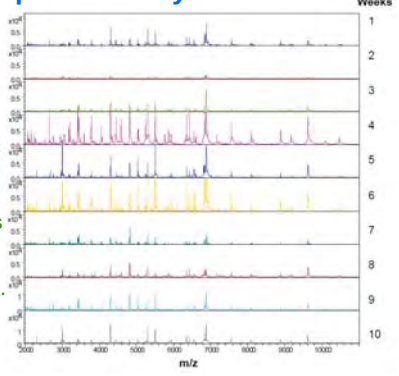
S. aureus
10^6 bacteria/well

Scores always 2.3-2.5

↓

Do not only rely on scores

- check spectra
- use other strains, other algorithms,…



Croxatto, Prod'hom & Greub, FEMS Microbiology Reviews 2011

2. Routine identification: cost analysis

Cost analysis (1371 isolates)

assuming cost of technician time of 1.2 €/min

	with MALDI-TOF*	without MALDI-TOF
mean cost	4.03€	9.43€
annual cost	71'921€	168'094€

➡ 2.34 fold reduction

Including acquisition (5 year amortization) & maintenance

132'521€ 168'094€

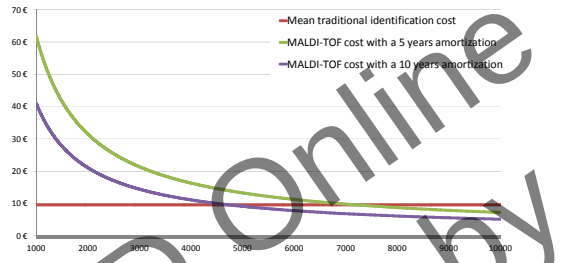
➡ 1.27 fold reduction

***identification by MALDI-TOF and when needed additional approaches**

Heininger, Prod'hom & Greub, in preparation

2. Routine identification: cost analysis

Cost analysis



Already cost saving when performing 5'000 to 7'000 tests per year

Heininger, Prod'hom & Greub, in preparation

Table

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7. Conclusions

3. Identification of "Difficult to identify strains"

Isolates identified at the species level by 16S rDNA sequencing (n=419)

- **No ID or incorrect ID (n=178) 42.5%**
  Streptomyces, Actinomyces and *Nocardia*: need a specific extraction protocol
- **Concordant ID at species level (n=241) 57.5%**
  - **score x ≥ 2.0 (n=182) 43.4%**
  - **score 1.7 ≤ x ≤ 2.0 (n=59) 14.1%**

Bizzini *et al.* J Clin Microbiol (2011)

3. Identification of "Difficult to identify strains"

Improved identification of Gram positive rods

- **Improved database**
- **Improved extraction protocol**
- **Changed cut-off (1.7 instead 2)**

Reduction of unidentified Gram positive rods from 12.1% to 5.6%

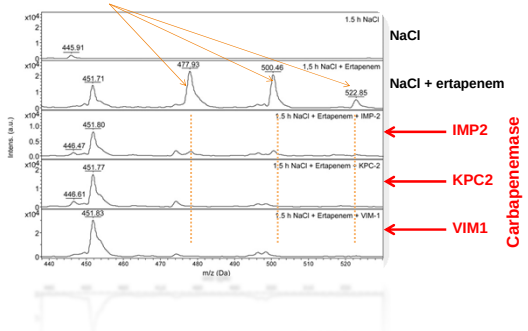
Schulthess B *et al.* J Clin Microbiol (2014)

Table

1. **What is Maldi-tof ?**
2. **Routine identification of bacterial isolate**
3. **Identification of « difficult to identify strains »**
4. **Detection of carbapenemases & toxins**
5. **Direct identification from positive blood cultures**
6. **Clinical impact of MALDI-TOF**
7. **Conclusions**

4. Detection of carabapenemases

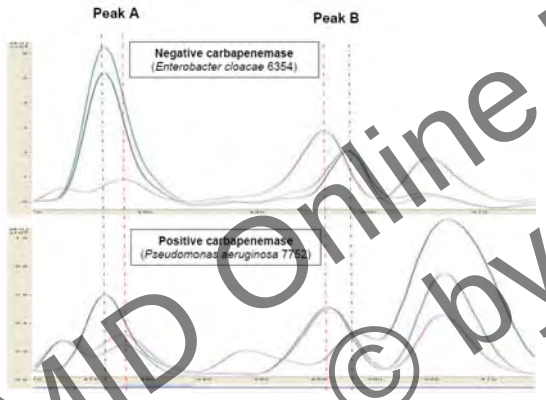
Loss of peaks



Loss of peaks at 476 Da,498 Da and 521 Da

Burckhardt *et al.*, J Clin Microbiol (2011) 49: 3321-4

4. Detection of carabapenemases



Vogne *et al.*, submitted

4. Detection of carabapenemases

Modified test

using a 10 microg ertapenem disk; only 1 hour incubation

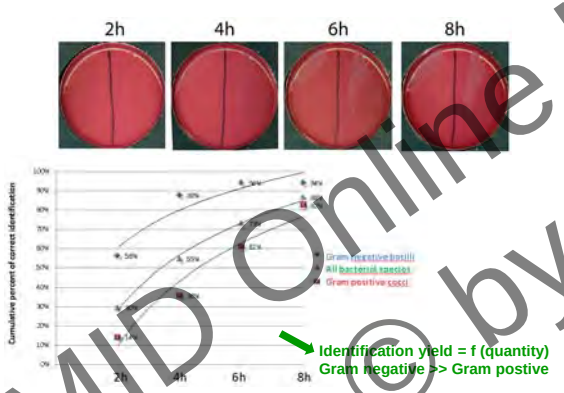
Diagnostic approach	Sensitivity	Specificity
Phenotypic detection (Modified Hodge test)	90.5% (19/21)	86% (24/28)
Phenotypic detection (IP/IPI)*	54.5% (6/11)	92.1% (35/38)
PCR-Check MDR Carba	90.5% (19/21)	100% (28/28)
Microarray	90.5% (19/21)	100% (28/28)
MALDI-TOF	100% (21/21)	100% (28/28)
MS/MS	100% (21/21)	100% (28/28)

*phenotypic detection of metallo-ß-lactamases (VIM, IMP, NDM)

Vogne *et al.*, submitted

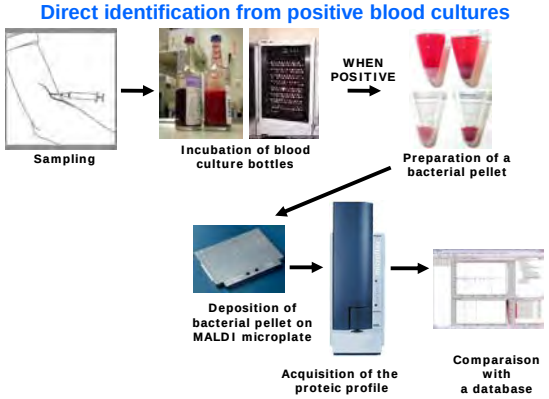
Table

5. Direct identification from positive blood cultures



5. Direct identification from positive blood cultures

Direct identification from positive blood cultures



5. Direct identification from positive blood cultures

Pellet preparation

Prod'hom et al. J Clin Microbiol (2010)

Patented

5. Direct identification from positive blood cultures

Results

Excellent accuracy

n=122

69(57%) > 2.0

96(79%) > 1.7

1 discordant

S. caprae vs *S. pasteuri*

Prod'hom et al. J Clin Microbiol (2010)

5. Direct identification from positive blood cultures

Overview of the litterature

Authors (year)	n	Concordant ID at species level
Prod'hom (2010)	126	78% (GN: 89%, GP: 72%)
Prod'hom (unpublished)	314	85% (GN: 89%, GP: 83%)
La Scola (2009)	599	66% (GN: 91%, GP: 49%*)
Ferreira 2010	300	43% (GN: 83%, GP: 32%)
Stevenson (2010)	212	80%
Ferroni (2010)	685	89% (312 spiked bottles)
Christner (2010)	277	94%
Ferreira (2010)	68	76%

*Improved extraction for *Staphyloccocus* spp.: 38 to 75%

Croxatto, Prod'hom & Greub. FEMS Microbiol Rev 2011

5. Direct identification from positive blood cultures

Applications of the pellet

Authors (year)	n	Concordant ID at species level
Prod'hom (2010)	122	78% (GN: 89%, GP: 72%)
Prod'hom (unpublished)	314	85% (GN: 89%, GP: 83%)

Prod'hom et al, in prep.

Patented

Vitek AST → **Major errors 0.4% for Gram neg. 0.8% for *Staphyloc.***

Prod'hom et al, J Med Microbiol 2013

GenXprt MRSA → **Randomized trial (18 months; impact on vancomycin use)**

Clerc et al, Clin Microbiol Infect 2013

5. Direct identification from positive blood cultures

Direct identification from blood cultures

Several studies from 2009-2012
- Adequate bacterial concentration is needed
- Removal of red blood cells, blood proteins, …
- Overall success rate ranging from 65 to 85% using SDS, saponin, Tween, MALDI- Sepsityper, …

Differential centrifugation protocol:
- identification available & reliable in :
  97.3 for Gram negative bacteria
  98.4% for Gram positive bacteria

March-Rosello et al.
Eur J Clin Microb Infect Dis 2013

5. Direct identification from positive blood cultures

«no reliable identification» for 26 bottles

Factors possibly affecting MALDI-TOF MS efficiency

Presence of a capsule
- *K. pneumoniae, H. influenzae, S. pneumoniae* (n=13)

Close relatedness of bacteria within streptococci
- *S. pneumoniae, S. oralis,* … (n=13)

Cell wall composition of Gram positive bacteria conferring high resistance to lysis (n=21)

Presence of residual blood proteins ?
Inadequate pocedure ?
} **quality control**

Prod'hom et al. J Clin Microbiol (2010)

5. Direct identification from positive blood cultures

Pre-MALDI-TOF era | **Post-MALDI-TOF era**

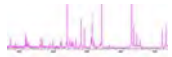

Gram + cocci arranged in chains


Gram + cocci arranged in clusters


Gram - bacilli


E. faecium


S. aureus


E. coli

Day 0

Overnight incubation

Day 1


S. aureus *E. coli*

5. Direct identification from positive blood cultures

Expected clinical impact:

- reduced time to results
- blood culture >> other identifications

- Gram positive bacteria in chains: — Possible impact on:
  - *Streptococci* vs *Enterococci* — Investigations, treatment
  - *E. faecium* vs *E. feacalis* — Treatment
- Gram positive cocci in cluster:
  - *S. aureus* vs *S. lugdunensis*, *S. epidermidis*, … — Clinical significance
- Gram negative bacteria
  - *E. coli* / *Klebsiella* vs Gram negative bacteria such as *Serratia marcescens*, *Morganella morganii* and *Enterobacter cloacae* encoding on their chromosome an inducible cephalosporinase
  - Non fermentative bacteria — Treatment

Table

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6. Clinical impact of MALDI-TOF

Impact of MALDI-TOF: retrospective study

- **Time to identification**
  1h35 with MALDI-TOF (versus 25h43 without)

Clinical impact:

- **20/157 results led to a treatment change (in adults)**
- **1/ 40 results led to a treatment change (in children)**

Martiny *et al.* Clin Microbiol Infect 2013

6. Clinical impact of MALDI-TOF

Impact of MALDI-TOF: retrospective study

- **Time to identification**
  1h35 with MALDI-TOF (versus 25h43 without)

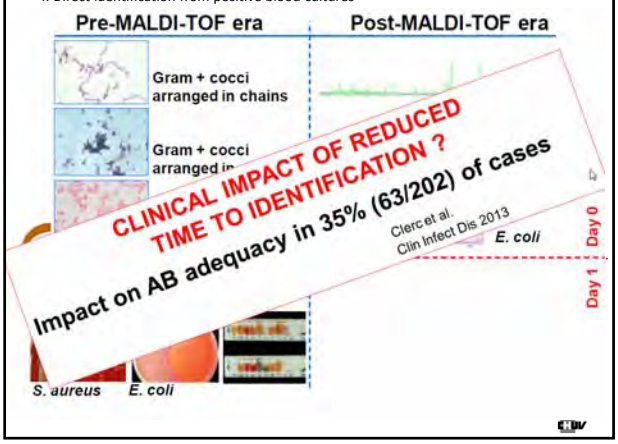
Clinical impact:

- **20/157 results led to a treatment change (in adults)**
- **1/ 40 results led to a treatment change (in children)**

- **New blood cultures (n=2)**
- **Catheter removal (n=4)**
- **Additionnal investigations (n=3)**
- **Documented sample exchange (n=1)**
- **Exclude a contamination**
  (38% in pediatry; 9% for adults)

Martiny *et al.* Clin Microbiol Infect 2013

4. Direct identification from positive blood cultures



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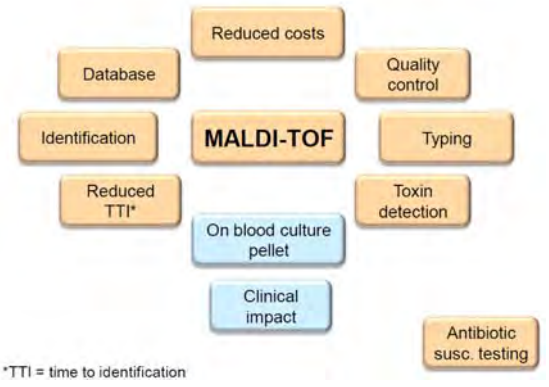
7. Conclusions

BACTERIAL IDENTIFICATION

is one of the major application of MALDI-TOF

- Excellent identification method
- Applicable to blood culture pellet
- Rapid (< 1h)
- Cheap and accurate
- Need for improved database
- Clinical impact

7. Conclusions



*TTI = time to identification

Thank you

Thank you

Sébastien Aeby
Alain Bizzini
Myriam Corthesy
Antony Croxatto
Christian Durussel
Philippe Hauser
Samuel Heiniger
Aude Leresche
Julia Lienard
Guy Prod'hom
Nathalie Ramirez
Anna Ruegger
Laurence Simon
Gizha Shklqim
Christelle Vogne
...

Impact of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry on the Clinical Management of Patients With Gram-negative Bacteremia: A Prospective Observational Study

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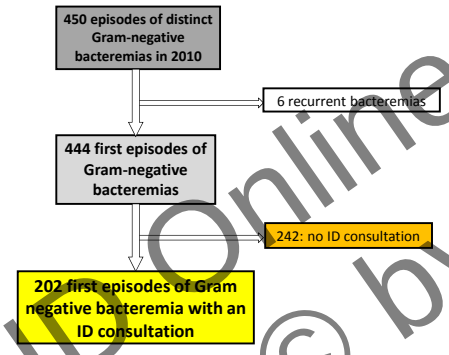
6. Clinical impact of MALDI-TOF



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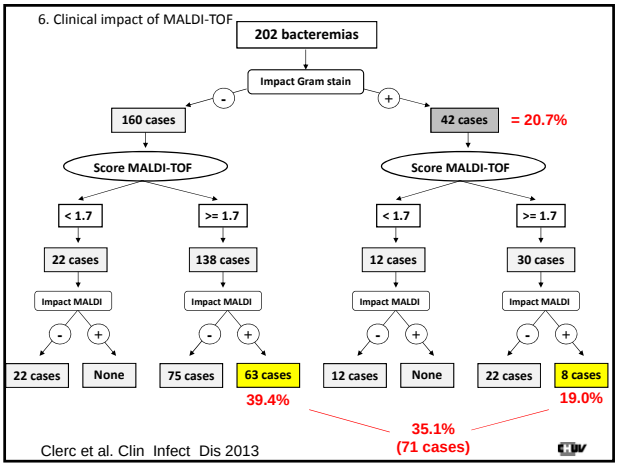
6. Clinical impact of MALDI-TOF



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6. Clinical impact of MALDI-TOF

Impact of the Sequential Reporting	N = 202
Gram stain	42 (20.8)
Streamlining	16 (7.9)
Spectrum broadening	16 (7.9)
Introduction of empirical antibiotic therapy	10 (5.0)
MALDI-TOF MS	71 (35.1)
Streamlining	22 (10.9)
Spectrum broadening	31 (15.3)
Introduction of focused empirical antibiotic therapy	18 (8.9)

Impact of MALDI-TOF on empirical antibiotic therapy in 35% of bacteremia

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6. Clinical impact of MALDI-TOF

MALDI-TOF MS and POCT-PCR for the Rapid Diagnosis of *Staphylococcus aureus* Bacteremia:

- **Randomized trial with/without POCT-PCR (n=197)**
- **95% of *S. aureus* identification in blood cultures with MALDI-TOF**

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GenXpert on blood cultures - 106 cases

24 episodes of MRSA (1 polymicrobial)
- All correctly identified
- No false-positive result

82 episodes of MSSA (1 polymicrobial)
- 81 correctly identified, 1 undetermined

Specificity 100%
Sensitivity 99%

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Time to result

- **Positive blood culture → MALDI-TOF**
  Median time 104 minutes
- **MALDI-TOF → GenXpert**
  Median time 97 minutes

201 minutes

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Clinical impact

	Intervention *N = 106*		Standard *N = 91*	
	MRSA n = 24	**MSSA** n = 82	**MRSA** n = 19	**MSSA** n = 72
MRSA carriers	18 (75)	1 (1.2)	16 (84.2)	1 (1.4)
Anti-MRSA treatment	22 (91.7)	14 (17.1)*	18 (94.7)	21 (29.2)*

* P = 0.09, Fisher's exact test

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6. Clinical impact of MALDI-TOF

Summary

- 95% of correct *Staph. aureus* identification in blood cultures with MALDI-TOF
- High performances of **GenXpert on positive blood cultures**
- **Clinical impact**:
  - Reduced unnecessary coverage of MRSA in cases of MSSA bacteremia (17% versus 29%)
  - Early diagnosis of unsuspected MRSA bacteremia

➡ **MALDI-TOF done routinely on all blood cultures since 2009**

➡ **POCT-PCR MRSA done on all *S. aureus* blood culture (from patients not known to be MRSA carriers)**

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