



Applications of Mass Spectrometry in medical microbiology

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There is no conflict of interest with any financial organization regarding the material information discussed in this presentation.

MALDI-TOF MS in microbiology



Matrix-Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS)

Main application in medical microbiology:
identification of bacteria, yeasts and fungi

First papers describing this application:

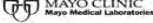
Claydon MA, Davey SN, Edwards-Jones V, Gordon DB.
The rapid identification of intact microorganisms using mass spectrometry.
Nat. Biotechnol. 1996;14(11):1534-6.

Krishnamurthy T, Ross PL, Rajamani U.
Detection of pathogenic and non-pathogenic bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
Rapid Commun. Mass Spectrom. 1996;10(8):883-8.

Holland RD, Wilkes JG, Rafii F, Sutherland JB, Parsons CC, Voorhees KJ, Lay JO Jr.
Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry.
Rapid Commun. Mass Spectrom. 1996;10(10):1227-32.

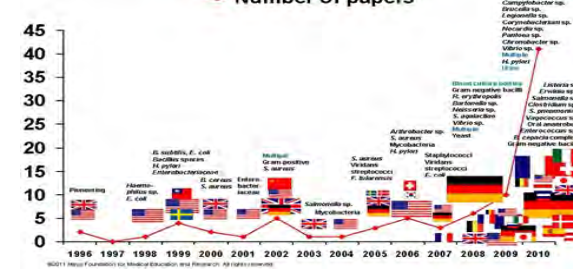
MALDI-TOF MS in microbiology

(early 2014: ~600 medline citations)



Bacterial Identification by MALDI TOF Mass Spectrometry¹

Number of papers



1. <http://www.mayocliniclaboratories.com/articles/topics/transcripts/201202-bact-h015.html>

Not only papers
but also books and Special Issues

MALDI-TOF: Microbiology Workflow

Dr. Thomas Maier, Bruker Biotech, Bremen, Germany

Main advantage of MALDI-TOF: rapid ID
Financial implications

Integrating Rapid Pathogen Identification and Antimicrobial Stewardship Significantly Decreases Hospital Costs

Katherine A. Pinner, PharmD; Rindoff L. Olson, MD, PhD; William L. Mouton, PharmD; Patricia L. Czirnoch, BS; James R. Davis, PhD; Geoffrey A. Land, PhD; Isai E. Pittman, PhD; James M. Moxon, MD, PhD

Context—Early diagnosis of gram-negative bloodstream infections, prompt identification of the infecting organism.

Results—The mean hospital length of stay in the preintervention group survivors (n = 100) was 11.9 versus 9.3 days in the intervention group survivors (n = 100).

- **Houston Methodist Hospital, Texas, USA**
- **Uses BD Bactec + (Culture based ID vs Bruker MALDI-TOF) + BD Phoenix**
- **Outcome:**
 - Length of stay: 11.9 days (classic ID) vs 9.3 days (MALDI)
 - Time saving: 2.6 days per patient
 - Reimbursement: DRG is independent of the length of stay!
 - Cost per patient: 45709 US \$ vs 26162 US \$
 - Cost savings per patient: 19547 US \$

compared to those in patients treated after implementation. Differences in length of hospitalization and hospital costs were assessed in survivors.

Arch Pathol Lab Med. doi: 10.5858/arpa.2012-0651-OA

Arch Pathol Lab Med—Vol 137, September 2013

MALDI for microbial typing

- MALDI-TOF is a very stable and accurate technique
 - Taxonomical Resolution comparable to 16S rDNA sequencing
 - Faster than conventional techniques
 - Cost-effective
- Not every MALDI-TOF system is suitable for routine diagnostic microbiology.
 - Dedicated machine with optimized features to provide ease of use, robustness and reliability
 - Dedicated software and database

MALDI for microbial typing



Current systems:
bioMérieux vs Bruker

bioMérieux

Current systems:
bioMérieux vs Bruker


VITEK MS RUO (AXIMA@SARAMIS™)

- Open database
- For research use only
- ~2000 species

VITEK MS

- IVD CE system
- Closed database
- ~800 species


*Formerly Shimadzu/Saramis



The slide shows two pieces of laboratory equipment. On the left is a tall, white VITEK MS RUO (AXIMA@SARAMIS™) system. On the right is a smaller VITEK MS system with a computer monitor displaying a software interface and a 'Prep Station'.

Current systems:
bioMérieux vs Bruker

Bruker



The slide is mostly blank with the word 'Bruker' centered in the middle. A large watermark 'ESCMID Online Lecture Library © by author' is overlaid diagonally across the slide.

Current systems:
bioMérieux vs Bruker

Bruker Microflex

Either as open system or CE IVD

Various databases

- General MSP library
- Security library
- Filamentous fungi library
- Mycobacterial library


Partnerships with Siemens and BD

for integrated Identification & Antimicrobial Susceptibility Testing workflow



The slide features a Bruker Microflex system, which includes a tall, dark blue vertical unit and a computer monitor displaying a software interface. To the left of the main image are four circular icons representing different aspects of the system: a blue molecular structure, a person working in a lab, a laboratory setting, and a circular diagram.

Choosing a machine



The image shows four small 3D figures standing in a line, each holding up a checkmark inside a colored square frame. From left to right, the frames are red, orange, yellow, and green. The figures are white and appear to be walking towards the right.

bioMérieux vs Bruker

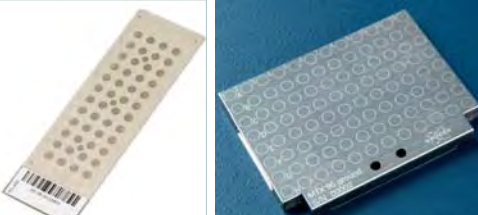
Main differences

	bioMérieux VITEK MS	Bruker Microflex
# Machines	200	1500

bioMérieux vs Bruker

Main differences

	bioMérieux VITEK MS	Bruker Microflex
# Machines	200	1500
Targets	Disposable only	Disposable, and reusable (ground steel, polished steel)



The image shows two target cards. On the left is a disposable target card, which is a rectangular strip with a grid of small circular wells. On the right is a reusable target card, which is a square plate with a grid of larger circular wells. The reusable card has a barcode and some text on it.

bioMérieux vs Bruker
Main differences

	bioMérieux VITEK MS	Bruker Microflex
# Machines	200	1500
Targets	Disposable only	Disposable, and reusable (ground steel, polished steel)
No Samples/target	48 + 3 control (x4)	96

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Create own DB entry	Difficult	Easy

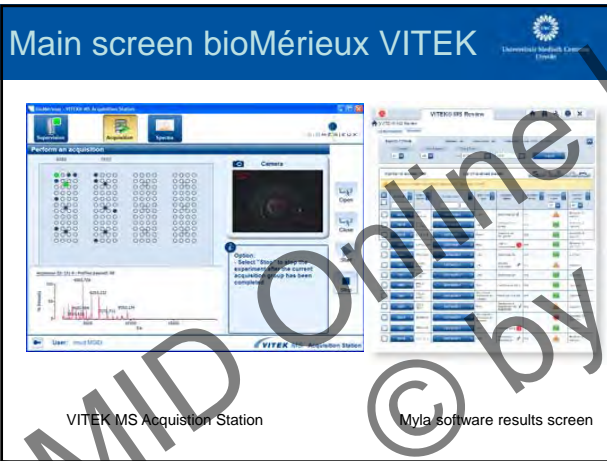
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Extra options	Few, if any	Many (direct blood ID, typing...)

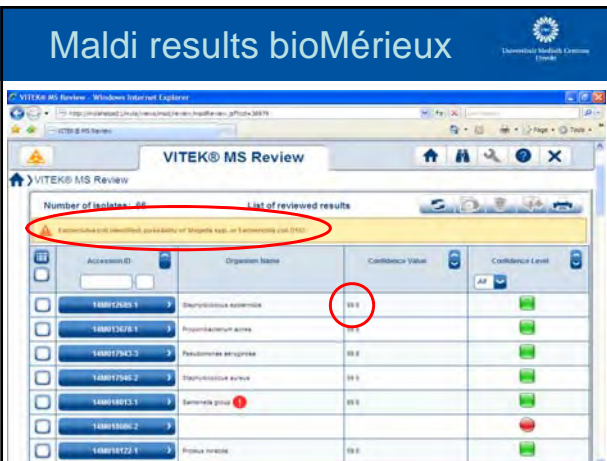
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Result output type	% reliable match	Top 10 match





Main screen Bruker Microflex

Maldi results Bruker Overview page

Project Info

Project Name: 131917.kim
 Project Description: MSF (low) 271.52.8C
 Project Creation Date/Time: 2011-03-17 11:42:48 CDT
 Project Analyte Class: 4
 Project Type: B250 (Bruker) GC/MS

Rank Overview

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
1	21181	<i>Enterococcus faecalis</i>	2100	<i>Enterococcus faecalis</i>	2099
2	141284	<i>Enterococcus faecium</i>	1999	<i>Enterococcus faecium</i>	1998
3	141284	<i>Enterococcus faecium</i>	1998	<i>Enterococcus faecium</i>	1997

Meaning of Score Values

Score	Interpretation	Rank	Color
2100	Highly similar to reference spectrum	1	Green
2000	Very similar to reference spectrum	2	Yellow
1700	Similar to reference spectrum	3	Orange
800	Not similar to reference spectrum	4	Red

Maldi results Bruker Detailed analyte page (top 10 match)

Analyte Name: HF
 Analyte Description: 141284
 Analyte ID: 2011-03-17 11:41:07 CDT
 Analyte Creation Date/Time: 2011-03-17 11:41:07 CDT
 Applied MSF Libraries: Bruker Libraries

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	<i>Enterococcus faecium</i> 20221_1 CDB	2100	1125
2 (++)	<i>Enterococcus faecium</i> 13697 CDB	2099	1125
3 (++)	<i>Enterococcus faecium</i> FX_21084508_22 MED	2098	1125
4 (++)	<i>Enterococcus faecium</i> DSM 17501 DSM	2097	1125
5 (++)	<i>Enterococcus faecium</i> DSM 2146 DSM	2096	1125
6 (++)	<i>Enterococcus faecium</i> DSM 2014 DSM	1999	1125
7 (++)	<i>Enterococcus faecium</i> DSM 11750 DSM	1998	1125
8 (++)	<i>Enterococcus faecium</i> DSM 6177 DSM	1997	1125
9 (-)	<i>Enterococcus faecium</i> VSE_F3_18884213 MED	1996	1125
10 (-)	<i>Enterococcus faecium</i> DSM 196817 JGI	1995	112851

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bioMérieux vs Bruker

Main differences

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Best machine: dependent on type of lab, expected use/workflow, & number of samples
Being an academic lab and early adopter we chose Bruker

Your MALDI-TOF arrived

Now what?




- Installation
- Connect to LIMS
- Validation routine ID
- Training of personnel
- Routine implementation
- Use it for 'fun stuff'
 - Direct ID from bloodculture
 - ID of yeasts and fungi
 - AB resistance determination
 - Typing (outbreak surveillance)

Picture: <http://www.kpack.nl>

Validation of most tests

normally one test has simple +/- result




Use simple test pannel → test OK if expected result is obtained

Picture: <http://www.martinvanoopen.nl>

Validation of MALDI-TOF ID


One test with many possible results



Impossible to test all options → is this a problem?

Picture: <http://iStockphoto.com>

Risk estimation



People generally are not inherently good in analysing risk. They tend to take decisions swayed by emotional response to a situation rather than an objective assessment of relative risk'

Validation from risk analysis and risk management perspective

Scale	Probability	Impact
Very low	Unlikely to occur	Negligible impact
Low	May occur occasionally	Minor impact on quality
Medium	Is as likely as not to occur	Notable impact on quality
High	Is likely to occur	Substantial impact on quality
Very high	Is almost certain to occur	Threatens the patient safety

Validation from risk analysis and risk management perspective

Known restrictions of MALDI identification

Shigella has to be considered as a part of the *E. coli* species and accordingly gives no different MALDI pattern.

Streptococcus pneumoniae is very closely related to the *S. mitis* group, there might occur misidentifications, most with low ID scores. A second test has to be used for confirmation (bile test preferred, alternatively optochin test; gold standard MLST – 16S rDNA cannot not distinguish).

Aeromonas spp. are closely related and consequently there might occur misidentifications. A second test has to be used for for confirmation (Gold standard sequencing of the *dnaJ* gene – 16S rDNA cannot not distinguish)

Training of personnel
Learning how to improve the quality

- **Sample preparation: less is more!**

Dr. Thomas Maier Bruker Daltonics, Bremen, Germany

Training of personnel
Learning how to improve the quality

- **Optimizing sample preparation**

Dr. Thomas Maier Bruker Daltonics, Bremen, Germany

Training of personnel
Learning how to improve the quality

- Studies show that use of different media do not affect results much
- Also different temperatures, medium composition hardly affect results.
- Even older cultures can be analyzed, without misidentification.

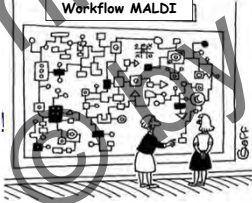
HOWEVER:

- Fresh cultures work best
- Rich media seem to provide better results
- Increasingly difficult: Gr⁻ < Gr⁺ < Yeasts < Filamentous fungi

Optimizing the workflow

- Microbial ID is a key laboratory procedure
- MALDI speeds up the ID procedure
- Direct ID OK or do we need extraction?
- Many 'new' species seen
- ID before AST!

Drastic change in workflow!



MALDI is a crucial component of the daily workflow BUT

Although MALDI is easy to master & use

- AND controls ARE included in recommended workflow
- And ID scores provide quick feedback on technical ability of personnel

There is a need for continuous monitoring of system & daily workflow

- Helps promote and maintain a efficiency culture
- Allow for quick and easy identification of problems and trends
- Allow to identify next steps in the continuous process improvement

NOTE THAT in spite of the crucial role of ID in your lab there is NO internal statistical evaluation in the commercial systems

Getting more out of your MALDI direct ID from blood cultures

Leidenhills Medisch Centrum
Eindhoven

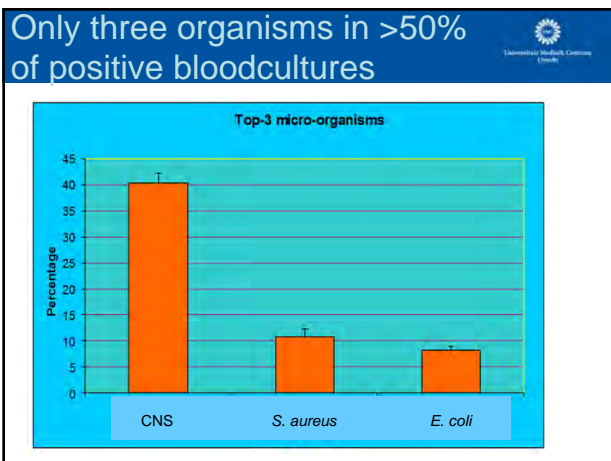
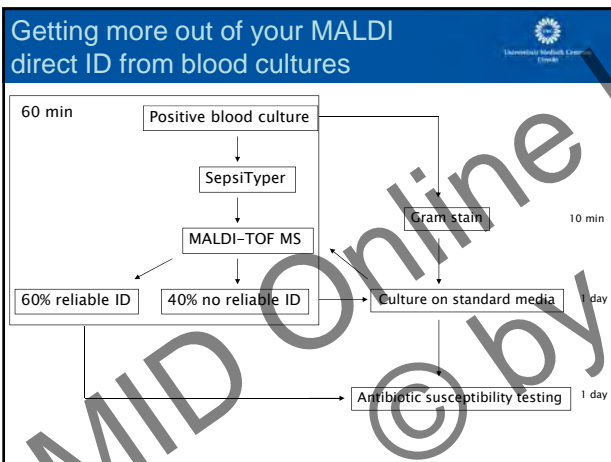
Open Access study available online
PLOS ONE

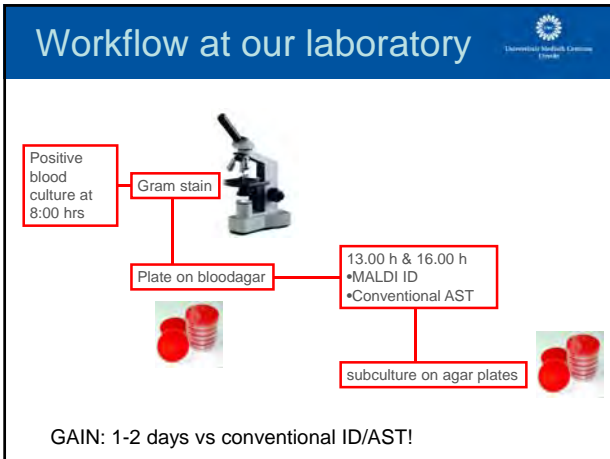
Direct Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry Improves Appropriateness of Antibiotic Treatment of Bacteremia

Anne L. M. Vlas*, Marc J. M. Bonten, C. H. Edwin Boel
Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands

Abstract
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) allows the identification of microorganisms directly from positive blood culture bottles. Use of the MALDI-TOF MS for rapid identification of microorganisms from blood culture bottles can reduce the turnaround time for identification and may lead to further appropriate treatment of bacteremia. During February and April 2010, direct MALDI-TOF MS was routinely performed on all positive blood cultures. During December 2009 and March 2010, no direct MALDI-TOF MS was used. Identification of antibiotic therapy was collected from the hospital and intensive care unit information systems from all positive blood cultures during the study period. In total, 253 episodes of bacteremia were included of which 89 during the intervention period and 164 during the control period. Direct identification of MALDI-TOF MS on positive blood culture bottles reduced the time to bacteremia identification by 28.6 h and was associated with an 11.3% decrease in the proportion of patients receiving appropriate antibiotic treatment 24 hours after blood culture positivity. In the control period, 75.3% in the intervention period (p<0.05). Routine implementation of this technique increased the proportion of patients on adequate antimicrobial treatment within 24 hours.

Introduction
The Bacter 3000 system (Biover Diagnostics, Sparks, USA) might allow faster implementation of appropriate antimicrobial therapy.







Getting more out of your MALDI Functional Betalactamase Test

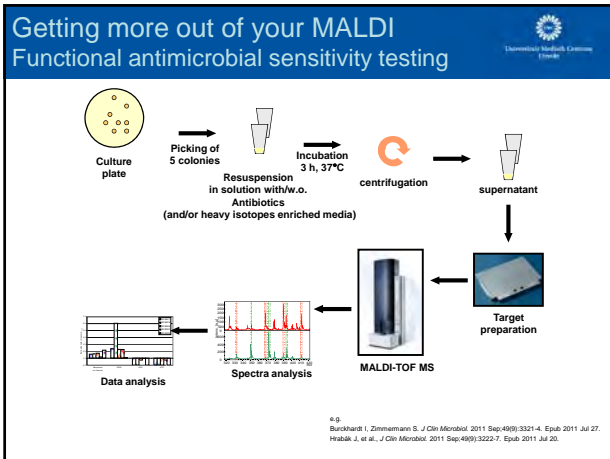
β -Lactamase

+ 18 Da

- 44 Da

$-\text{CO}_2$

- Several publications about the functional testing of betalactamase activity by MALDI-TOF mass spectrometry in 2011/2012
- In all cases, data evaluation and measurement was mainly visual/manual
- For practical application, but also further evaluation studies, automation of the analysis is necessary



MALDI-TOF MS for subtyping

Contents lists available at ScienceDirect
 International Journal of Medical Microbiology
 journal homepage: www.elsevier.com/locate/ijmm

MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant *Staphylococcus aureus* lineages
 Manuel Wolbers^a, Holger Rohde^{a,*}, Thomas Maier^b, Gerhard Gohar-Gumpo^a, Stefan Franke^a, Stefanie Scherpe^a, Martin Apfelbacher^a, Martin Grotzinger^a

Outbreak analysis with MALDI-TOF Mass Spectrometry – potentials and limits – an outlook
 A Burckhardt^a, S. Schmitt^a, J. Meier^a, S. Zimmermann^a, C. Weidert^a
^aInstitute for Hygiene, Department for Microbiology and Hygiene, University of Heidelberg, Heidelberg, Germany, ^bInstitute for Diagnostics, Germany

Direct bacterial profiling by means of MALDI-TOF mass spectrometry as a method of rapid differentiation of *Staphylococcus aureus* strains
 Maria Komienko^a, Elena Iliina^a, Alexandri Borovskaya^a, Mikhail Steletskii^a, Marina Sukhorukova^a, Markus Kretzschmar^a, Dimitry Gorovoy^a

Pasteurella multocida

Casus

- 2 patients
 - Both post-OR infection with *P. multocida* on same day

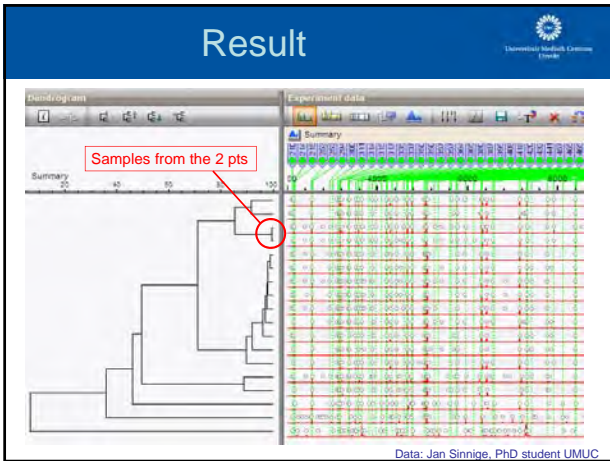
Question:

- Is this coincidence or result of infection during OR

Setup

- Samples from these two patients
- 15 unrelated isolates
- 1 Control (outlier)
- Software: **BioNumerics**

Data: Jan Sinnige, PhD student UMUC



Staphylococcus hominis

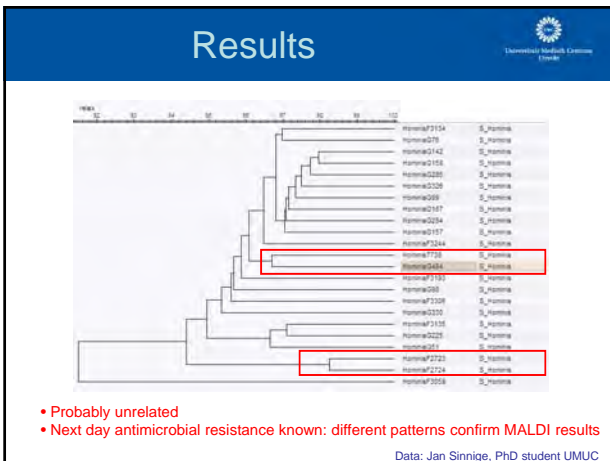
Casus

- November 2012
 - Patient with prosthetic hip & positive blood culture for *S. hominis*
 - Treated with succes (neg blood cultures)
- February 2013
 - Again positive blood culture for *S. hominis*

• Are these two *S. hominis* isolates related?

• Infection/colonization of prosthetic hip?

Data: Jan Sinnige, PhD student UMUC



MALDI-TOF MS for subtyping

- MALDI-TOF MS profiles of different **subtypes/subspecies** of a microorganism can differ tremendously – or by only a single peak shift (or not at all). Sometimes even related **species** are hard to distinguish!
- The highest degree of **intra-species diversity** can be in parallel with a differentiation of interest – or not related at all
- **Bioinformatics** has to be adopted to the respective requirements
- Necessity of **standardization** of cultivation and measurement have to be evaluated
- Spectra of **high quality** have to be acquired, stringent **QC**
- **Careful investigation** – not only by statistics – of any differences is mandatory. Do not just believe in bioinformatics!
- To differentiate: investigation and utilization of subtypes!

CONCLUSIONS

- MALDI-TOF is a revolution in microbial ID
 - Faster, cheaper, more reliable, etc...
 - Databases grow rapidly and this improves hitrate
- ID of bacteria and yeasts works fine
- ID of filamentous fungi needs tweaking
- ID directly from blood cultures in ~60% OK
- Fast anti-microbial testing and typing still experimental but promising

Acknowledgements

People at

 My colleagues at

Workgroup MALDI-TOF 

And all those that I borrowed ideas, slides and pictures from!

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