


MALDI-TOF MS

Applications 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

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

Main application in medical microbiology:
identification of bacteria & yeasts/fungi

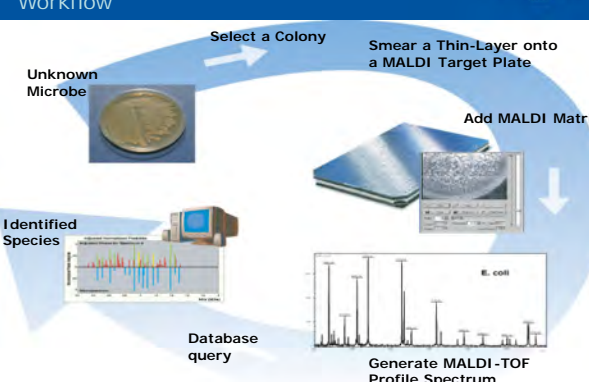
First papers describing routine microbial ID applications in 1996

- Claydon e.a. Nat. Biotechnol. 1996;14(1):1564-6.
- Krishnamurthy e.a. Rapid Commun. Mass Spectrom. 1996;10(8):883-8.
- Holland RD e.a. Rapid Commun. Mass Spectrom. 1996;10(10):1227-32.

Jan 2015: ~1500 PubMed citations on microbial MALDI-TOF MS

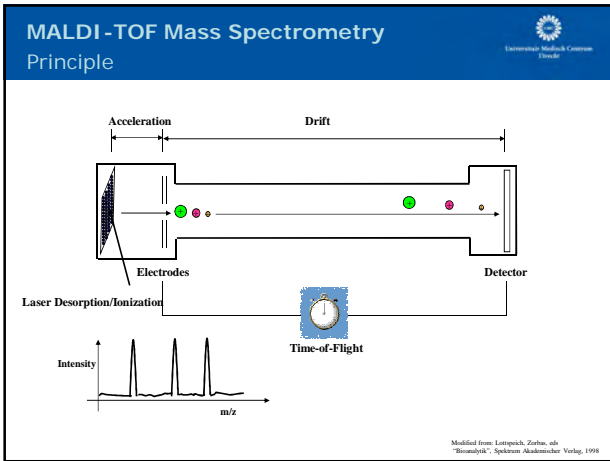
- Majority (~1000) on bacterial ID
- Rest on yeast & fungal ID, typing, AB resistance, economics, comparing different methods and machines, etc

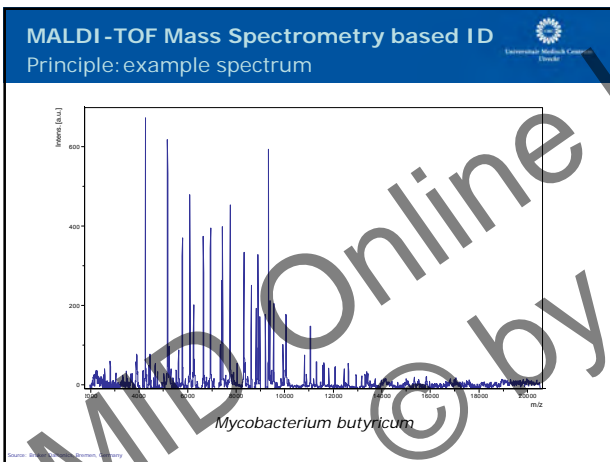
MALDI-TOF Mass Spectrometry Workflow

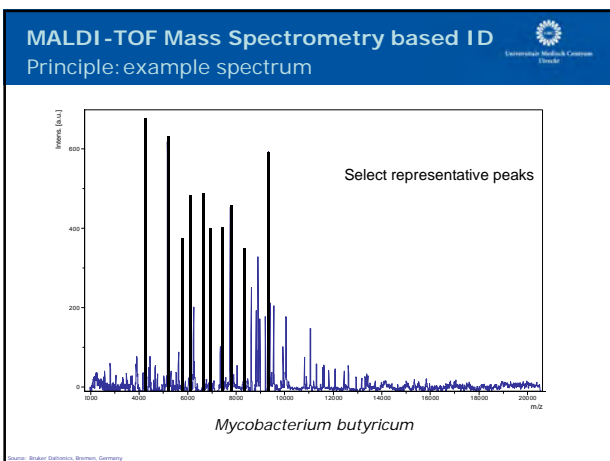


The diagram illustrates the MALDI-TOF mass spectrometry workflow. It starts with an 'Unknown Microbe' (represented by a coin) which is used to 'Select a Colony'. This colony is then 'Smear a Thin-Layer onto a MALDI Target Plate'. 'MALDI Matrix' is added to the smear. The resulting sample is analyzed to 'Generate MALDI-TOF Profile Spectrum', shown as a mass spectrum for *E. coli*. This spectrum is used for a 'Database query' to identify the 'Identified Species'.

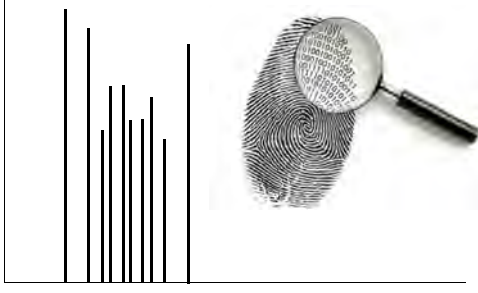
Source: Dr. Thomas Meier, Bruker Daltonics, Bremen, Germany







MALDI-TOF Mass Spectrometry based ID
Principle: example spectrum




Mycobacterium butyricum

Source: <http://microsoft-mass.com/signetics-acquires-velocity-fingerprint-sensor-technology-company>

MALDI-TOF MS for microbial ID

- Routine MALDI-TOF based microbial identification
 - Represents a reliable, 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-TOF MS for microbial ID




WHAT'S OUT THERE?
A Book About Space

By Lynn Wilson
Illustrated by Paige Billis-Evans

Commercial MALDI-TOF MS systems
bioMérieux

- VITEK MS RUO (AXIMA@SARAMIS™)**
 - Open database
 - For research use only
 - ~2000 species
- VITEK MS**
 - IVD CE system
 - Closed database
 - ~800 species
 - Separate bacterial/yeast DBs




Formerly Shimadzu/Saramis

Commercial MALDI-TOF MS systems
Bruker

- Bruker Microflex**
 - Either as open system or CE IVD (3 versions)
 - Various databases (can be combined in single run)
 - General MSP library
 - Security library
 - Filamentous fungi library
 - Mycobacterial library
 - (In total ~2,500 distinct species in combined DBs)
- Partnerships with e.g. Siemens and BD**
 - for integrated Identification & Antimicrobial Susceptibility Testing workflow



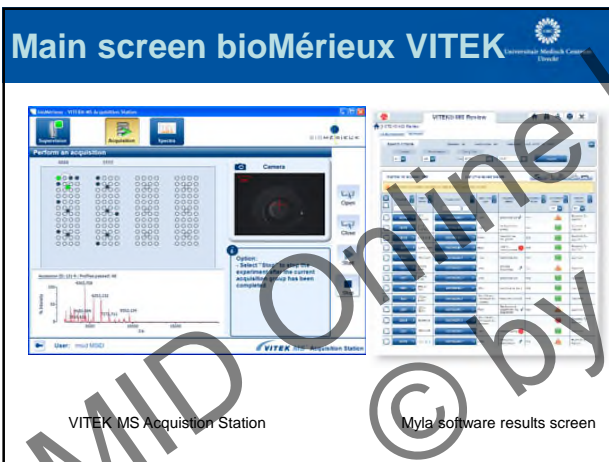
Choosing a machine

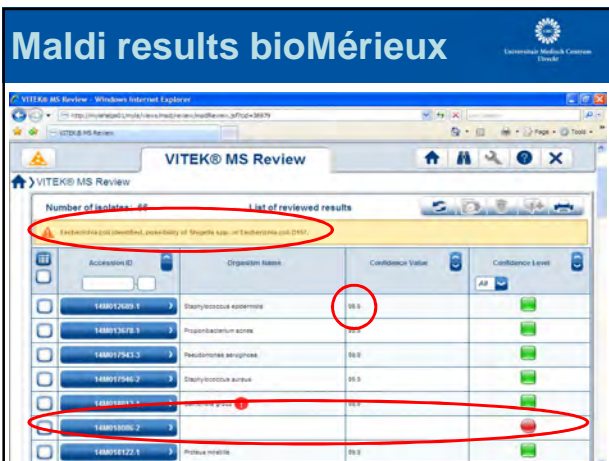


bioMérieux vs Bruker

Main differences

	bioMérieux VITEK MS	Bruker Microflex
# Machines	~500	~2000
Targets	Disposable only	Disposable, and reusable (ground steel, polished steel)
No Samples/target	48 + 3 control (x4)	96
Time to start of run	~10 minutes	~1 minute
Database	~800 species as superspectra	~2500 species As >7000 individual entries
Create own DB entry	Difficult	Easy
Extra options	Few, if any	Many (direct blood, typing, etc.)
LIMS connectivity	Proprietary middleware (Myla)	Direct or middleware
Result output type	% reliable match	Top 10 match





Main screen Bruker Microflex

More info when clicked

Rank	Species	Score	Decision
1	Escherichia coli	1375	species (genus-only)
2	Escherichia coli	1365	species (genus-only)
3	unreliable identification	1363	species (genus-only)
4	Streptococcus anginosus	1302	species (genus-only)
5	Streptococcus epidermidis	2152	species (genus-only)
6	no reliable identification	1427	species (genus-only)
7	no reliable identification	1156	species (genus-only)
8	Burkholderia fungorum	3349	species (genus-only)
9	Burkholderia fungorum	3188	species (genus-only)

Maldi results Bruker

Detailed analyte page (top 10 match)

Analyst Name: JP
 Analyte Description: M1596
 Analyte Creation Date-Time: 2013-09-17T14:13:07.042
 Applied MSP Library(s):
 Applied Taxonomy: Tree Bruker Taxonomy

Rank (Quality)	Matched Pattern	Score Value	N% MS Identifier
1 (++)	Enterococcus faecalis 20211_1_CMB	1352	1352
2 (++)	Enterococcus faecalis 13037_CMB	1352	1352
3 (++)	Enterococcus faecalis 13037_CMB	1352	1352
4 (++)	Enterococcus faecalis DSM 17050 DSM	1352	1352
5 (++)	Enterococcus faecalis DSM 2146 DSM	1352	1352
6 (++)	Enterococcus faecalis DSM 20134 DSM	1352	1352
7 (++)	Enterococcus faecalis DSM 11569 DSM	1352	1352
8 (++)	Enterococcus faecalis DSM 6177 DSM	1352	1352
9 (++)	Enterococcus faecalis VRE_PK_16064218_MLD	1347	1352
10 (++)	Enterococcus faecalis DSM 154977_2_0	1346	112804

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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.6stock.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.martinvanleeuwen.nl>

Validation of MALDI-TOF ID


One test with many possible results



Impossible to test all options → is this a problem?

Picture: <http://800px.com/800px.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 risks

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

Identify risk & perform 'testing intensity' accordingly

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)

Bordetella spp. are closely related and cannot be safely discriminated from each other by MALDI-TOF analysis alone.

..... And more in other words while really good it is not perfect

Training of personnel
Learning how to improve the quality

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

Dr. Thomas Maler | Bruker Daltonics, Bremen, Germany

Training of personnel
Learning how to improve the quality

- **Optimizing sample preparation**

Dr. Thomas Maler | 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 risk of misidentification.

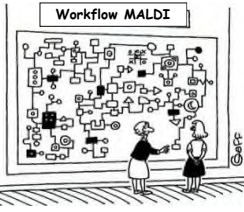
HOWEVER:

- 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
- Accurate 'universal method'
- 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 the system & daily workflow

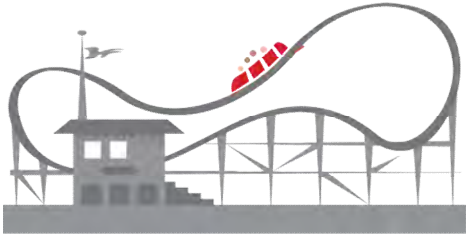
- Helps promote and maintain an efficient workflow
- Allow for quick and easy identification of problems and trends
- Allow to identify next steps in the continuous process improvement

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

MALDI-TOF

Beyond bacterial ID only

Fun Stuff!



Getting more out of your MALDI
Functional Betalactamase testing

CC1(C)SC(=O)NC1C(=O)R
 $\xrightarrow{\beta\text{-Lactamase}}$
CC1(C)SC(O)NC1C(=O)R
 $\xrightarrow{-CO_2}$
CC1(C)SC(O)N1C(=O)R

+ 18 Da
- 44 Da

- Several publications on the functional testing of betalactamases activity by MALDI-TOF mass spectrometry
- In most cases data evaluation and measurement was visual/manual
- For practical application full automation of the analysis is necessary

Getting more out of your MALDI
MALDI-TOF MS for subtyping/outbreak analysis

Contents list 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 Wolters^a, Holger Bahde^{a,*}, Thomas Mayer^a, Frank Heesbrun-Genster^a, Stefan Franke^a, Stefanie Scheppe^a, Martin Applebacher^a, Martin G. Klauer^a

Outbreak analysis with MALDI-TOF Mass Spectrometry – potentials and limits – an outlook
 A. Krasova^a, A. Nisetti^a, J. Astarf, A. Zappavigna^a, C. Wenzel

*Institute for Hygiene, Department for Microbiology and Hygiene, University of Heidelberg, Heidelberg, Germany; †Clinic 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 Ilina^a, Alexandru Borovskaya^a, Mikhail Belostanov^a, Marina Sukhorukova^a, Markus Kretzschmar^a, Vladimir Govorun^a

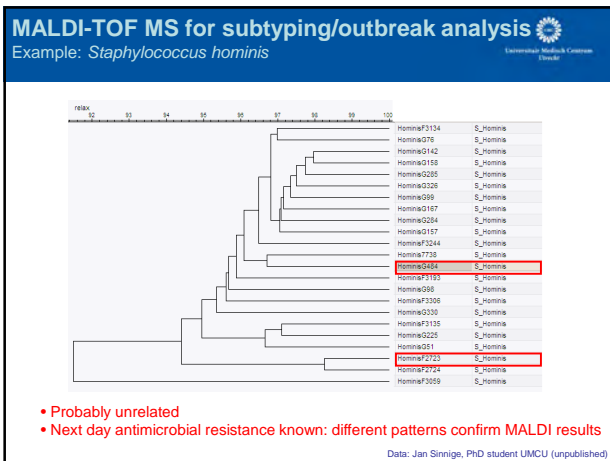
MALDI-TOF MS for subtyping/outbreak analysis
Example: *Staphylococcus hominis*

Casus

- November 2012
 - Patient with prosthetic hip & positive blood culture for *S. hominis*
 - Presumably treated successfully (Negative blood cultures)
- February 2013
 - Again positive blood culture for *S. hominis*

Is this an ongoing chronic infection (unsuccessful treatment) or an unrelated novel infection??

Data: Jan Sinnige, PhD student UMCU



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 ~80% OK
- Fast anti-microbial testing and typing still experimental but promising

Acknowledgements

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Workgroup MALDI-TOF

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