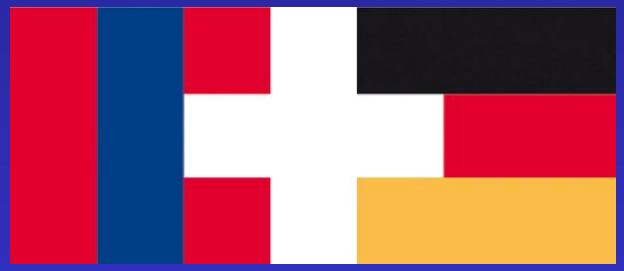
MVZ DORTMUND - Dr.Eberhard u. Partner - MICROBIOLOGY

MALDI-TOF (MS)

evaluation for routine diagnostics

MIKROBIOLOGY ŏ www.labmed.de / mikro@labmed.de



Medizinisches Versorgungszentrum
Dr. Eberhard & Partner Dortmund

Mongolia ŏ September 2012

accreditation since april 2003 (DIN EN ISO 15189; DACH)

Deutscher Akkreditierungs Rat

Medizinisches Versorgungszentrum / MVZ
Dr. Eberhard & Partner
Laboratoriumsmedizin Dortmund
Germany

founded in 1977 (private/independent)

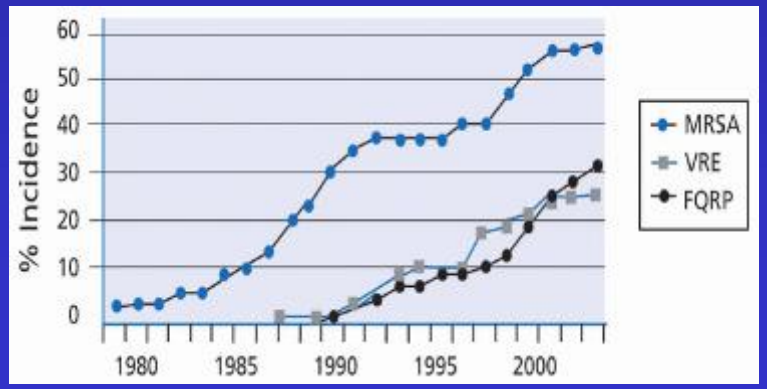
laboratory for hospitals and physicians in the surrounding area

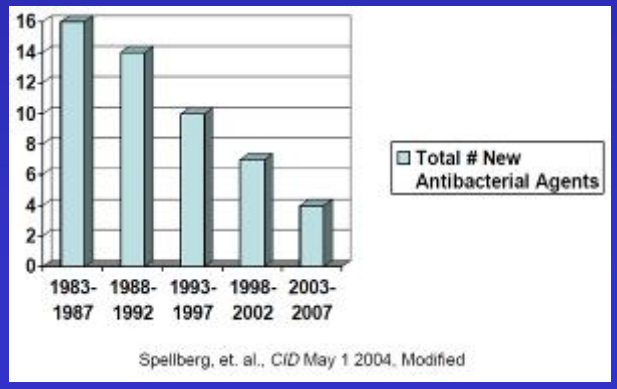
current projects in medical microbiology:

- **ESBL-Microarrays**
- **MALDI-TOF-MS**
  (additional: blood culture and yeasts)
- **FISH**
  (fluorescence-in-situ-hybridisation)

Multi-Drug-Resistent bacteria - a challenge

E nterococcus faecium	**VRE**
S taphyloc. aureus	**MRSA**
K lebsiella pneumoniae	**ESBL**
A cinetob. baumannii	**Carbapenemases, MDR**
P seudom. aeruginosa	**Carbapenemases, MDR**
E nterobacter cloacae	**ESBL+AmpC, MDR**





IDSA:

Bad bugs

–

No drugs

identification / determination of resistance

... methods currently used in our laboratory routine ...

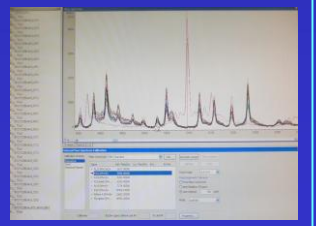
culture
streaking manually or by Innova (automated)




identification
VITEK 2/XL (biochem.) or MALDI-TOF (mass-spectrom.)





resistance
agar-diffusion or VITEK 2/XL (MIC)




MALDI-TOF (Mass-Spectrometry) - objectives

objectives for the evaluation of mass spectrometry:

ŏ **improving our diagnostics:**
- **higher quality of identification and faster results**

ŏ **integration into routine diagnostics:**
- **to work with the system in an efficient way - workflow?**
- **new perspectives: blood-cultures, Mycobacteria and yeasts?**

tests of the system:

ŏ **comparison of routine identification methods vs. MALDI-TOF:**
- **Vitek, Microscan Walkaway, api / Rapid ID**
- **16S Sequencing**
- **quality of identification in different groups of bacteria**

ŏ **double testing in MALDI-TOF**

introducing the method of MALDI-TOF (MS)

theoretic advantages of mass-spectrometry-method in a medical microbiology laboratory:

- **method for identification of cultural grown microorganisms**
- **significant faster than the biochemical identification methods**
  - **biochemistry: hours / days**
  - **MALDI-TOF-MS: few minutes (!)**
- **in some cases more precise identification possible?**
  - **based on identification of ribosomal proteins (!)**

improvement of microbiological diagnostics?

... faster and more precise !?

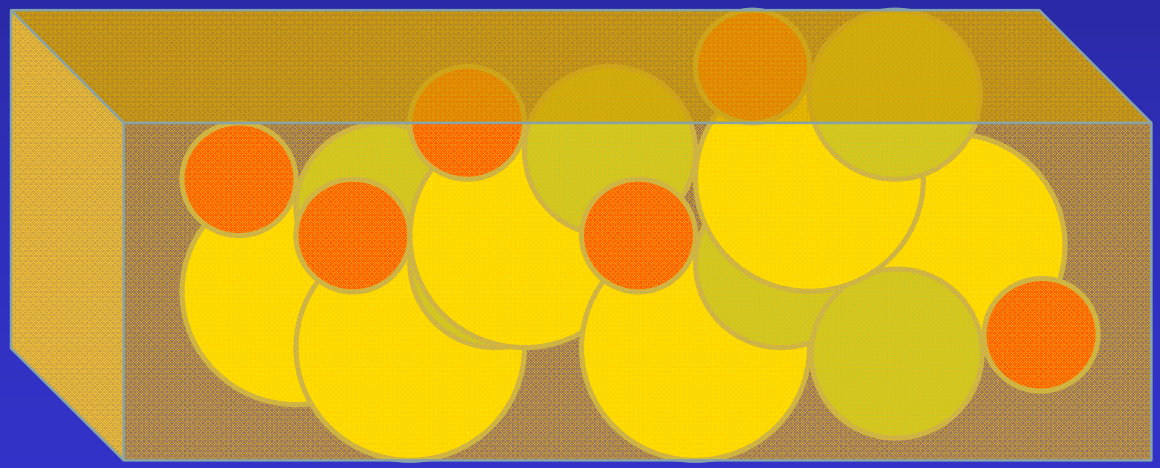
... what means „MALDI-TOF-MS“?

M atrix
A ssisted
L aser
D esorption /
I onisation
-
T ime
O f
F light
-
M ass
S pectrometry

Matrix Assisted:

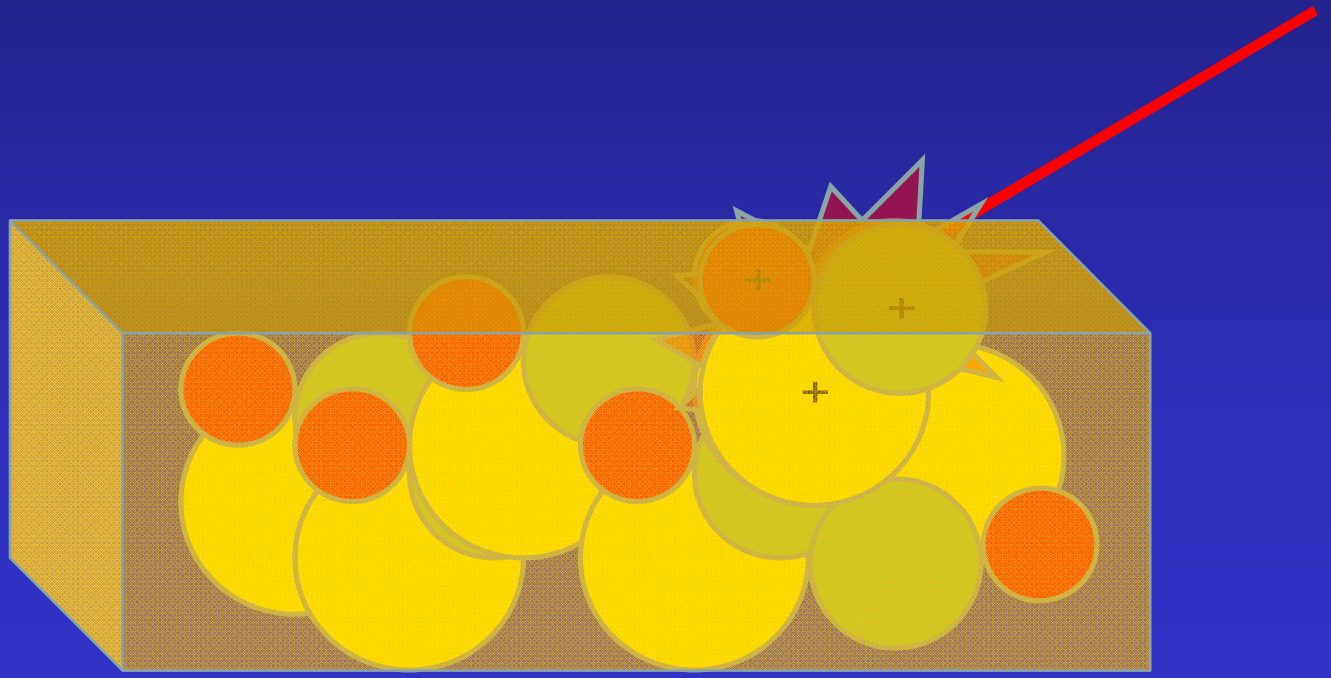
- **proteins that should be analysed are embedded in the matrix and crystallise - the analytes are not destroyed**

- **substance of the matrix consists of small organic molecules:**
  - **they absorb energy (e.g. laser-beam)**
  - **they deliver protons (charged positive)**
  - **e.g. benzoe acid-derivates, formic acid**



Laser Desorption/Ionisation

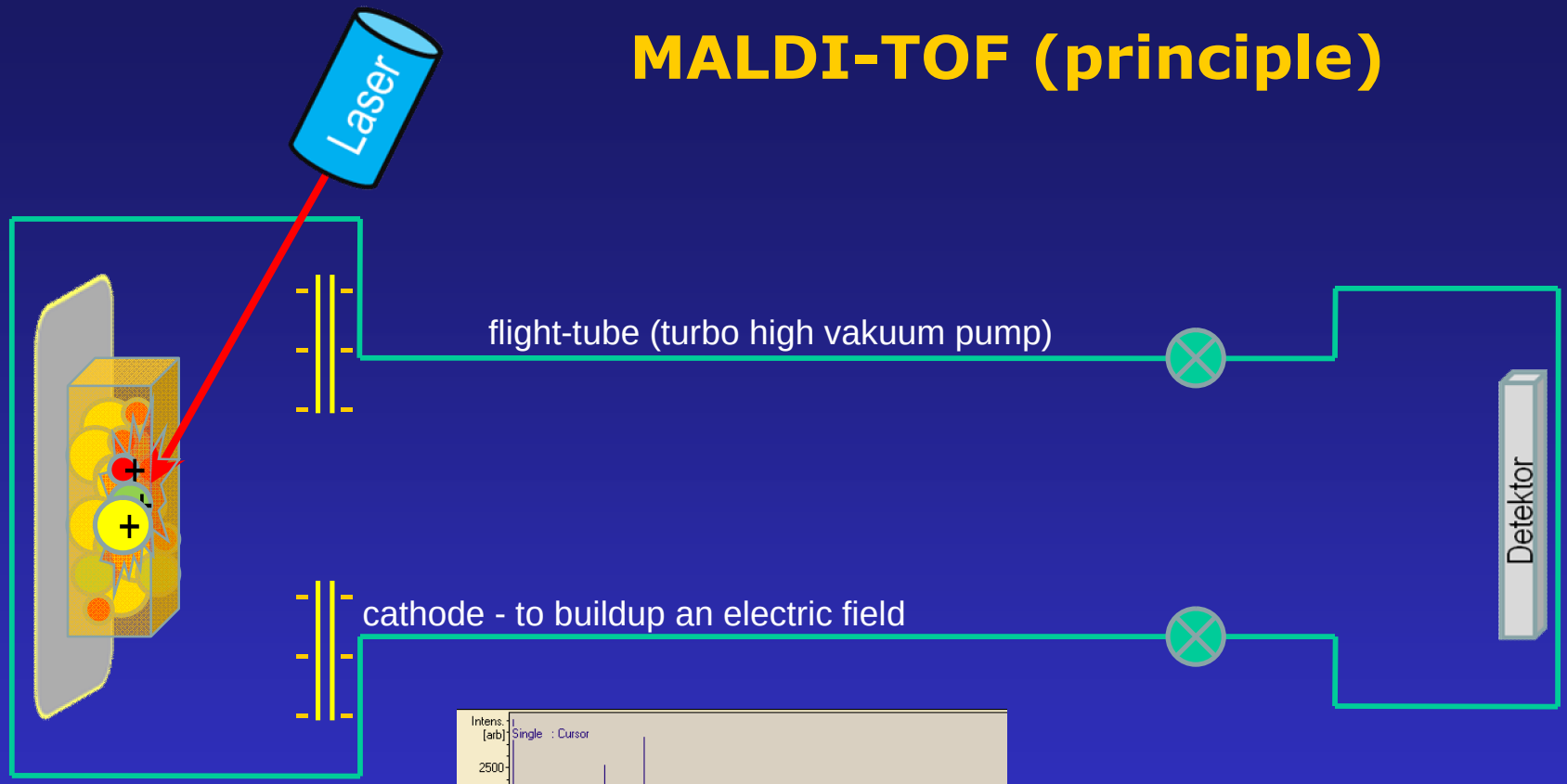
- **Desorption: moleküles leave the surface of the solid object**
  - **... in contrast to "adsorption"**
- **Ionisation: molecules became electrical charged particles**
  - **... e.g. by coming close to protons**

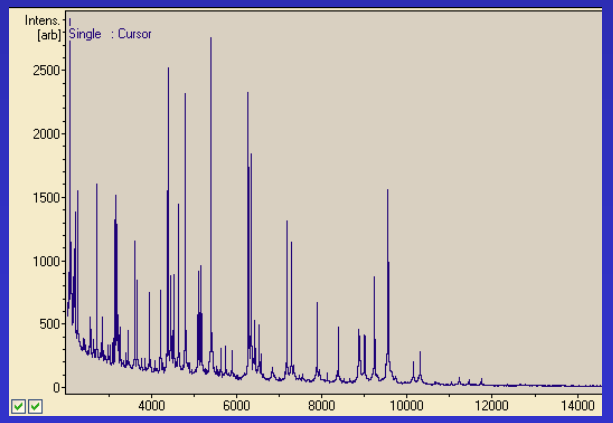


Time Of Flight

- ionized molecules were accelerated in an electric field
- are flying through a flight-tube (high-vacuum)
  - time of flight is dependent of mass and the kind of charge (large ʒ slower)
- at the end of the flight-tube a detector is located

MALDI-TOF (principle)





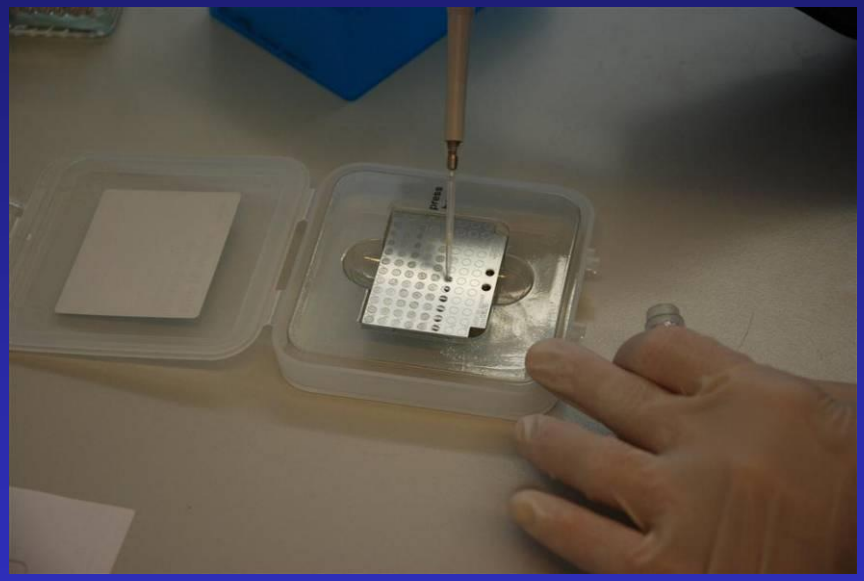
specific spektrum of masses - compared to database ...

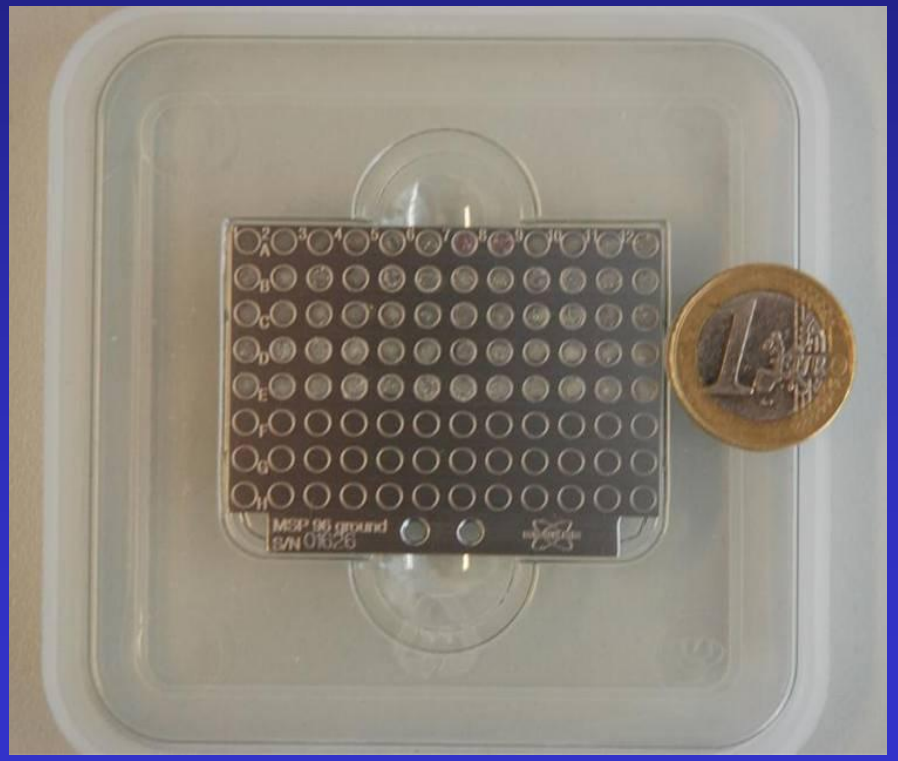
workflow – identification via MALDI-TOF:

cultural growth of a microorganism and preparation

… parallel: preparing test for resistance-determination

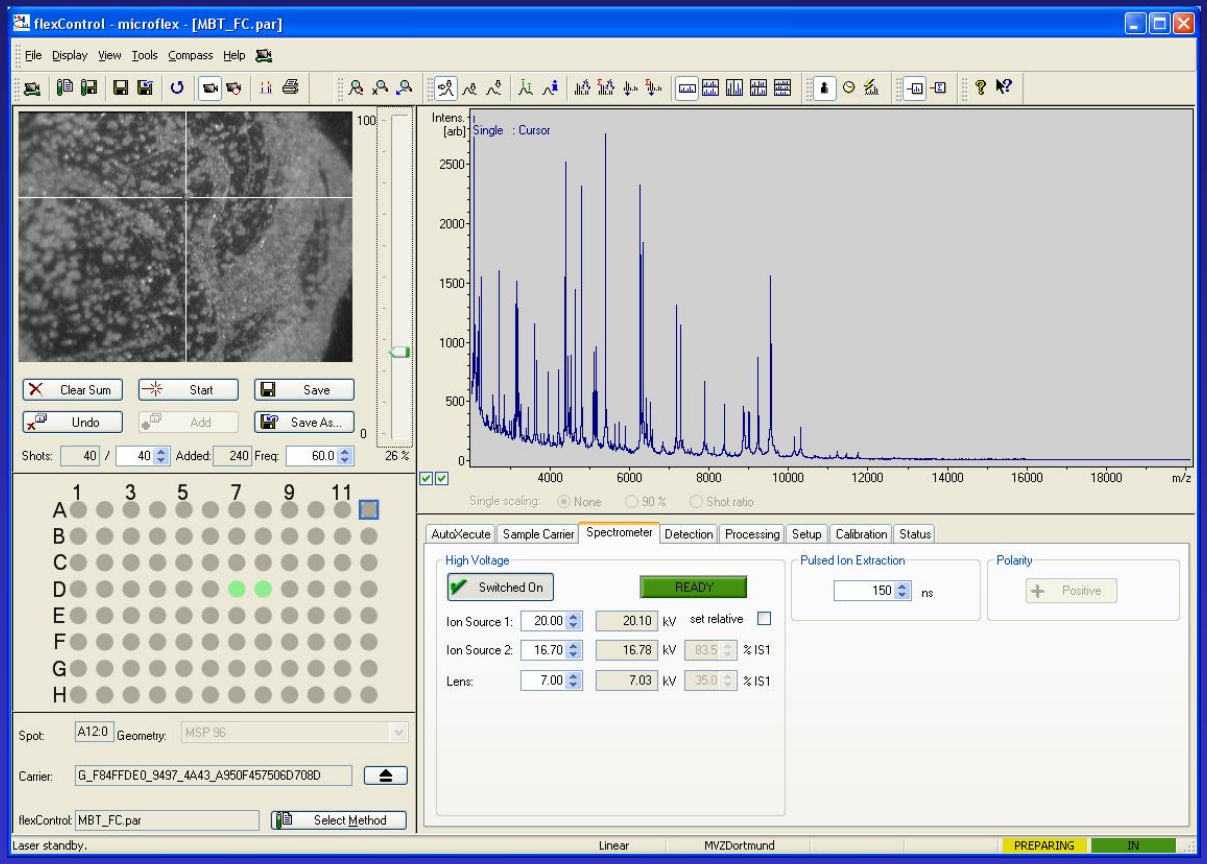
inoculation of colonies onto the metal target (precoated with a matrix-solution)





measurement in MALDI-TOF (mass spectrometry) (creation of a spectrum of masses)

comparison with reference-spectres in data base (similarity / probability is calculated)



3 identifikation possible within minutes ...

reported results

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Klebsiella oxytoca ATCC 700324 THL	2.408	571
2 (+++)	Klebsiella oxytoca ESBL 30298 PFM	2.394	571
3	Klebsiella oxytoca DSM 5175T HAM	2.14	571
(+)			
8 (+)	Raoultella ornithinolytica DSM 7464T HAM	1.751	54291
9 (+)	Raoultella planticola DSM 3069T DSM	1.741	575
10 (-)	Enterobacter aerogenes 15282_1 CHB	1.659	548

Meaning of Score Values

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

results of our first evaluation:

about 1.000 isolates tested / identified in 2010:

- **overall good identification results for our routine samples**
- **enterobacteriaceae: good results with high scores**
  - **problems with species closely related; E. coli/Shigella**
- **anaerobes: good results with high scores**
  - **better than biochemical methods**
- **candida: ID with lower scores, extraction helps**
  - **taxonomy sometimes difficult**
- **streptococci: good results for ß-haemol. Streptococci**
  - **problems with viridans streptococci and pneumococci**
- **„difficult" bacteria: Actinobaculum, Acidovorax, Pandorea**

MALDI-TOF in diagnostics of blood cultures:

until now: more than 50.000 isolates tested / identified:

10.800 enterobacteriaceae (incl. Salmonella, Campylobacter, Yersinia)

6.000 candida

5.600 staphylococci

3.800 anaerobic bacteria (Bacteroides, Prevotella Clostridium)

2.800 nonfermenters (Pseudomonas, Acinetobacter)

3.600 streptococci (ß-haemol., pneumococci, viridans)

2.900 enterococci

MALDI-TOF - an option for sepsis-diagnostics?

- comparable to molecularbiological methods?

- detection directly from blood cultures?

diagnostic of blood cultures - usual timeline in the routine:

- collecting specimen (patient)
- transport of specimen to the laboratory as soon as possible
- incubation: up to 7 days

positive 3 :

- gram-staining + culture (immediately)
- 1. day: preparing identification and resistance determination (in some cases at the same day: ID and parts of the RESI)
- 2. day final results (ID + Resi)

established method but complex and time-consuming...

3 faster methods available?

MALDI-TOF directly from blood cultures possible?

- method is spreading in microbiological laboratories
- data bases became better and better ... and are growing
- fast and shure identification of microorganisms

... initial calculated antimicrobial therapy is in a lot of cases earlier possible!

main task:

- disturbing proteines / molekules from the media of blood cultures must be eliminated !

actually:

- trying to evaluate an optimized preparation method for diagnostics of blood cultures, Mycobacteria and yeasts via MALDI-TOF
  (special extraction protocol)

conclusion:

- **evaluation of MALDI-TOF (MS) was successful !**
- **Workflow has to be planned thoroughly**
  (… then a high troughput is possible)
- **Very fast method**
  (… early identification can be very helpful in chosing the antimicrobial therapy)
- **Very promising method with the potential to replace some of the biochemical methods**
  (… optimisation of preparation-/extraction-methods leads to more specific results - even for blood cultures, Mycobacteria, yeasts etc.)

but: Susceptibility testing with conventional systems is necessary further on

... according to all medical issues it is useful to act as qualified, effective and innovative PARTNERS ...

... working hand in hand, and ...

... remembering hand disinfection!

(... the most important vehicle for microorganisms!)

Please support your hygiene-management !

Thanks for your attention!