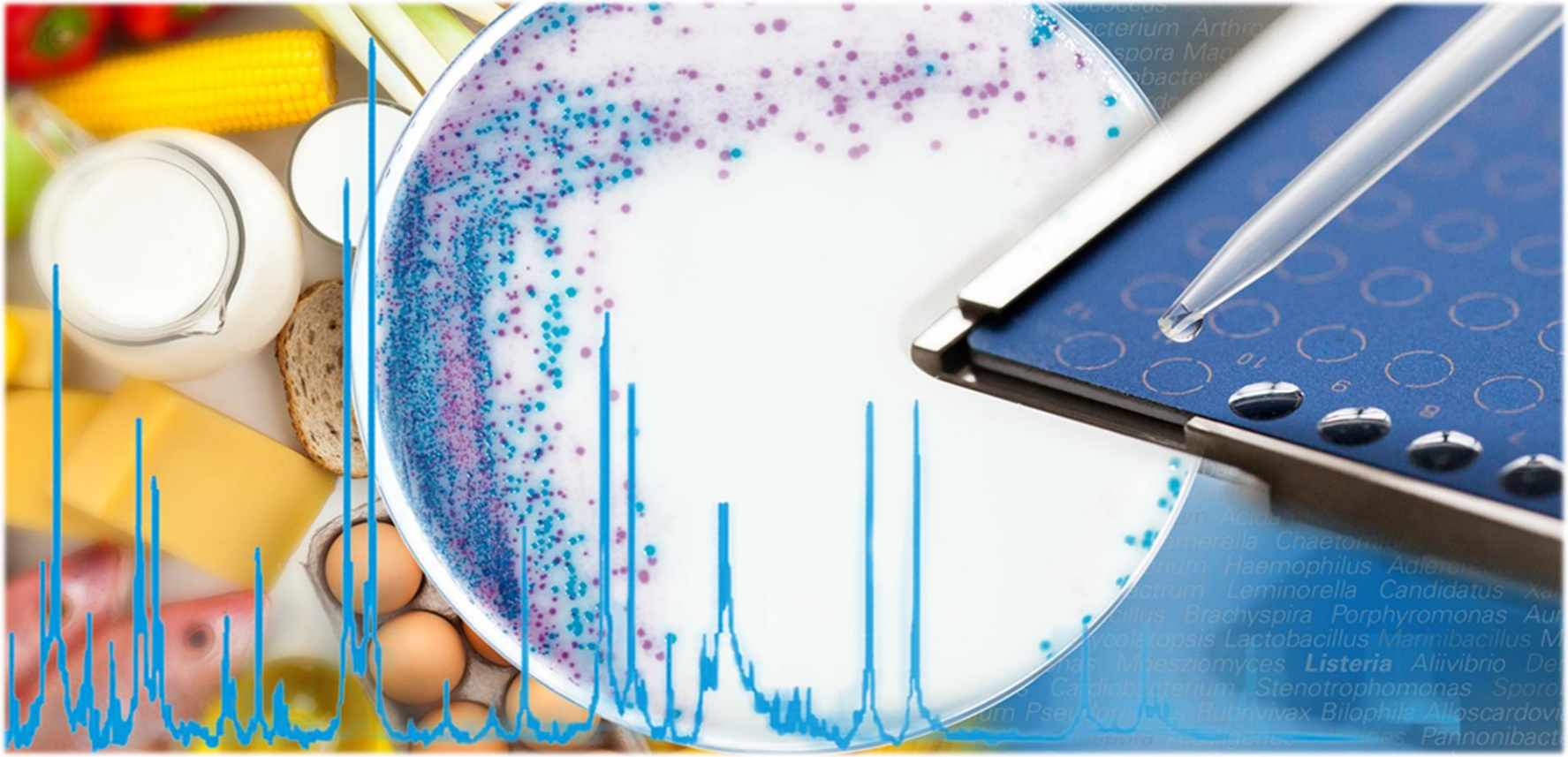
MALDI Biotyper – Changing Microbiology



4. Freiburger MALDI-Meeting – 25 Juni 2019

Dr. Gerold Schwarz - Sales and Support - Bruker Microbiology & Diagnostics Bremen

Bruker Corporation and MALDI Biotyper

- 1960 Foundation of the **Bruker** company
- 1992 Introduction **first MALDI-TOF** mass spectrometer
- 2004 Launch of **MALDI Biotyper** as a research tool
- 2008 First MALDI Biotyper in **routine laboratories**
- 2009 Launch of the MALDI Biotyper **IVD system**
- 2013 **FDA Clearance** of the MALDI Biotyper CA system
- 2017/18 **AOAC**-approvals, **ISO 16140**-part 6 validation

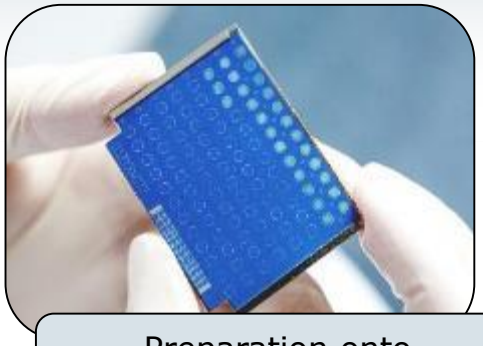
1988 ➔ MALDI technique by Karas M., Hillenkamp F. *Anal Chem. 1988 Oct 15; 60 (20): 2299-2301*

2002 ➔ Nobel Prize in chemistry (for soft ionization techniques)

MALDI Biotyper

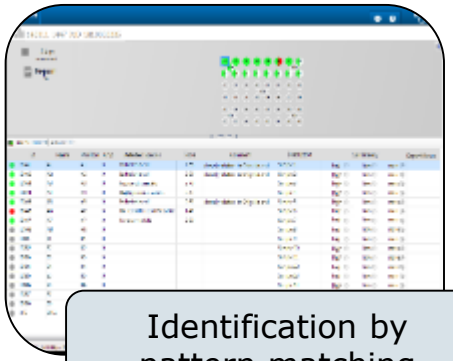
Microorganism Confirmation/Identification by MALDI-TOF MS: Workflow

Selection of colony of unknown microorganism



Preparation onto MALDI target plate

Acquisition of MALDI-TOF profile spectrum

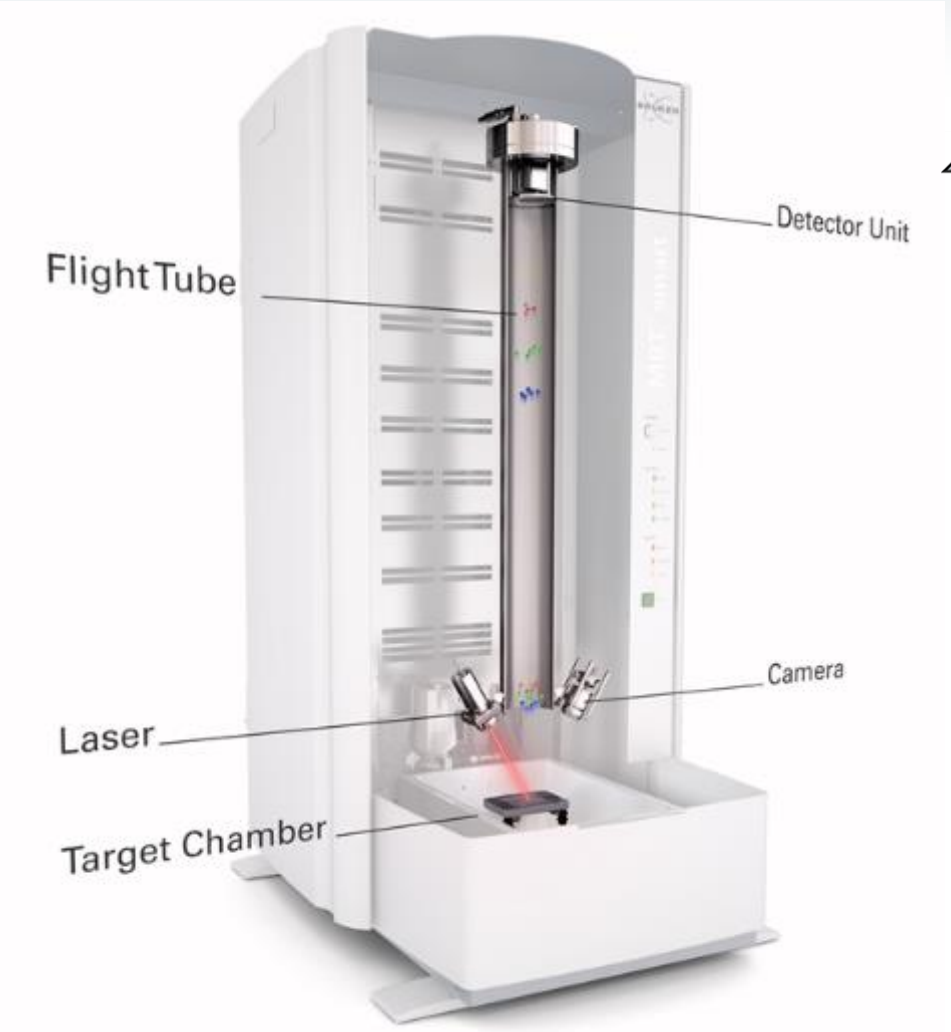


Identification by pattern matching

96 samples identified in ~60 minutes

MALDI Biotyper - Basics

Matrix Assisted Laser Desorption / Ionization Time Of Flight Mass Spectrometry

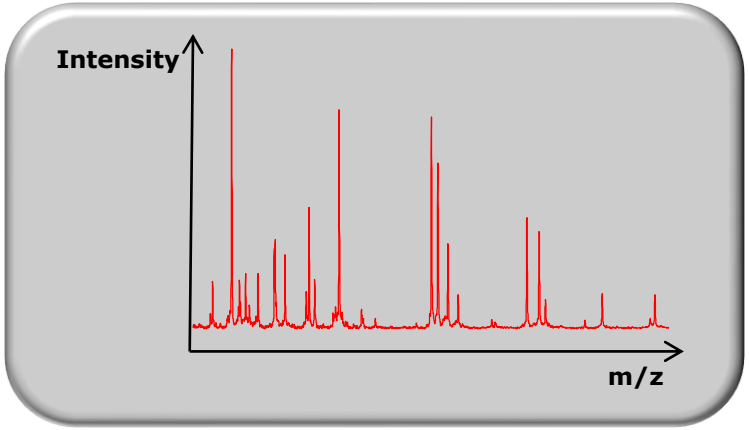


Time-of-Flight

$v = d/t$

$E_{kin} = mv^2/2$

m/z values



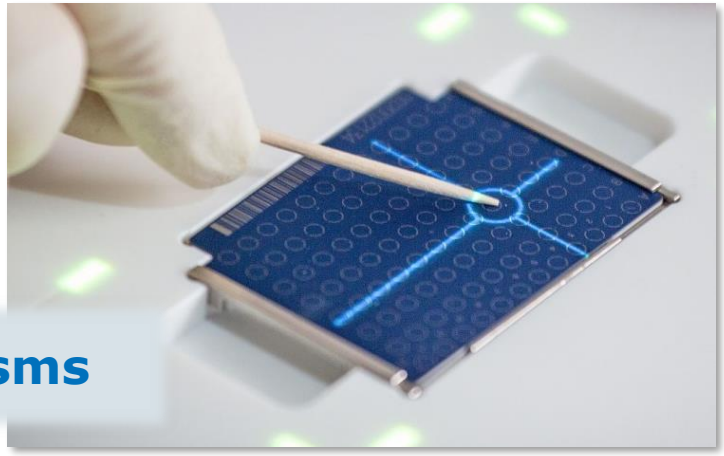
MALDI Biotyper
Sample preparation procedures

Direct transfer (for 90-95% of microorganisms)
- Direct transfer of biological material
- add MALDI Matrix
- Gram negative and most Gram positive microorganisms

Extended direct transfer (e.g. for yeast)
- Direct transfer of biological material
- Add drop of formic acid
- Dry at room temperature
- Add matrix

Extraction (for MSPs, better ID..)
- Enrich "targeted" molecules from isolated colonies

➔ Special protocols for special organisms



Direct transfer using a tooth pick

MALDI Biotyper

Robust pattern matching to MBT Compass Library

Updates on Libraries and Modules

MALDI Biotyper
Reference library

Bruker MBT Compass Library (version April 2019)

8468 MSPs
2950 different species

	MSP	Genus	Species
Gram -	3525	258	1266
Gram +	4070	210	1450
Yeast	806	45	210
Filamentous Fungi	67	27	43
∑	8468	540	2969

Every Microorganism could be of Relevance

Abiotrophia *defective* ***Acetobacter*** *aceti Acetobacter cerevisiae Acetob*
Achromobacter xylosoxidans ***Acidaminococcus*** *fermentans Acidaminoc*
Acinetobacter bouvetii Acinetobacter calcoaceticus Acinetobacter gerneri
Acinetobacter schindleri Acinetobacter sp Acinetobacter tandoii Acinetob
Actinobacillus ureae ***Actinobaculum*** *massiliense Actinobaculum schaalii*
denticolens Actinomyces europaeus Actinomyces funkei Actinomyces ge
Actinomyces nasicola Actinomyces neuii Actinomyces odontolyticus Actino
viscosus Actinomyces weissii ***Adlercreutzia*** *equolifaciens* ***Advenella*** *ince*
caviae Aeromonas encheleia Aeromonas enteropelogenes Aeromonas eucr
sobria Aeromonas sp[2] Aeromonas veronii ***Afipia*** *broomeae Afipia felis*
humatus Agromyces italicus Agromyces lapidis Agromyces mediolanus A
contaminans Alicyclobacillus cycloheptanicus Alicyclobacillus fastidiosus
vulcanalis ***Aliivibrio*** *fischeri* ***Alishewanella*** *fetalis* ***Alistipes*** *finegoldii Alis*
coloradensis Amycolatopsis fastidiosa Amycolatopsis japonica Amycolatop
Anaerobiospirillum *succiniciproducens* ***Anaerococcus*** *hydrogenalis An*
migulanus ***Aquincola*** *tertiaricarbonis* ***Arcanobacterium*** *canis Arcanobac*
nitrofigilis Arcobacter skirrowii ***Aromatoleum*** *alkani Aromatoleum anae*
Aromatoleum toluvorans ***Arsenicicoccus*** *bolidensis Arsenicicoccus derm*
citreus Arthrobacter creatinolyticus Arthrobacter crystallopoietes Arthrob
Arthrobacter monumenti Arthrobacter mysorens Arthrobacter nasiphocae A
protophormiae Arthrobacter psychrolactophilus Arthrobacter psychrophenc
tumbae Arthrobacter uratoxydans Arthrobacter ureafaciens Arthrobacter
Avibacterium gallinarum Avibacterium volantium ***Azoarcus*** *communis Az*
arsenicus Bacillus asahii Bacillus atrophaeus Bacillus azotoformans Bacillu
Bacillus cohnii Bacillus decolorationis Bacillus drentensis Bacillus endophy
hemicellulosilyticus Bacillus horikoshii Bacillus horneckiae Bacillus horti
Bacillus mannanilyticus Bacillus marisflavi Bacillus megaterium Bacillus m
Bacillus pseudofirmus Bacillus pseudomycoides Bacillus psychrosaccharo
Bacillus subterraneus Bacillus subtilis Bacillus thermoamylovorans Bacillu
coagulans Bacteroides coprocola Bacteroides coprophilus Bacteroides eg
plebeius Bacteroides pyogenes Bacteroides salyersiae Bacteroides sterco
angulatum Bifidobacterium animalis Bifidobacterium asteroids Bifidobacte
longum Bifidobacterium magnum Bifidobacterium merycicum Bifidobac

MALDI Biotyper

Libraries new entries ....

ECCMID 2019:

- ➔ **Standard 9.0 8468 MSPs** (8326 IVD)
- ➔ **Mycobacteria 6.0** – (952 MSPs + IVD identical)
- ➔ **Filamentous Fungi 3.0** (new - 577 MSPs)
- ➔ SR library (IVD extension - BBFV)
- ➔ Third claim US-FDA (July 2017 - 424 bacteria)
- ➔ April 2018 US-FDA (candida auris added – 425 bacteria)
- ➔ Third parties (MALDI-Up, D-MASS, CDC-Microbenet, ..)

MBT Subtyping Module

One workflow - Two calculations

MBT Subtyping Module
Introduction

- Supporting now:

➢ *Bacteroides fragilis* typing for detection of carbapenem resistance

➢ *Staphylococcus aureus* typing for MRSA detection

2016

➢ *Listeria monocytogenes* typing to confirm species

➢ *Mycobacterium chimaera/intracellulare* differentiation

➢ bla_{KPC} plasmid peak detection in *Klebsiella pneumoniae*

2017

Expanding the application range of the MALDI Biotyper from identification to instant specific marker detection

MBT Subtyping Module

Listeria monocytogenes
species confirmation

MBT Subtyping Module
Listeria monocytogenes

- *Listeria monocytogenes* group members are closely related (*L. monocytogenes, L. ivanovii, L. innocua*, *L. welshimeri* and *L. seeligeri)*
- **Mainly *L. monocytogenes* is pathogenic for humans**
- *L. monocytogenes* is one of the most virulent foodborne pathogens, causing listeriosis
- The infection is most likely to sicken pregnant women and their newborns, adults aged 65 or older, and people with weakened immune systems

➢ **Rapid and accurate identification** of *Listeria* strains is essential for appropriate management and control of food safety

MBT Subtyping Module

Listeria monocytogenes

Result in MBT Compass: Report

- ***Listeria monocytogenes* typing to confirm species**

  → **directly** from direct transfer (and extended direct transfer)

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
0_E1 (+++) (A)	15015734-1 (LM) TSA_ALOA 24 h Art EDT3 (standard)	Listeria monocytogenes typed as L. monocytogenes	2.30	Listeria monocytogenes	2.29
0_E2 (+++) (A)	15015734-1 (LM) TSA_ALOA 24 h Art EDT4 (standard)	Listeria monocytogenes typed as L. monocytogenes	2.31	Listeria monocytogenes	2.30

ID performed on DT => ID > 2.0 => Subtyping performed

NO additional steps required

MBT Subtyping Module

bla$_{KPC}$ plasmid detection in *K. pneumoniae*

Result in MBT Compass: Report

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
0_A7 (+++) (A)	KP-M-93 chrom (standard)	Klebsiella pneumoniae presumptive KPC	2.47	Klebsiella pneumoniae	2.38
0_A8 (+++) (A)	KP-M-94 chrom (standard)	Klebsiella pneumoniae presumptive KPC	2.49	Klebsiella pneumoniae	2.40
0_A9 (+++) (A)	KP-M-94 chrom (standard)	Klebsiella pneumoniae presumptive KPC	2.48	Klebsiella pneumoniae	2.37

Subtyping Result	Comment
KPC	classification as KPC on the basis of specific marker peaks (11109)

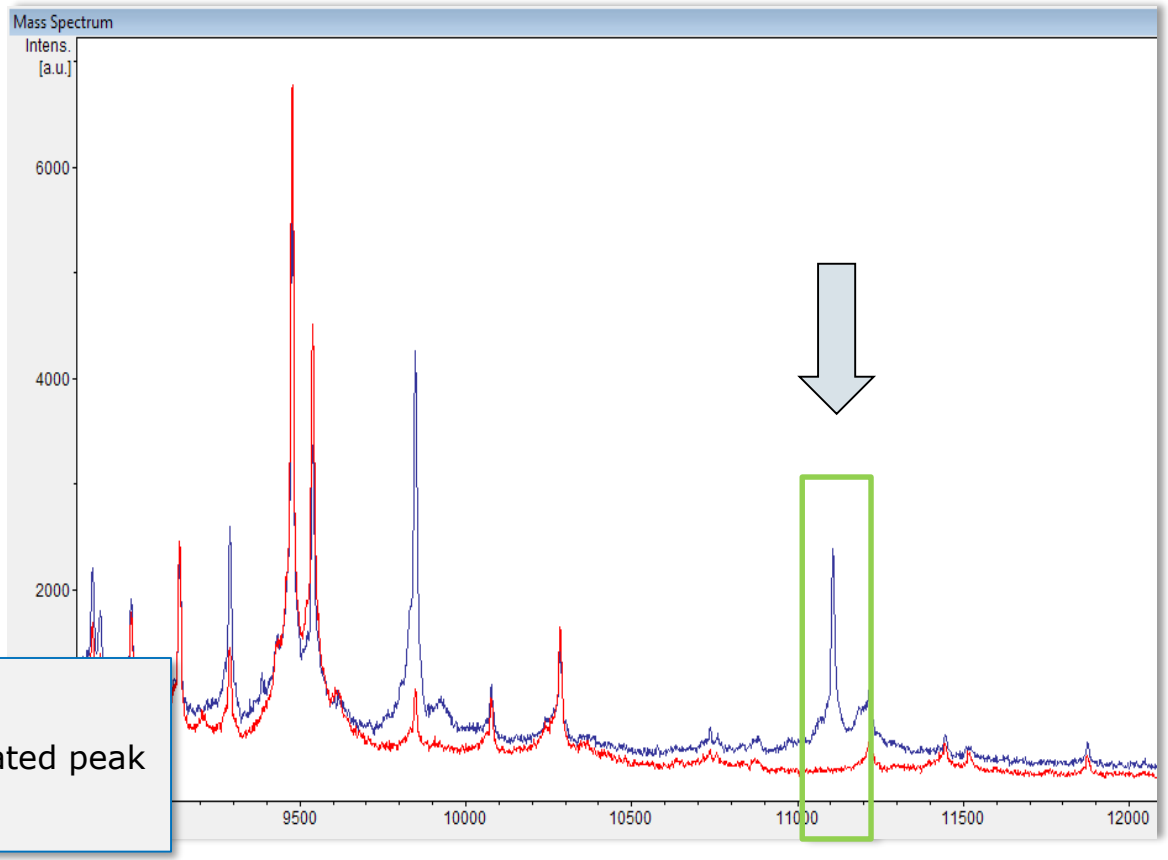
ID performed on DT => ID > 2.0 => Subtyping performed

NO additional steps required

MBT Subtyping Module
bla$_{KPC}$ plasmid detection in *K. pneumoniae*

bla$_{KPC}$ plasmid peak detection at m/z 11,109

- Good detection rate
- Very specific peak



Klebsiella pneumoniae:

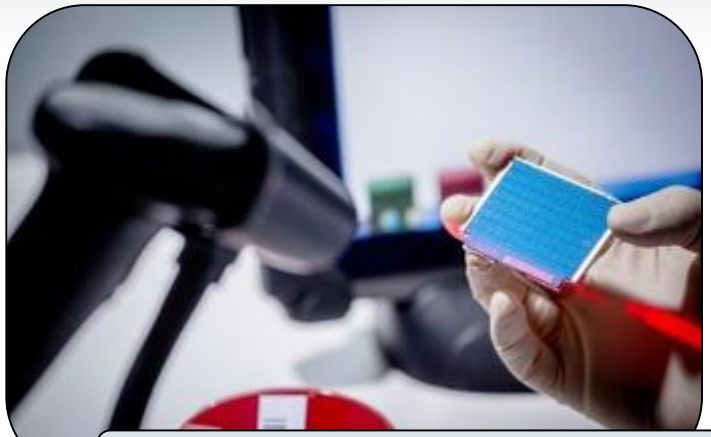
- BLUE: KPC with *bla*$_{KPC}$ related peak
- RED: ESBL

Automation

MBT Pilot and Galaxy

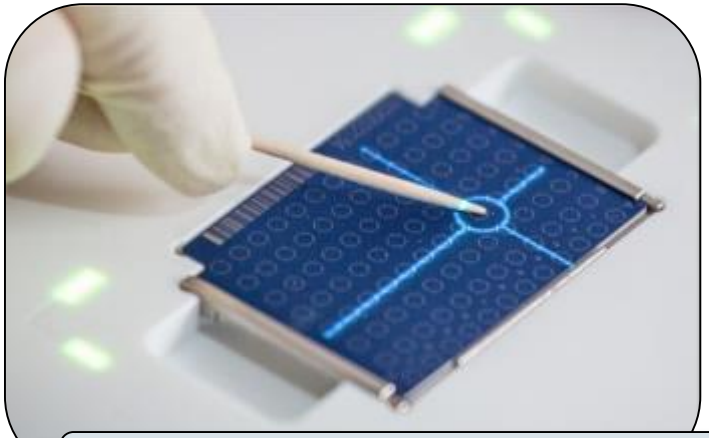
MALDI Biotyper

Paperless and traceable workflow



Define worklist by barcode

Include samples into worklist



Prepare samples; add matrix

Load worklist by barcode

MALDI Biotyper

Paperless and traceable workflow
MBT Satellite software

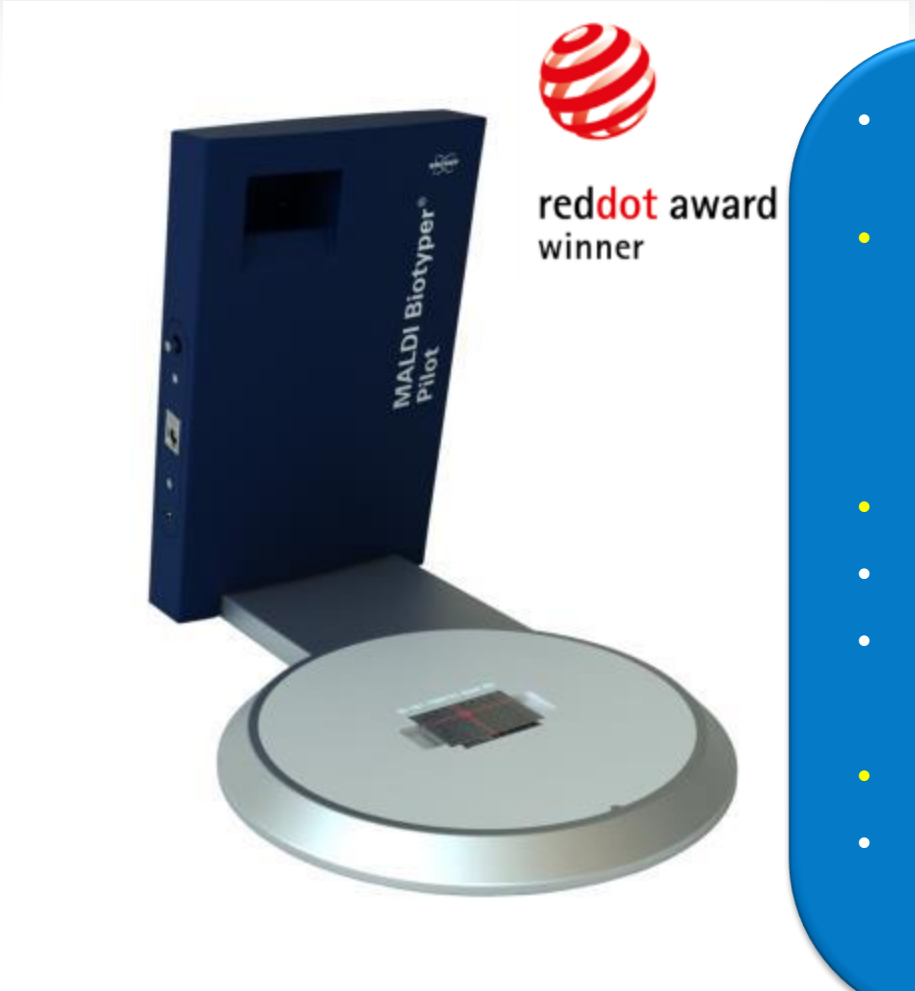
For convenient sample prep on the work benches:

- Software module for project creation and set-up of corresponding working lists

- Also optionally available pre-installed on a tablet-PC (Display # 9.7")

MBT Pilot

Traceability, safety and confidence



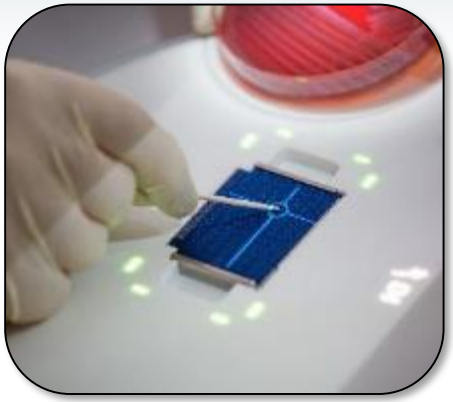
- Complements the MBT Satellite software to a **barcoded and paperless workflow**
- **Micro-projection technology**
  - For optically guided preparation
  - No laser reflection
  - Patent pending
- **Cross hairs** to indicate next position
- Preparation spot **not covered**: no glare
- Process is fully **traceable**

- **Ergonomic**
- Tool for improved workflow and quality control

MBT Pilot
Guided Target Preparation

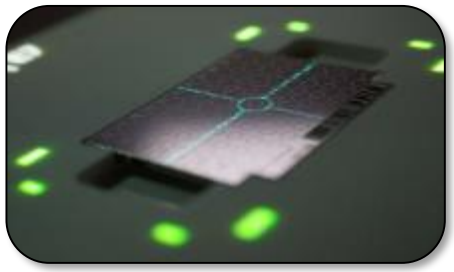
1. Scan barcode of MALDI target

2. Insert target into MBT Pilot target holder, the next free position is indicated on the target



3. Apply sample material at indicated position

4. Scan sample barcode

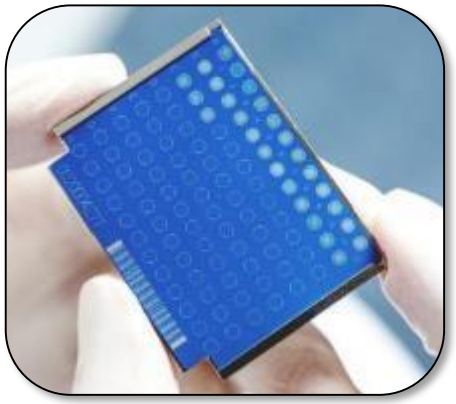


5. Prepare additional samples by repeating steps 3 and 4

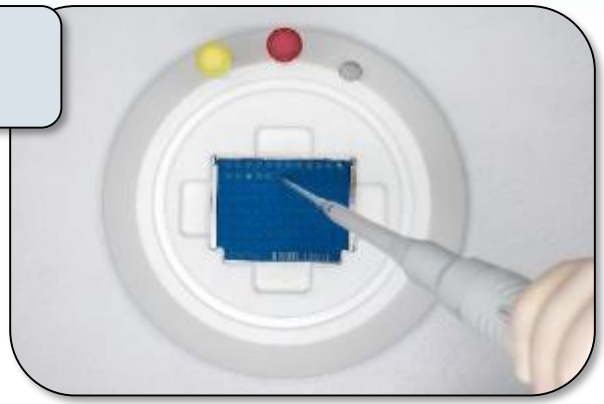
MALDI Biotyper

Subsequent sample preparation steps

After transfer of all samples to the MALDI target



Add FA/matrix manually



OR

Add FA/matrix automatically by **MBT Galaxy**!

MBT Galaxy

For automated application of matrix and formic acid

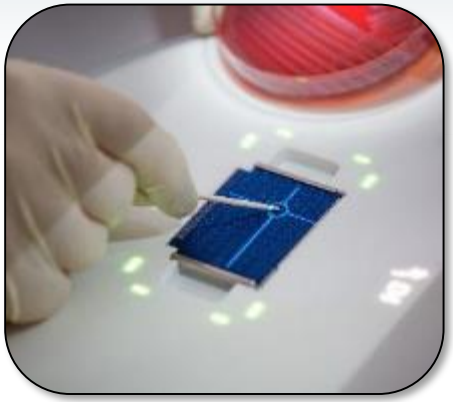
- **Frees time for laboratory personnel**
- **Ensures highest preparation quality**
- **Improves traceability**
- **Less waste**

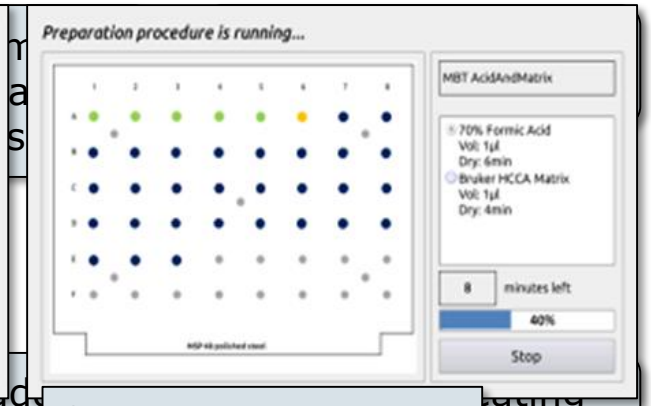
- Frees laboratory personnel from pipetting
- Onboard barcode reading automatically loads the work list from MBT server
- Automated formic acid / matrix preparation
- Contact free droplet application
  - ✓ eliminates carry-over
  - ✓ no pipette tips → less waste
- Ensures highest preparation quality by control of:
  - Previous matrix preparation on the spot
  - Droplet volume
  - Drying atmosphere

- Improves traceability in a paperless workflow

MBT Pilot and MBT Galaxy

Combined workflow





1. Press Start

→ Insert target

2. Select preparation

→ Target identification
→ Spotlist incl. BTS submitted by MBT Server

3. Target preparation

MALDI Biotyper
Combined workflows - other modules

- Fungi Library 3.0 and MBT filamentus Fungi Module
- Mycobacteria 6.0 and MBT Mycobacteria Module
- Subtyping Module
- Food Report Option (AOAC, MicroVal, Confirmation)
- Sepsityper Module (direct blood culture analysis)
- STAR BL Module (Selective Testing of Antibiotic Resistence)
- IVD- mode, RUO- mode, GP (industrial microbiology),

MALDI Biotyper
Automation..

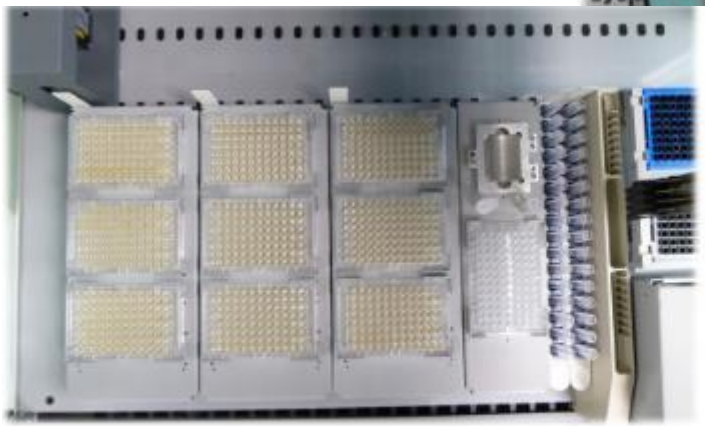
Automation

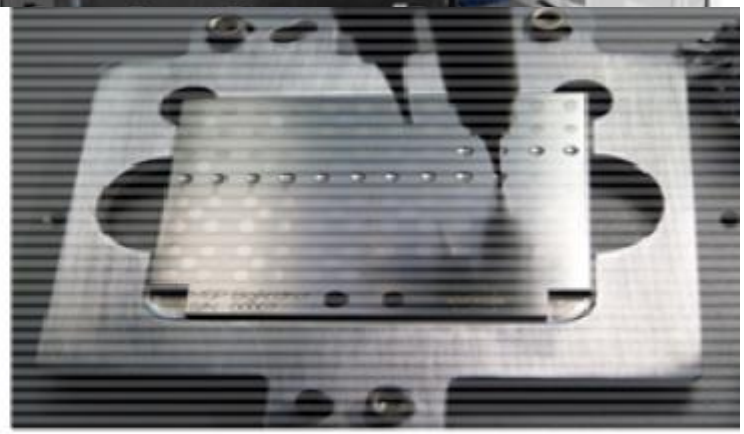
Colony picker, robotic..

MALDI Biotyper
Micronaut ASTroid

MICRONAUT ASTroID

Workflow MICRONAUT / MALDI-TOF fully automated





Inoculation of MICRONAUT MIC plates and MALDI-TOF targets from a uniform bacteria suspension

MALDI Biotyper
BD Kiestra Lab Automation

IDENTIFICATION | MALDITOFA

Automatic Colony Picking

Transferring colonies to a MaldiTOF target plate is both labour intensive and error sensitive. It is now possible to automate this vital process with the MalditofA, which helps you to improve quality and efficiency of the identification step. The automatic preparation of the target plate will ensure that the right amount of sample material and the right amount of matrix and acid fluids will be added for direct and extended-direct transfer.

Automatic Suspension preparation

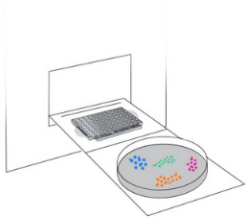
The system will automatically prepare a suspension fluid for AST with a desirable McFarland concentration. An integrated density meter will ensure the right concentration is prepared. The prepared AST suspension tubes can be placed in the InoculA for automatic inoculation and spreading. This will result in a perfect and fast prepared confluent spread.

MALDI Biotyper
MALDI Colonyst..

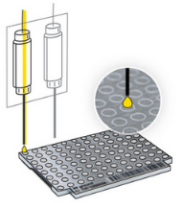
BIOTECON Diagnostics

MALDI Colonyst®

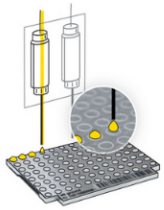
MALDI Biotyper
MALDI Colonyst..



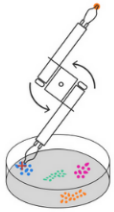
MALDI target and Petri dish inserted for transfer of colonies



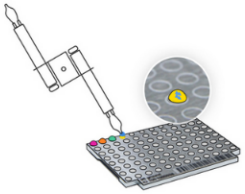
Pre-deposition of formic acid using liquid deposition needle



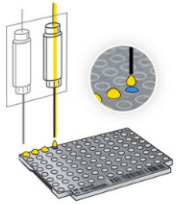
Formic acid deposited only on preselected positions



Each selected colony precisely picked...



...and smoothly deposited into formic acid droplet



After drying of all colonies, MALDI matrix is deposited

with feeder

MALDI Biotyper
Latest News

Bruker Launches MALDI Biotyper Sirius at ASM Microbe Conference

… new MALDI Biotyper *sirius*™ system with additional negative-ion mode to support MALDI research and RUO validation studies, e.g. for colistin-resistance testing

US introduction of Micronaut™ products for true minimum inhibitory concentration (MIC) antibiotic susceptibility testing (AST) in veterinary medicine

US introduction of RUO versions of MALDI Biotyper-based rapid Selective-Testing of Antibiotic Resistance (MBT-STAR™) assays for validation studies on carbapenem resistance and cephalosporine resistance

MALDI Biotyper
Latest News

The new RUO colistin-resistance assay uses lipid analysis in negative-ion mode, and has been developed at Imperial College London, UK

Larrouy-Maumus et al., presented at ECCMID 2019, (submitted for publication).

Article | OPEN | Published: 15 November 2018

Rapid detection of colistin resistance in *Acinetobacter baumannii* using MALDI-TOF-based lipidomics on intact bacteria

Laurent Dortet, Anais Potron, Rémy A. Bonnin, Patrick Plesiat, Thierry Naas, Alain Filloux & Gerald Larrouy-Maumus

Scientific Reports **8**, Article number: 16910 (2018) | Download Citation

MALDI Biotyper
Official Method of Analysis by AOAC International

- The MBT has been certified according to the

  ***Official Method of Analysis* program (OMA) of the AOAC International**

  for the **confirmation and identification of:**

  - ✓ ***Salmonella* spp.**
    ***Cronobacter* spp.**
    ***Campylobacter* spp.**
    and other gram-negative bacteria
  - ✓ ***Listeria* spp.**
    Listeria monocytogenes
    and other gram-positive bacteria

First Action AOAC Official Method^{SM} 2017.09

First Action AOAC Official Method^{SM} 2017.10

MALDI Biotyper
ISO 16140-Part 6 Certification by MicroVal

- The MBT is the very **FIRST and ONLY** confirmation method certified by MicroVal according to the NEW **ISO 16140-part 6 standard**

for the **confirmation of:**

- ✓ ***Cronobacter* spp.**
- ✓ ***Salmonella* spp.**
- ✓ ***Campylobacter* spp.**
- ✓ ***Listeria spp. and Listeria monocytogenes***

Certificate N° 2017LR72

Certificate N° 2017LR73

Certificate N° 2017LR74

Certificate N° 2017LR75

Thank you for your attention!

Dr. Gerold Schwarz
Applications Support Team Bremen

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Bild: Bruker Daltonik GmbH Bremen