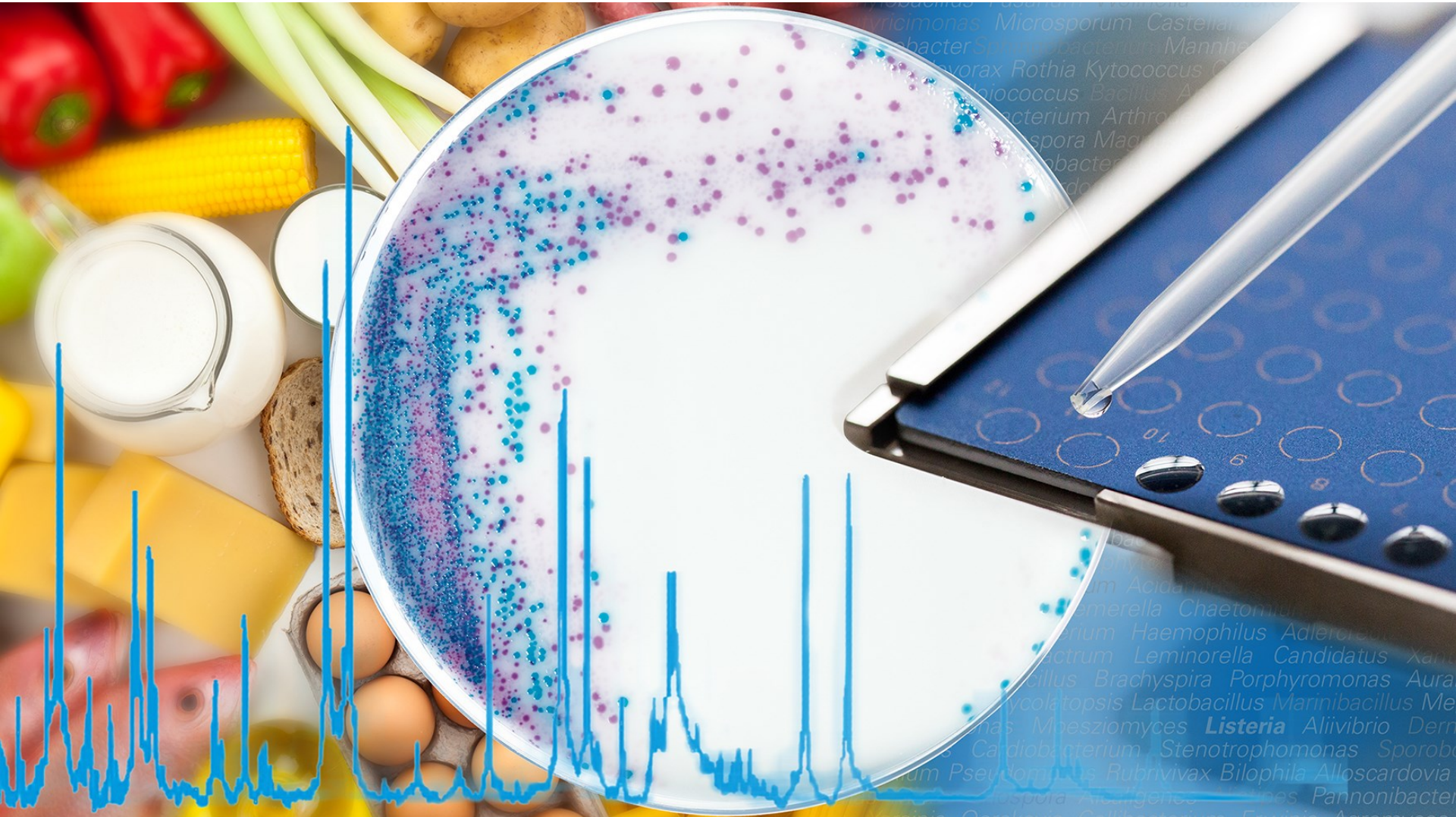
MALDI Biotyper

Tömegspektrometria a mikrobiológiában

Pál Róbert

Flextra-Lab Kft.



Bruker Today
Global Corporation

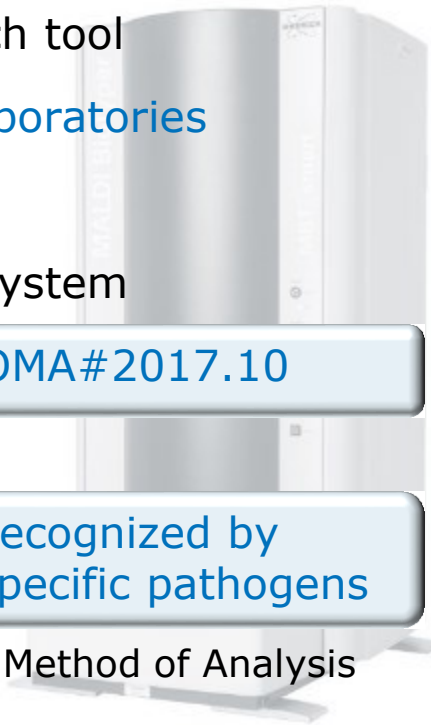
- Worldwide sales and service network with over 6.000 employees
- Global market leadership in many segments

Bruker Corporation
Élelmiszeripari analitikai alkalmazások

- **Bruker offers solutions for**
  - A nyersanyagok azonosítása (FT-IR, FT-NIR and handheld Raman)
  - Elem összetétel elemzés (FT-NIR and XRF)
  - Olaj és zsír jellemzése (FT-NIR and TD-NMR)
  - Csomagoló anyagok jellemzése (FT-IR, XRF, LC/MS and GC/MS)
  - Eltarthatósági vizsgálat (EPR)
  - A szennyezőanyagok és a maradékanyagok gyors vizsgálata (LC/MS and GC/MS)
  - Élelmiszer-hitelesség és az élelmiszer-hamisítás (FT-NMR FoodScreener)
  - A kórokozók és a higiéniai indikátorok gyors azonosítása és nyomon követése (**MALDI Biotyper** and IR Biotyper)
  - Technológiai törzsek és mikrobiális spoilerek ellenőrzése, elemzése (**MALDI Biotyper** and IR Biotyper)

MALDI Biotyper
Timeline

- 1960 Foundation of the Bruker company
- 1988 Establishment of the mass spectrometry business in Bremen, Germany
- 1991 Introduction of the first MALDI-TOF mass spectrometer
- 2004 Launch of the MALDI Biotyper as a research tool
- 2008 First MALDI Biotyper systems in routine laboratories
- 2009 Launch of the MALDI Biotyper IVD system
- 2013 FDA Clearance of the MALDI Biotyper CA system
- 12/2017 AOAC-OMA* approvals, OMA#2017.09 & OMA#2017.10
- 4/2018 > 2,900 MALDI Biotyper systems sold
- 2/2018 ISO 16140-part 6 validation by MicroVal, recognized by the EU 2073 as an alternative to confirm specific pathogens



*AOAC-OMA: American Organisation of Analytical Chemists - Official Method of Analysis

MALDI Biotyper
Általános jellemzők

- **Sebesség => A patogének és más baktériumok (élesztők) nagyon gyors azonosítása**
- **Rendkívül pontos azonosítási eredmények, összehasonlíthatóak a szekvenálással => Megbízható**
- **Könnyű használat => Gyors és egyszerű betanítás**
- **Ugyanaz a munkafolyamat és fogyóeszköz minden baktérium és élesztő számára**
- **Kisebb költség mintánként, mint a hagyományos módszerek**
- **Rendszeres könyvtárfrissítések => Az azonosítási teljesítmény folyamatos bővítése**
- **Saját hivatkozási könyvtárak létrehozásának lehetősége => Rugalmas**

MALDI Biotyper System
Élelmiszer mikrobiológiai alapegységek

MALDI Biotyper Mass Spectrometer
- Benchtop instrument
- Unattended Operation

Dedicated MBT Compass Software for
- Automated data acquisition
- Automated data processing
- Automated pattern matching
- Report creation

MBT Compass Reference Library
- 2,748 species entries
- Continuously maintained & updated

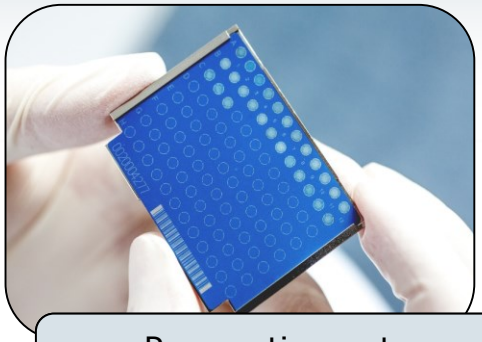
MBT Subtyping Module
- Fast & easy *Listeria* species differentiation
- Resistance marker detection

Consumables
- Bruker HCCA Matrix
- Bruker Bacterial Test Standard
- MBT Biotarget 96

MALDI Biotyper

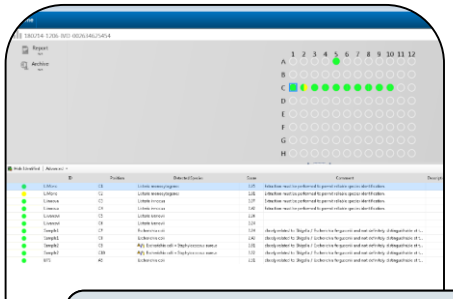
Microorganism Confirmation/Identification by MALDI-TOF MS: Workflow

Selection of colony of unknown microorganism



Preparation onto MALDI target plate

Acquisition of MALDI-TOF profile spectrum

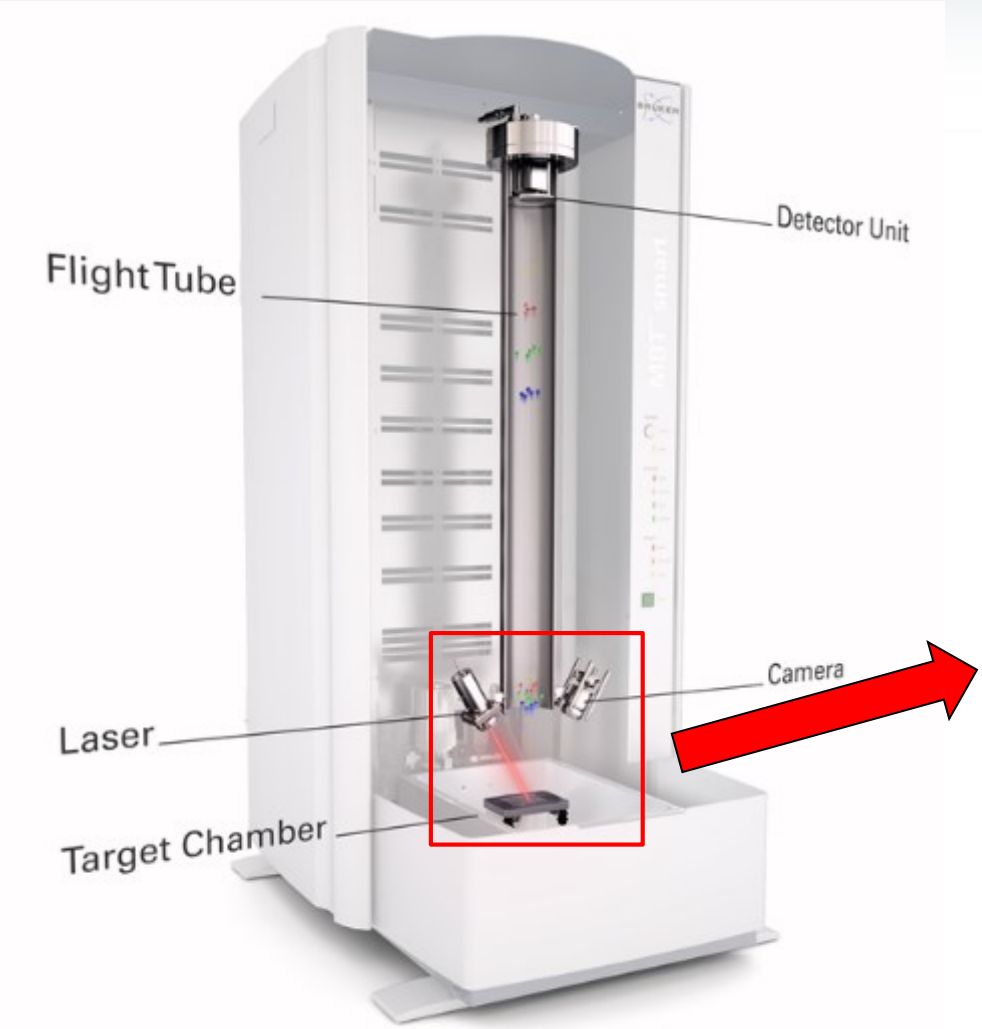


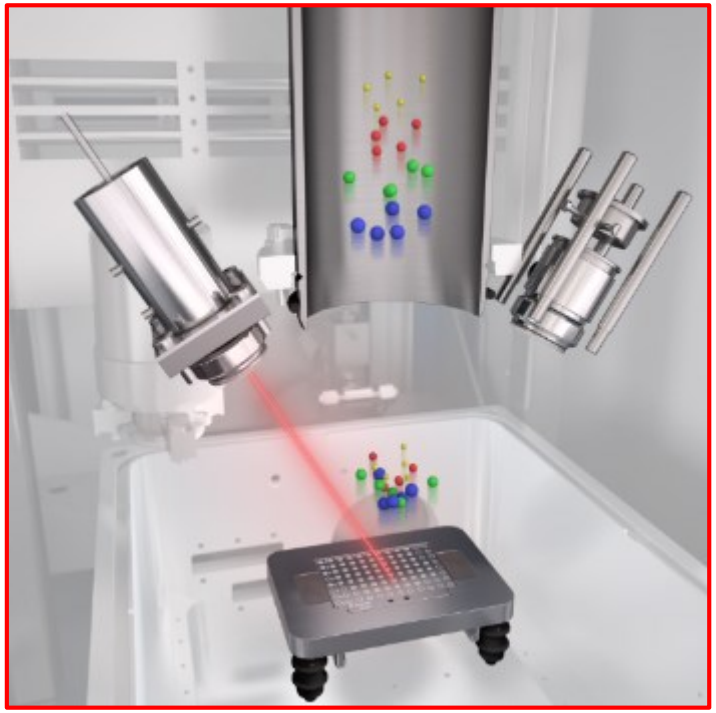
Identification by pattern matching

96 samples identified in ~60 minutes

MALDI Biotyper - Basics

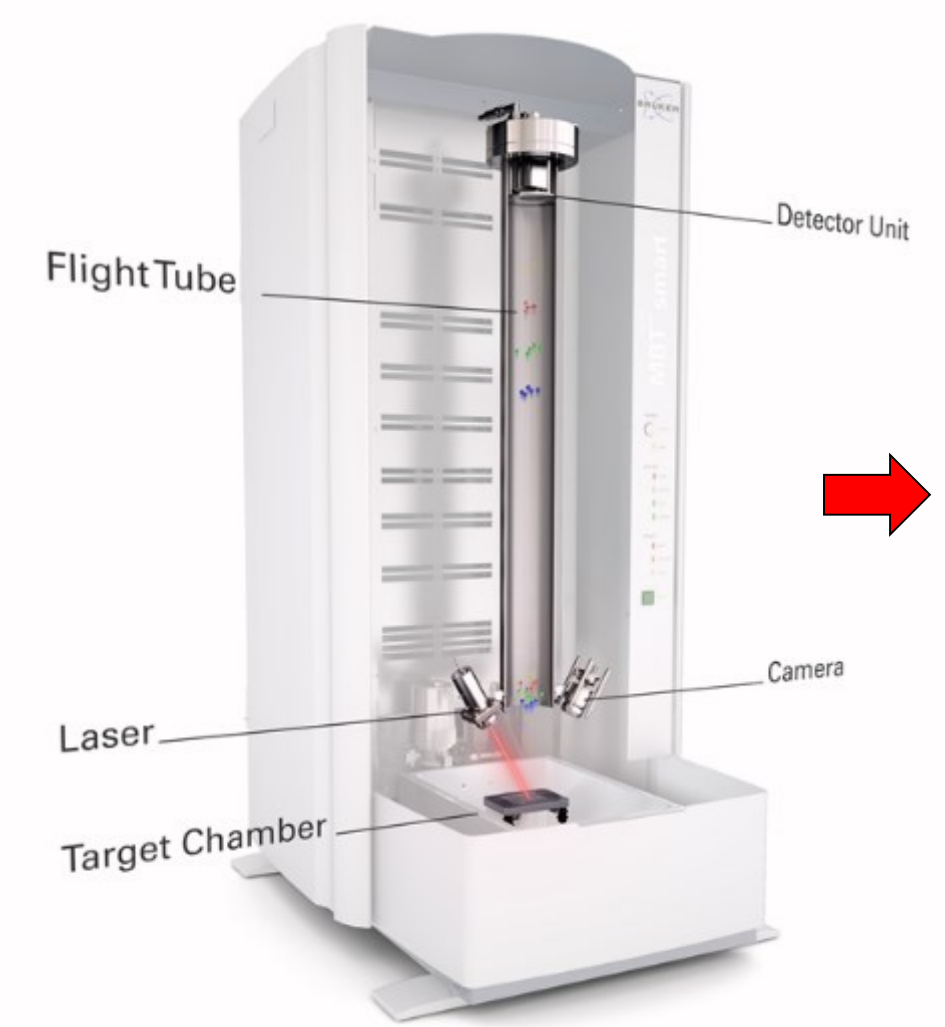
Matrix Assisted Laser Desorption / Ionization Time Of Flight Mass Spectrometry

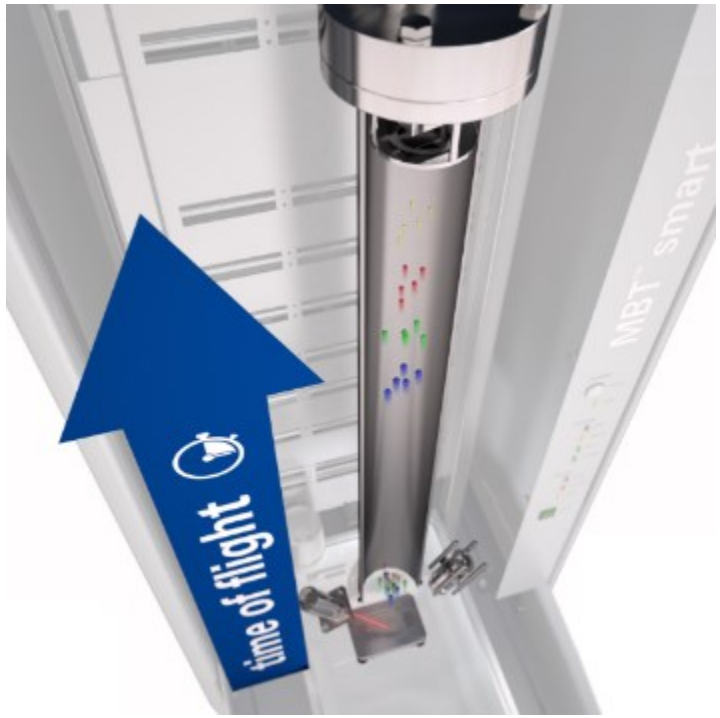




MALDI Biotyper - Basics

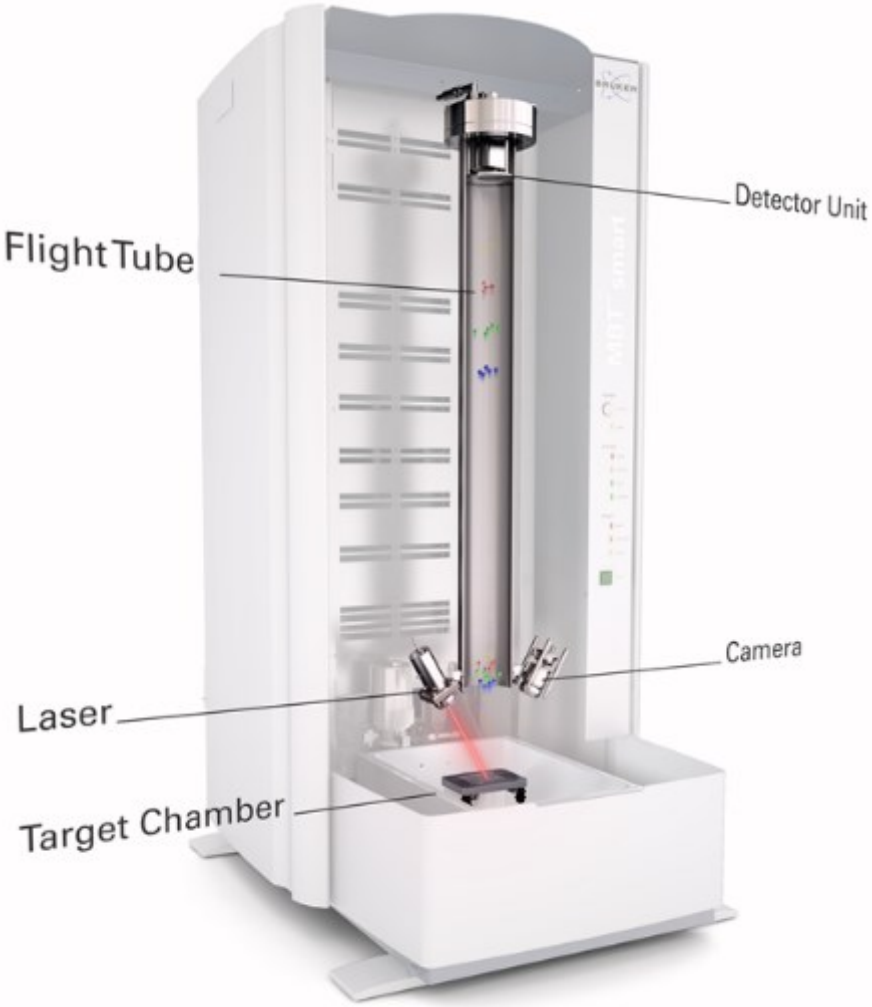
Matrix Assisted Laser Desorption / Ionization Time Of Flight Mass Spectrometry

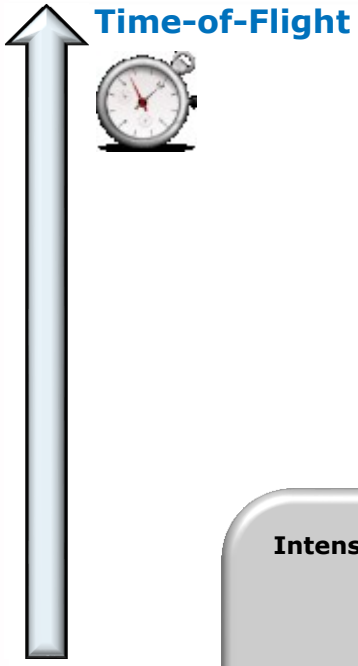




MALDI Biotyper - Basics

Matrix Assisted Laser Desorption / Ionization Time Of Flight Mass Spectrometry

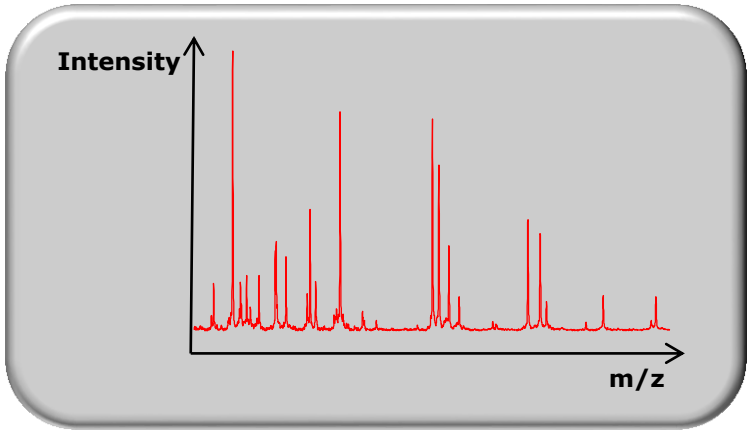




$v = d/t$

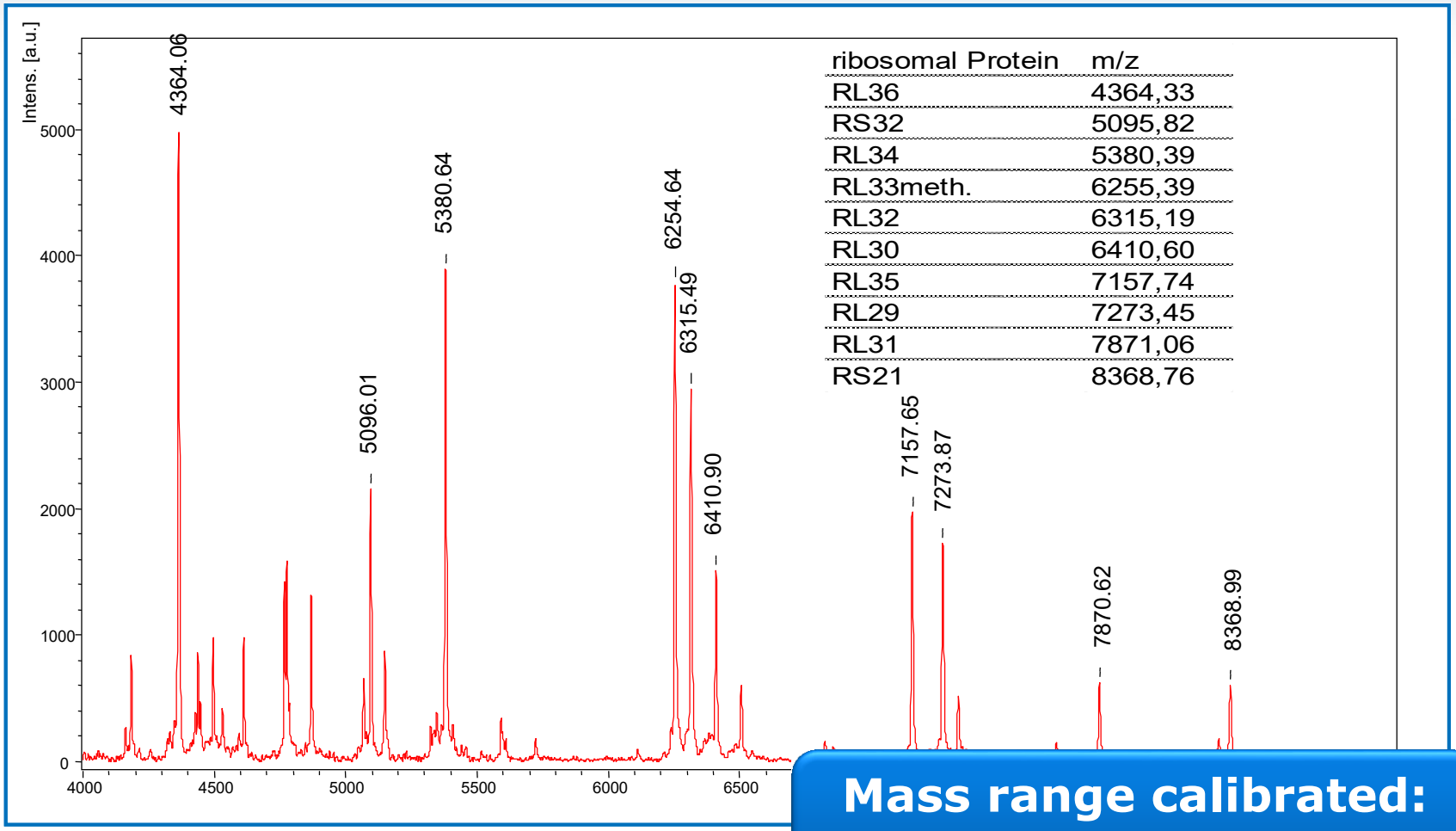
$E_{kin} = mv^2/2$

m/z values



MALDI Biotyper - Basics

Robust identification method, as it relies on highly abundant proteins



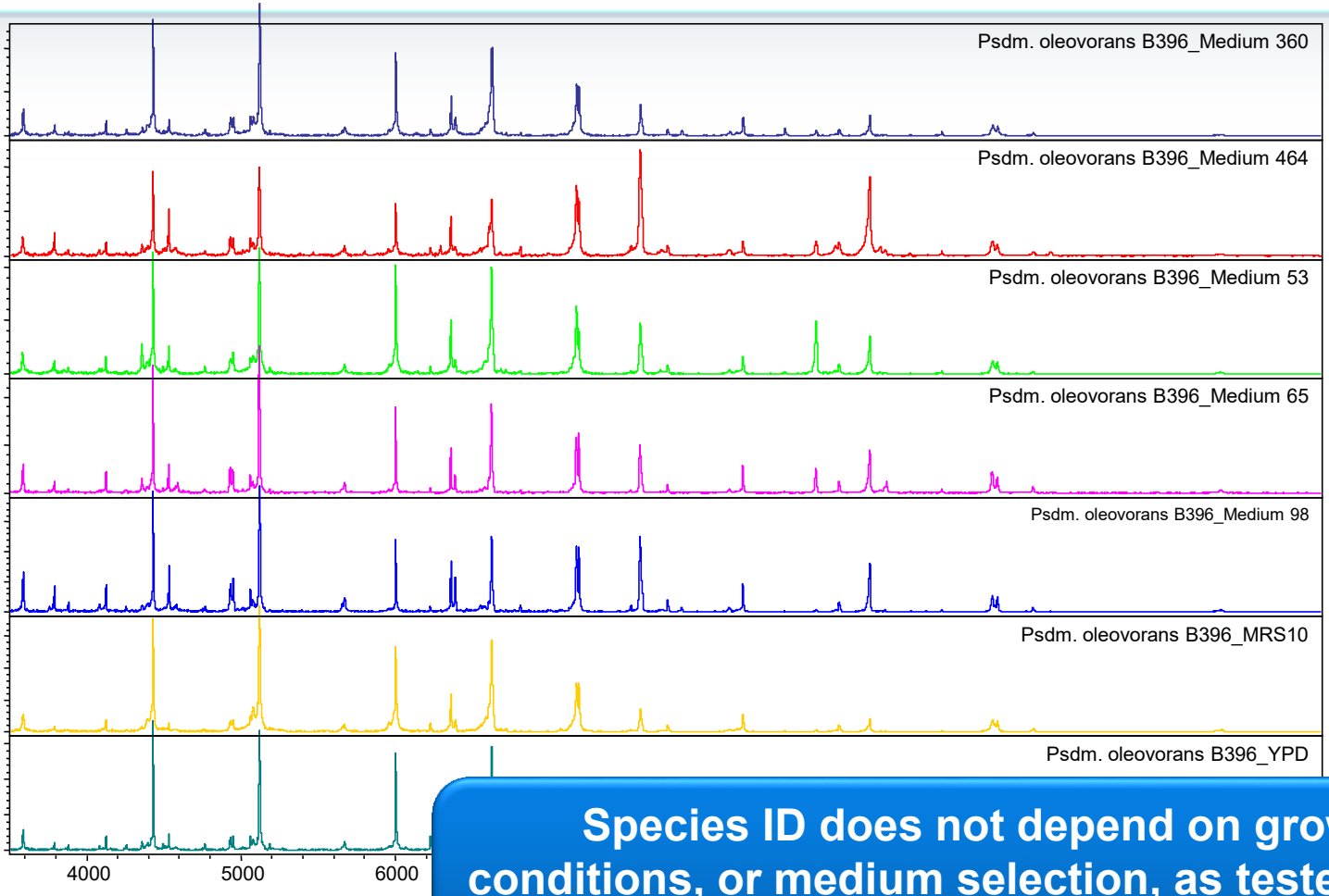
ribosomal Protein	m/z
RL36	4364,33
RS32	5095,82
RL34	5380,39
RL33meth.	6255,39
RL32	6315,19
RL30	6410,60
RL35	7157,74
RL29	7273,45
RL31	7871,06
RS21	8368,76

E. coli

Mass range calibrated: 2,000 – 20,000 Da

MALDI Biotyper

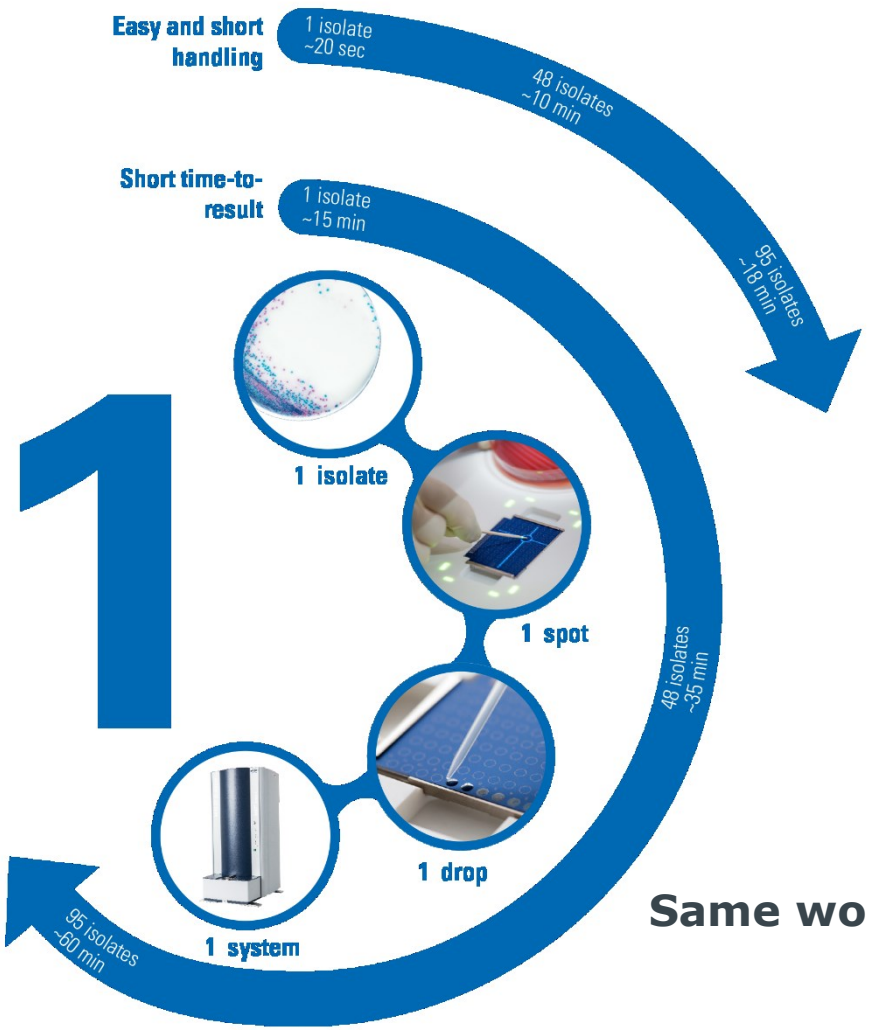
Workflow independent from culture medium



Pseudomonas o

Species ID does not depend on growth conditions, or medium selection, as tested during the certification studies presented later

MALDI Biotyper
1 System – 1 Workflow: summary



Short time-to-result, including

- Sample transfer
- Bacterial Test Standard for QC
- Drying of the HCCA matrix
- Mass spectrum acquisition
- Spectra matching

N° of isolates	TTR
1 isolate	~ 15 min
48 isolates	~ 35 min
95 isolates	~ 60 min

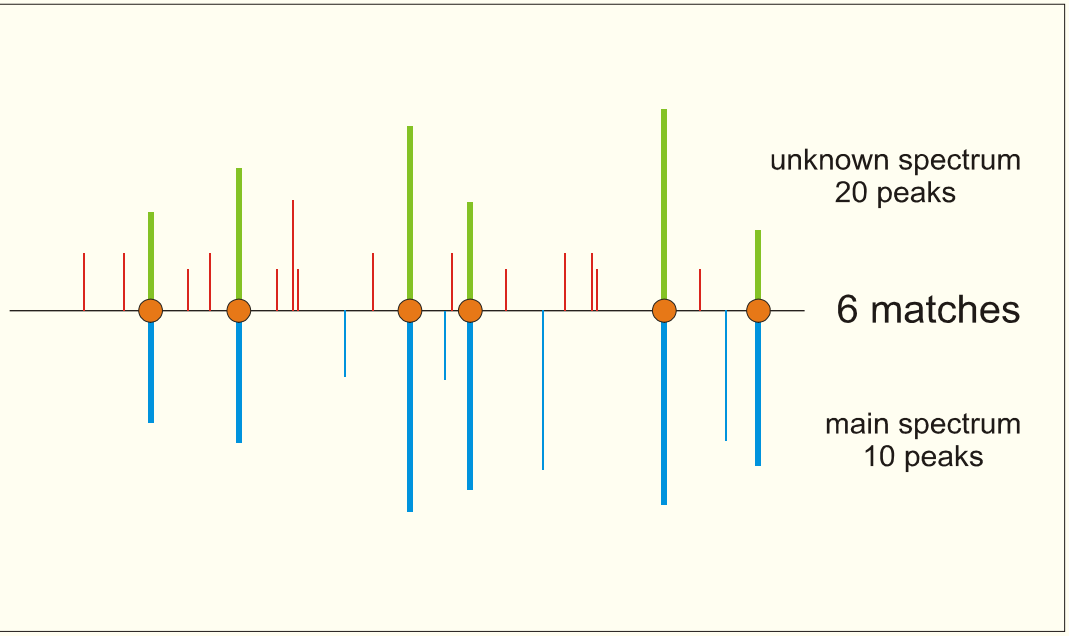
Same workflow for bacteria, yeasts and molds

5 to 10% alternative sample prep methods,
i.e. Extended Direct Transfer or Extraction method

MALDI Biotyper - Bioinformatics

Unsupervised Main Spectra (MSP) concept

Score base pattern matching



Unknown microorganism is matched against each Main Spectrum in the library

Calculation of a matching score based on:

Matches MSP to unknown

% matches of the reference spectrum (e.g. 6/10)

Matches unknown to MSP

% matches of the unknown spectrum (e.g. 6/20 = 3/10)

Correlation of intensities

value of intensity correlation

Range	Interpretation	Symbols	Color
2.000 - 3.000	High Confidence Identification	(+++)	green
1.700 - 1.999	Low Confidence Identification	(+)	yellow
0.000 - 1.699	No Organism Identification Possible	(-)	red

MALDI Biotyper

RUO Reference library

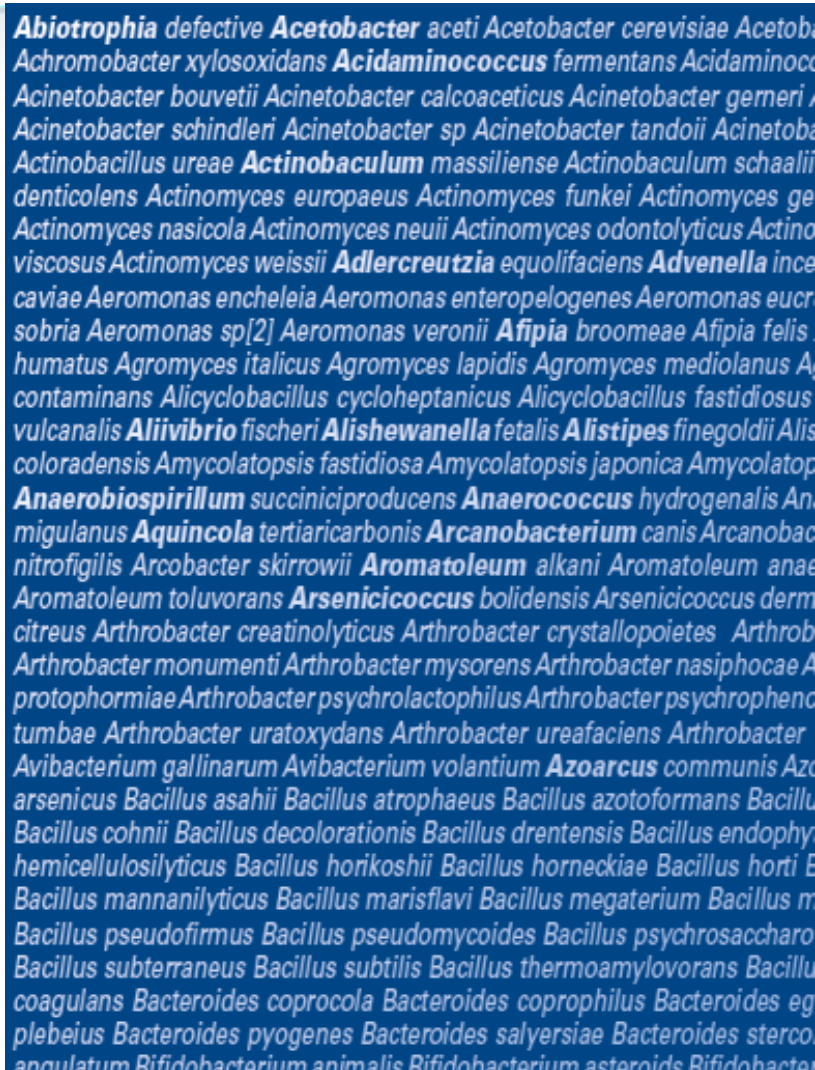
Every Microorganism could be of Relevance

Bruker MBT Compass Library: (version April 2018)

✓ 2,748 different species

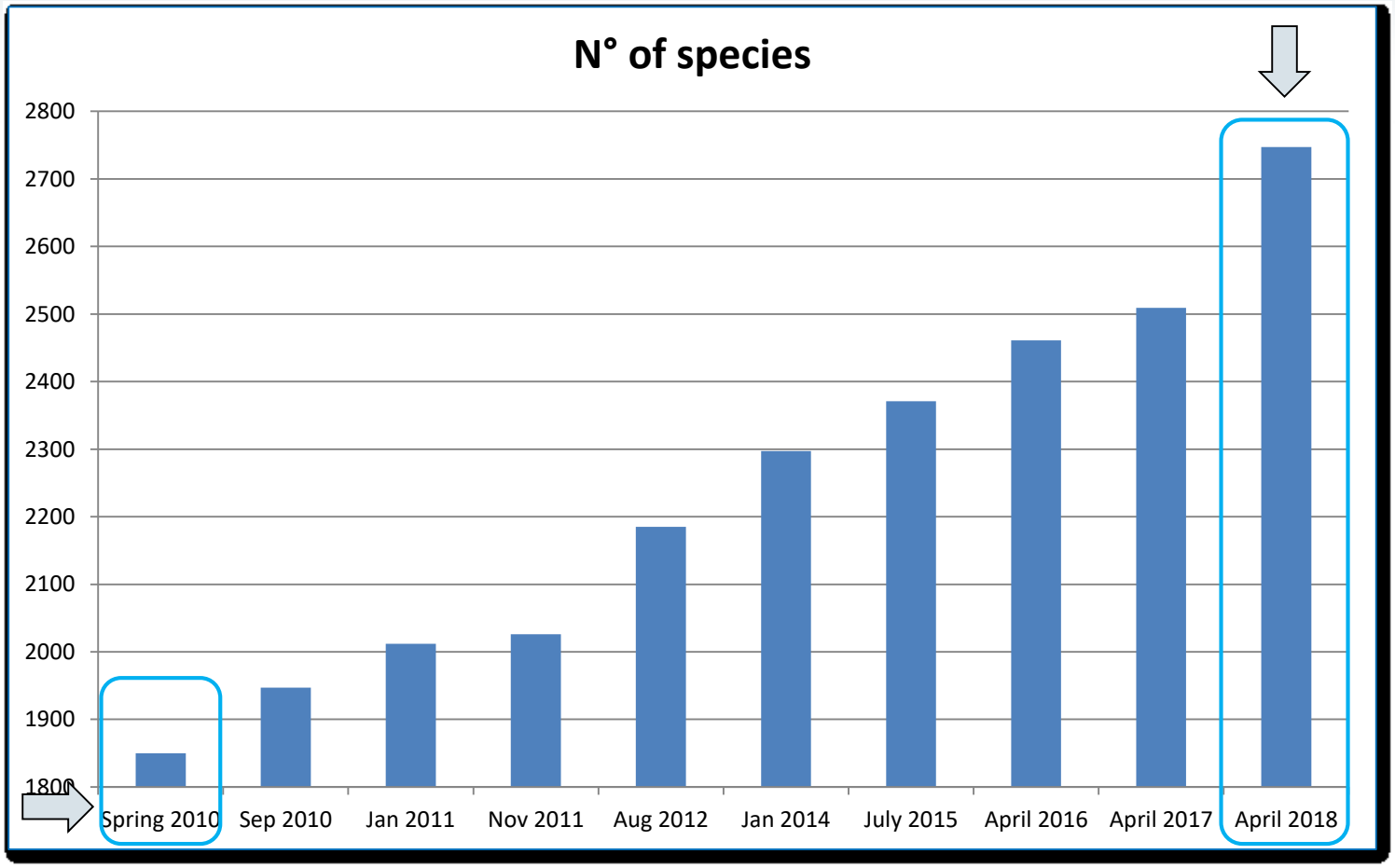
Including:

Salmonella spp.
Cronobacter spp.
Listeria spp.
Campylobacter spp.
...



MALDI Biotyper RUO library

Updates since 2010



Easy validation of library updates

MBT Filamentous Fungi Library

152 species / species groups

152 species / species groups

Absidia caerulea	*Aspergillus tamarii*	*Exophiala dermatitidis*
Absidia glauca	*Aspergillus terreus*	*Fusarium aquaeductuum*
Acaulium acremonium	*Aspergillus tritici*	*Fusarium aveanceum*
Alternaria alternata	*Aspergillus unguis*	*Fusarium cerealis_culmorum_group*
Arthrinium arundinis	*Aspergillus ustus*	*Fusarium chlamydosporum*
Arthrinium phaeospermum	*Aspergillus versicolor*	*Fusarium delphinoides*
Arthroderma amazonicum	*Aspergillus westerdijkiae*	*Fusarium dimerum*
Arthroderma eboreum	*Aureobasidium melanogenum*	*Fusarium equiseti*
Arthroderma flavescens	*Aureobasidium pullulans*	*Fusarium incarnatum*
Arthroderma gloriae	*Beauveria bassiana*	*Fusarium oxysporum*
Arthroderma lenticulare	*Botrytis cinerea*	*Fusarium petroliphilum*
Arthrographis kalrae	*Byssochlamys fulva*	*Fusarium proliferatum*
Aspergillus calidoustus	*Byssochlamys nivea*	*Fusarium solani*
Aspergillus clavatus	*Byssochlamys spectabilis*	*Fusarium* sp
Aspergillus flavus_oryzae_group	*Chaetomium globosum*	*Fusarium verticillioides*
Aspergillus fumigatus	*Chrysosporium keratinophilum*	*Isaria farinosa*
Aspergillus glaucus	*Chrysosporium shanxiense*	*Lasiodiplodia* sp
Aspergillus iizukae	*Cladosporium cladosporioides*	*Lichtheimia corymbifera*
Aspergillus japonicus	*Cladosporium herbarum*	*Lomentospora prolificans*
Aspergillus lentulus	*Cladosporium* sp	*Metarhizium marquandii*
Aspergillus minisclerotigenes	*Cladosporium sphaerospermum*	*Microascus melanosporus*
Aspergillus montevidensis	*Coniochaeta hoffmannii*	*Microsporum canis*
Aspergillus nidulans	*Coniochaeta mutabilis*	*Monascus ruber*
Aspergillus niger	*Cunninghamella elegans*	*Monilinia laxa*
Aspergillus nomius	*Curvularia clavata*	*Mucor circinelloides*
Aspergillus ochraceus	*Curvularia lunata*	*Nannizzia fulva*
Aspergillus parasiticus	*Curvularia pallescens*	*Nannizzia gypsea*
Aspergillus penicillioides	*Curvularia verruculosa*	*Nannizzia incurvata*
Aspergillus pseudoglaucus	*Dichotomopilus funicola*	*Nannizzia persicolor*
Aspergillus pulvinus	*Didymella aurea*	*Nannizzia praecox*
Aspergillus sclerotiorum	*Epicoccum nigrum*	*Paraphyton cookei*
Aspergillus sp[4]	*Epicoccum sorghinum*	*Penicillium brevicompactum*
Aspergillus sydowii	*Epidermophyton floccosum*	*Penicillium chrysogenum*

152 species / species groups, for which contributions have been received from over 20 laboratories across 8 countries

152 species / species groups

Penicillium citrinum	*Penicillium verrucosum*	*Scytalidium* sp
Penicillium commune	*Phaeoacremonium cinereum*	*Sporothrix schenckii*
Penicillium corylophilum	*Phialemoniopsis curvata*	*Stachybotrys chartarum*
Penicillium crustosum	*Phoma herbarum*	*Syncephalastrum racemosum*
Penicillium digitatum	*Plectosphaerella cucumerina*	*Talaromyces pseudostromaticus*
Penicillium expansum	*Pseudogymnoascus pannorum*	*Talaromyces ruber*
Penicillium fellutanum	*Purpureocillium lilacinum*	*Talaromyces rugulosus*
Penicillium glabrum	*Rasamsonia argillacea*	*Talaromyces* sp
Penicillium italicum	*Rhizomucor pusillus*	*Thanatephorus cucumeris*
Penicillium menonorum	*Rhizopus microsporus*	*Trichoderma hamatum*
Penicillium nalgiovense	*Rhizopus oryzae*	*Trichoderma longibrachiatum*
Penicillium namyslowskii	*Rhizopus sexualis*	*Trichoderma orientale*
Penicillium olsonii	*Rhizopus stolonifer*	*Trichoderma* sp[3]
Penicillium onobense	*Sarocladium kiliense*	*Trichophyton benhamiae*
Penicillium oxalicum	*Scedosporium apiospermum*	*Trichophyton equinum*
Penicillium pimiteouse	*Scedosporium aurantiacum*	*Trichophyton erinacei*
Penicillium roqueforti	*Scedosporium dehoogii*	*Trichophyton interdigitale*
Penicillium singorense	*Scedosporium prolificans*	*Trichophyton rubrum*
Penicillium sp[2]	*Schizophyllum commune*	*Trichophyton tonsurans*
Penicillium sp[6]	*Scopulariopsis brevicaulis*	*Trichophyton violaceum*
Penicillium turbatum	*Scytalidium lignicola*	*Trichurus* sp

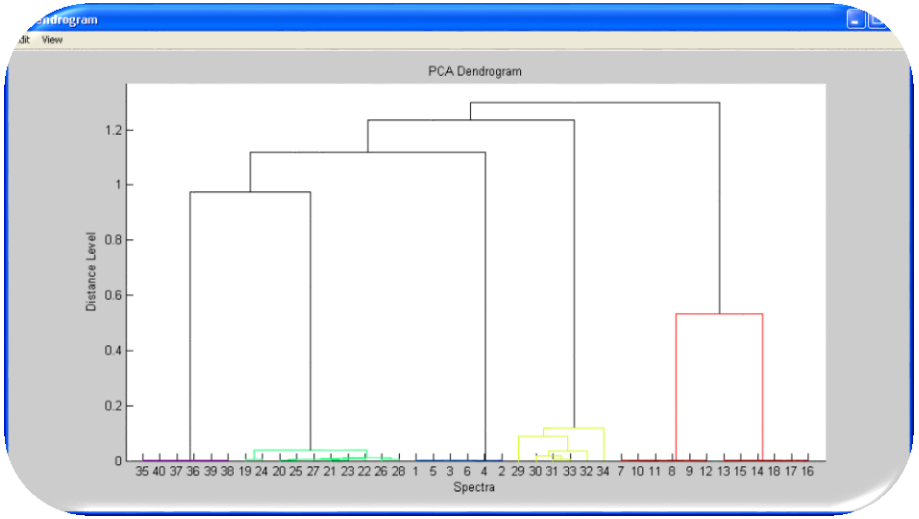
MALDI Biotyper
Saját referencia könyvtár létrehozása

Az opcionális **MBT Explorer** modul használata:

- Egyszerű és automatizált referenciakönyvtárbejegyzés
- Ugyanazzal a bioinformatikával, mint a Bruker könyvtárak
- Lehetővé teszi az MSP-k exportját / importálását az MBT-vel együttműködő más laboratóriumokba: ossza meg a cég referencia spektrumát
- Lehetővé teszi a Bruker könyvtárak és a házi készítésű könyvtár egyidejű használatát

Ezek lehetnek könyvtárak, amelyek helyspecifikus izolátumokkal és / vagy fontos termeléshez használt bejegyzésekkel rendelkeznek.

Használja a szoftvereszközöket a testreszabott mikroorganizmus-bejegyzések egyszerű összeállításához és az ujjlenyomatok összehasonlításához



MALDI Biotyper

Connect to online available libraries

e.g. MicrobeNet from CDC Atlanta

- Free available
- Internet connection in the lab needed
- Possible because of the MBT open microbiology concept

- Status May 2018:

  1004 MSPs, all on rare and unusual species, to complement the Bruker MBT Compass Library, continuous expansion

- Future Expansions: rare pathogens, BT agents, moulds

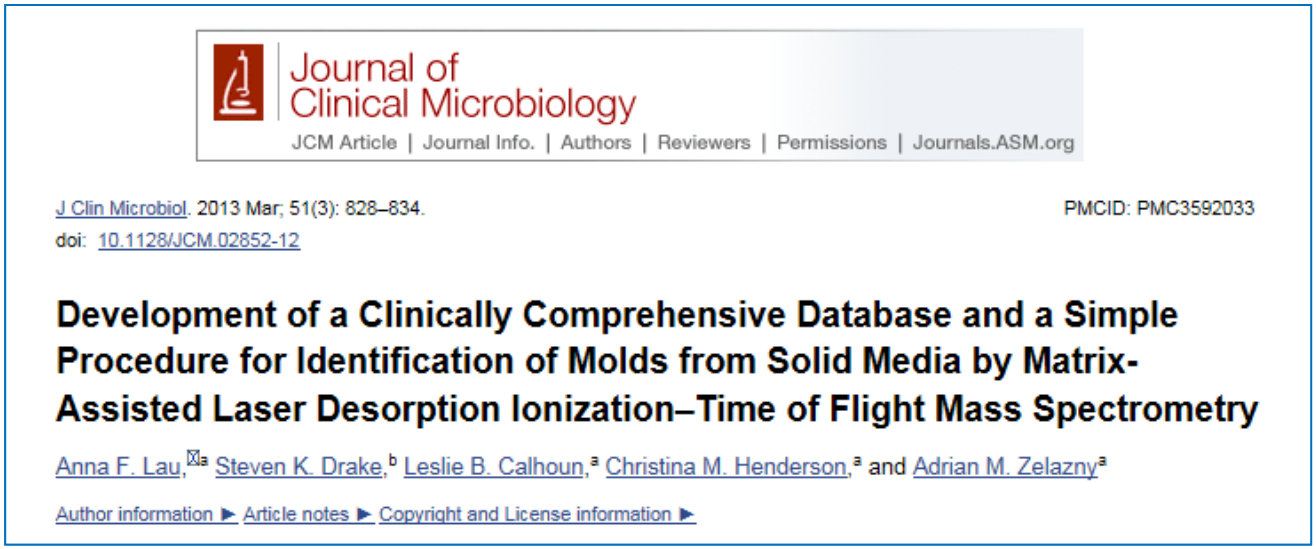
- Easy access and easy GUI, result overview similar to the MBT software
- Comes with comprehensive microbiology knowledge base

MALDI Biotyper

Download available libraries

e.g. National Institute for Health

NIH provides a library to be downloaded in addition to the Bruker MBT Libraries



Journal of Clinical Microbiology

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J Clin Microbiol. 2013 Mar; 51(3): 828–834. PMCID: PMC3592033

doi: 10.1128/JCM.02852-12

Development of a Clinically Comprehensive Database and a Simple Procedure for Identification of Molds from Solid Media by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Anna F. Lau,[a] Steven K. Drake,[b] Leslie B. Calhoun,[a] Christina M. Henderson,[a] and Adrian M. Zelazny[a]

Author information ▶ Article notes ▶ Copyright and License information ▶

The MBT "Open Microbiology" concept enables this initiative!

MBT Subtyping Module
Listeria monocytogenes

- **Characteristic peaks** in the Listeria spectra are used to confirm the identification, using direct transfer sample preparation:

Method	Accuracy for genus ID	Accuracy for species ID
Direct Transfer	100%	96.5%
Extended Direct Transfer	100%	95.4%
Formic Acid Extraction	100%	100%
Direct Transfer + MBT Subtyping Module	100%	100%

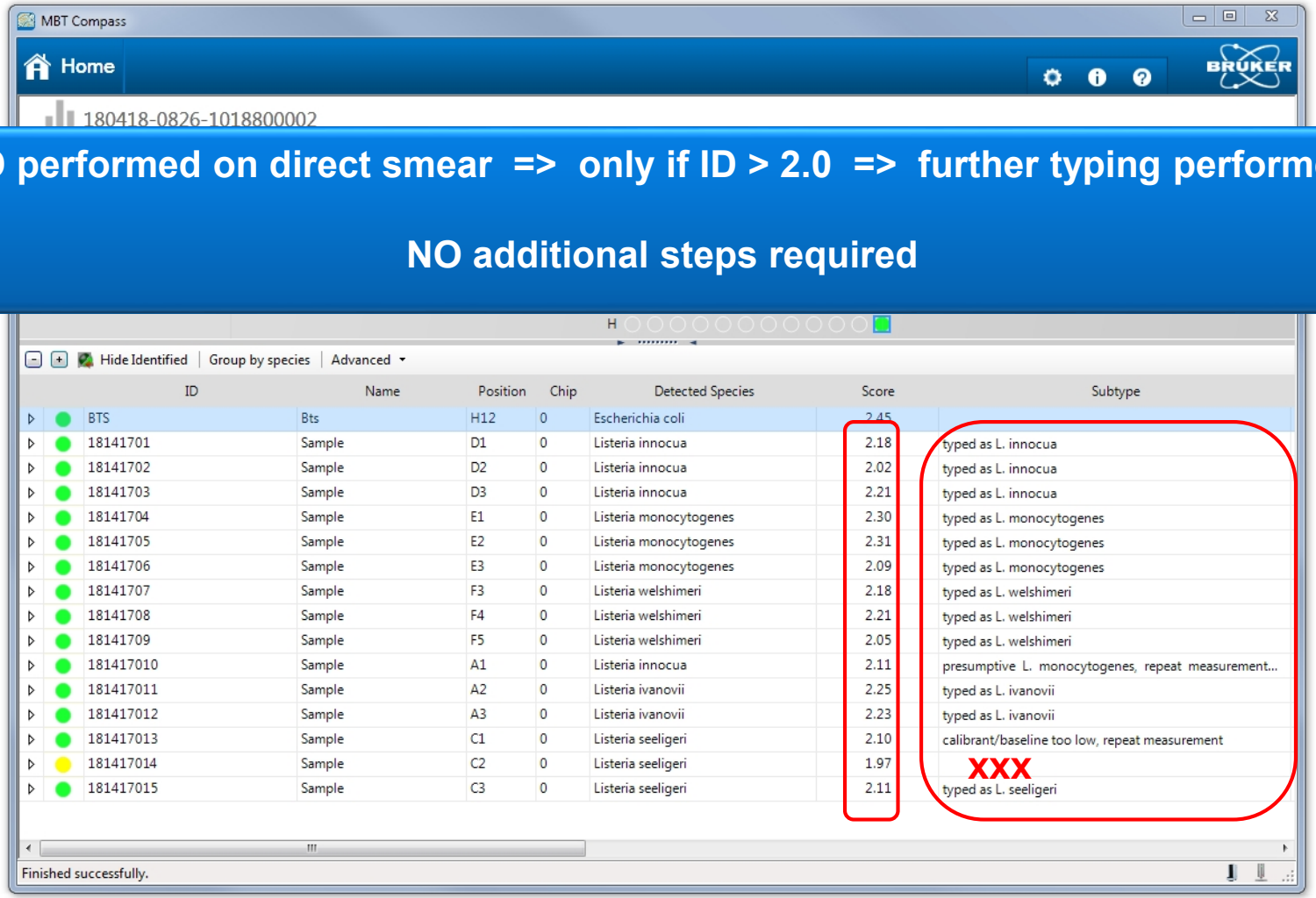
Without the MBT Subtyping Module the optimal way to achieve 100% ID is through extraction

With the MBT Subtyping Module 100% correct ID is obtained after direct transfer

MBT Subtyping Module

Listeria identification

Result in MBT Compass

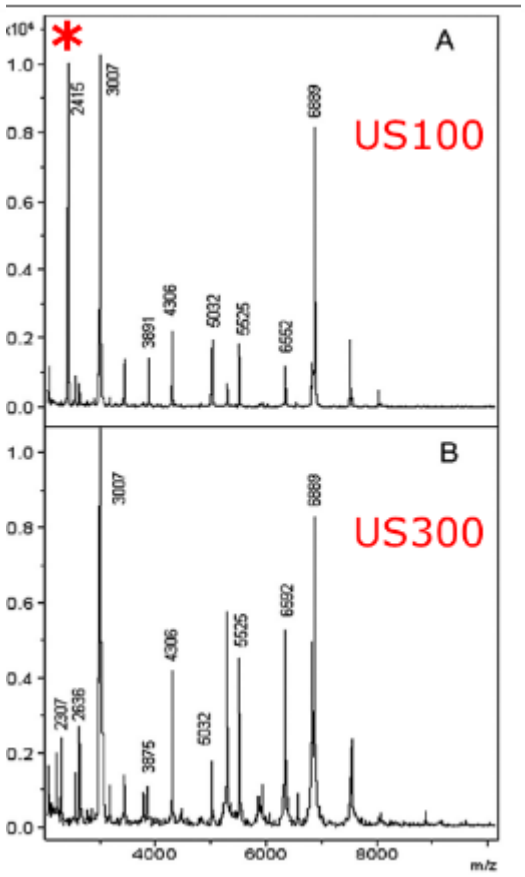


MBT Subtyping Module

Detection of specific antibiotic resistance e.g. *S. aureus* typing for MRSA detection

Meticillin Rezistens Staphylococcos Aureus

- The *S. aureus* subtyping application looks to detect the PSM-mec peptide peak present in a part of MRSA strains
  - If PSM-mec present: MRSA
    (Very high % of correct detections)
  - If not, does not mean it is not a MRSA
- The proportion of MRSA with the PSM-mec peak is variable, depending on local epidemiology
  - ➢ Success rate varies from <10-90% depending upon region
- The *S. aureus* subtyping allows to detect immediately a part of MRSA strains, with no extra effort!



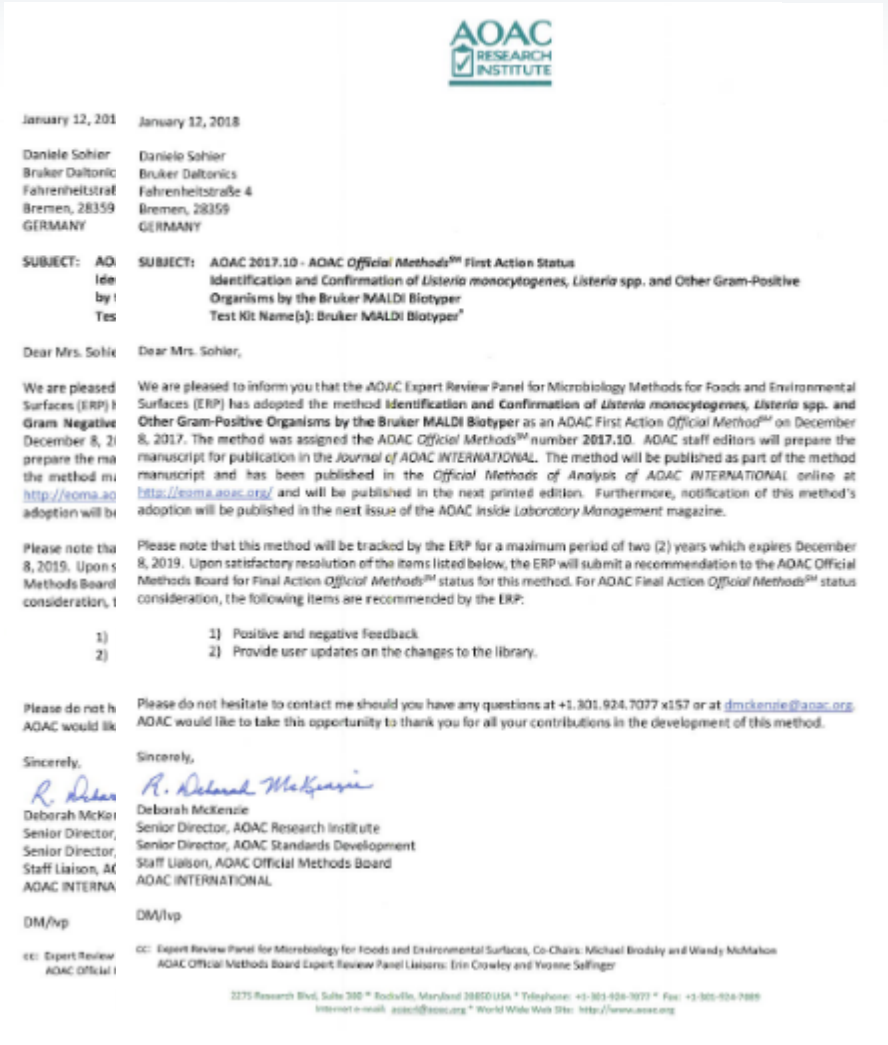
Early Warning System for Cattle & Raw Milk

MALDI Biotyper
Official Method of Analysis by AOAC International

USA tanúsítvány

AOAC First Action *Official Method*^{SM} 2017.09
Confirmation and Identification of *Salmonella* spp., *Cronobacter* spp.
and other **gram-negative organisms** by the Bruker MALDI Biotyper Method

AOAC First Action *Official Method*^{SM} 2017.10
Confirmation and Identification of *Listeria monocytogenes*, *Listeria* spp.
and other **gram-positive organisms** by the Bruker MALDI Biotyper method



AOAC RESEARCH INSTITUTE

January 12, 2018

Daniele Sohier
Bruker Daltonics
Fahrenheitstraße 4
Bremen, 28359
GERMANY

SUBJECT: AOAC 2017.10 - AOAC *Official Methods*^{SM} First Action Status
Identification and Confirmation of *Listeria monocytogenes*, *Listeria* spp. and Other Gram-Positive Organisms by the Bruker MALDI Biotyper
Test Kit Name(s): Bruker MALDI Biotyper®

Dear Mrs. Sohier,

We are pleased to inform you that the AOAC Expert Review Panel for Microbiology Methods for Foods and Environmental Surfaces (ERP) has adopted the method **Identification and Confirmation of *Listeria monocytogenes*, *Listeria* spp. and Other Gram-Positive Organisms by the Bruker MALDI Biotyper** as an AOAC First Action *Official Method*^{SM} on December 8, 2017. The method was assigned the AOAC *Official Methods*^{SM} number **2017.10**. AOAC staff editors will prepare the manuscript for publication in the *Journal of AOAC INTERNATIONAL*. The method will be published as part of the method manuscript and has been published in the *Official Methods of Analysis of AOAC INTERNATIONAL* online at http://eoma.aoac.org/ and will be published in the next printed edition. Furthermore, notification of this method's adoption will be published in the next issue of the AOAC *Inside Laboratory Management* magazine.

Please note that this method will be tracked by the ERP for a maximum period of two (2) years which expires December 8, 2019. Upon satisfactory resolution of the items listed below, the ERP will submit a recommendation to the AOAC Official Methods Board for Final Action *Official Methods*^{SM} status for this method. For AOAC Final Action *Official Methods*^{SM} status consideration, the following items are recommended by the ERP:

1) Positive and negative Feedback
2) Provide user updates on the changes to the library.

Please do not hesitate to contact me should you have any questions at +1.301.924.7077 x157 or at dmckenzie@aoac.org. AOAC would like to take this opportunity to thank you for all your contributions in the development of this method.

Sincerely,

R. Deborah McKenzie

Deborah McKenzie
Senior Director, AOAC Research Institute
Senior Director, AOAC Standards Development
Staff Liaison, AOAC Official Methods Board
AOAC INTERNATIONAL

DM/lvp

cc: Expert Review Panel for Microbiology for Foods and Environmental Surfaces, Co-Chairs: Michael Brodsky and Wendy McMahon
AOAC Official Methods Board Expert Review Panel Liaisons: Erin Crowley and Yvonne Salfinger

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AOAC-OMA: Association of Official Analytical Chemists - Official Method of Analysis

MALDI Biotyper
ISO 16140-Part 6 Certification by MicroVal

EU tanúsítvány

MICROVAL® NEN

Certificate n° 2017LR73
Confirmation of *Salmonella* spp.
by the Bruker MALDI Biotyper method

Certificate n° 2017LR72
Confirmation of *Cronobacter* spp.
by the Bruker MALDI Biotyper method

Certificate n° 2017LR75
Confirmation of *Listeria* spp. and
Listeria monocytogenes
by the Bruker MALDI Biotyper method

Lloyd's Register LRQA

MICROVAL® NEN

CERTIFICATE OF COMPLIANCE

LLOYD'S REGISTER QUALITY ASSURANCE

hereby declares that the certification assessment has demonstrated that

MALDI Biotyper®

Complete Solution for the confirmation of *Listeria* spp. and *Listeria monocytogenes*

Manufactured and supplied by:
Bruker Daltonik GmbH
Fahrenheitstraße 4
D-28359 Bremen
GERMANY

has been validated and revealed to be at least equivalent to the reference method as demonstrated by the validation study report. The summary of the validation report is available on the MicroVal website: www.microval.org

Reference methods:
1. ISO 11290-1 (2017): Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp. - Part 1: detection method;
2. ISO 11290-2 (2017): Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp. - Part 2: enumeration method.

Scope: Confirmation of *Listeria* spp. and *Listeria monocytogenes* from colonies isolated on Ottaviani & Agosti Agar, Palcam, Oxford, Modified Oxford and RAPID'L. mono, and any non-selective nutrient agars

The validation and certification has been performed in accordance with ISO/DIS 16140-6:2017 and the MicroVal Rules and Certification Scheme version 8.

Certificate no.: 2017LR75
First approval date: 26 April 2018
Expiry date: 25 April 2022

ISSUED BY: Lloyd's Register Nederland B.V.
Rotterdam, The Netherlands

Certificate no.: 2017LR75 03-05-2018 Page 1 of 1

K.P. v.d. Mandelelaan 41a, 3062 MB Rotterdam, The Netherlands. Kvk nr.: 24247948
This approval is carried out in accordance with the LRQA assessment and certification procedures and monitored by LRQA

Confirmation workflow: comparison

Biochemical tests – MALDI Biotyper

Methods	Analysis step	*Listeria*	*L. monocyto-genes*	*Salmonella*	*Cronobacter*
Reference FDA, USDA, ISO methods	Purification step on a non-selective agar (+ 24 hrs)	• Required	• Required	• Required except in the ISO 6579	• Required
	Confirmation of characteristic colonies (up to 5)	• Gram staining • Catalase • (Motility)	• Gram staining • Catalase • Hemolysis • Biochemical tests • (Motility)	• Biochemical tests • Serological tests	• Oxidase • Biochemical tests
	Final result if presence of characteristic colonies on selective media	**Day 1**	**Day 2**	**Day 2 to 3**	**Day 2**
	Handling time*	Not available	**> 10 min**	**4 min**	**3,5 min**
MBT	**Direct Transfer from isolated colonies on selective agars**	• **No purification step required** • **Same workflow for all the pathogens** • **Result in 15 min to 1 hour (1 to 95 isolates)** • **20 sec handling time / isolate**			

*handling time from ISO 16140 Validation reports

Workflow: comparison

16S rDNA sequencing – MALDI Biotyper

Mikrobiológiai teszt

16S rDNA workflow

- Sample prep
  Dedicated micropipettes & area
- Prepare PCR mix
  Dedicated micropipettes & area
- PCR run on Thermocycler
- Prepare sequencing mix
  Dedicated micropipettes & area
- Sequencing and data analysis

Electrophoresis *(Optional)*

Time to result (one sample or batch) : half a day
Handling time for 30 samples: 70 min + 20 min

Time ..... to ..... result

Time to result

MBT workflow

- Sample prep
- MBT: spectrum acquisition and data analysis

Time to result : 1 hr / 95 samples
Handling time : 20 min / 95 samples

MBT validated AOAC-OMA & ISO 16140-part 6

MALDI Biotyper

Basic Bruker consumables
Quality controlled

- MALDI matrix:

  HCCA Matrix

- Standard for QC:

  Bacterial Test Standard

- Disposable MALDI target:

  MBT Biotarget 96

Compared to traditional ID methods, significantly reduced consumables budget needed for standard ID!

MALDI Biotyper
Az élelmiszer-mikrobiológia legfontosabb jellemzői

AOAC OMA és ISO 16140-part 6 tanúsítvány:

- Élelmiszer alapú kórokozók és minőségi indikátorok ellenőrzése
- A bakteriális izolátumok azonosítása

Gyors
- Percek alatti eredmény

Egyszerű
- Ugyanaz az egyszerű munkafolyamat minden mintához
- Gyors betanítás
- Nincs szükség külön helyiségre

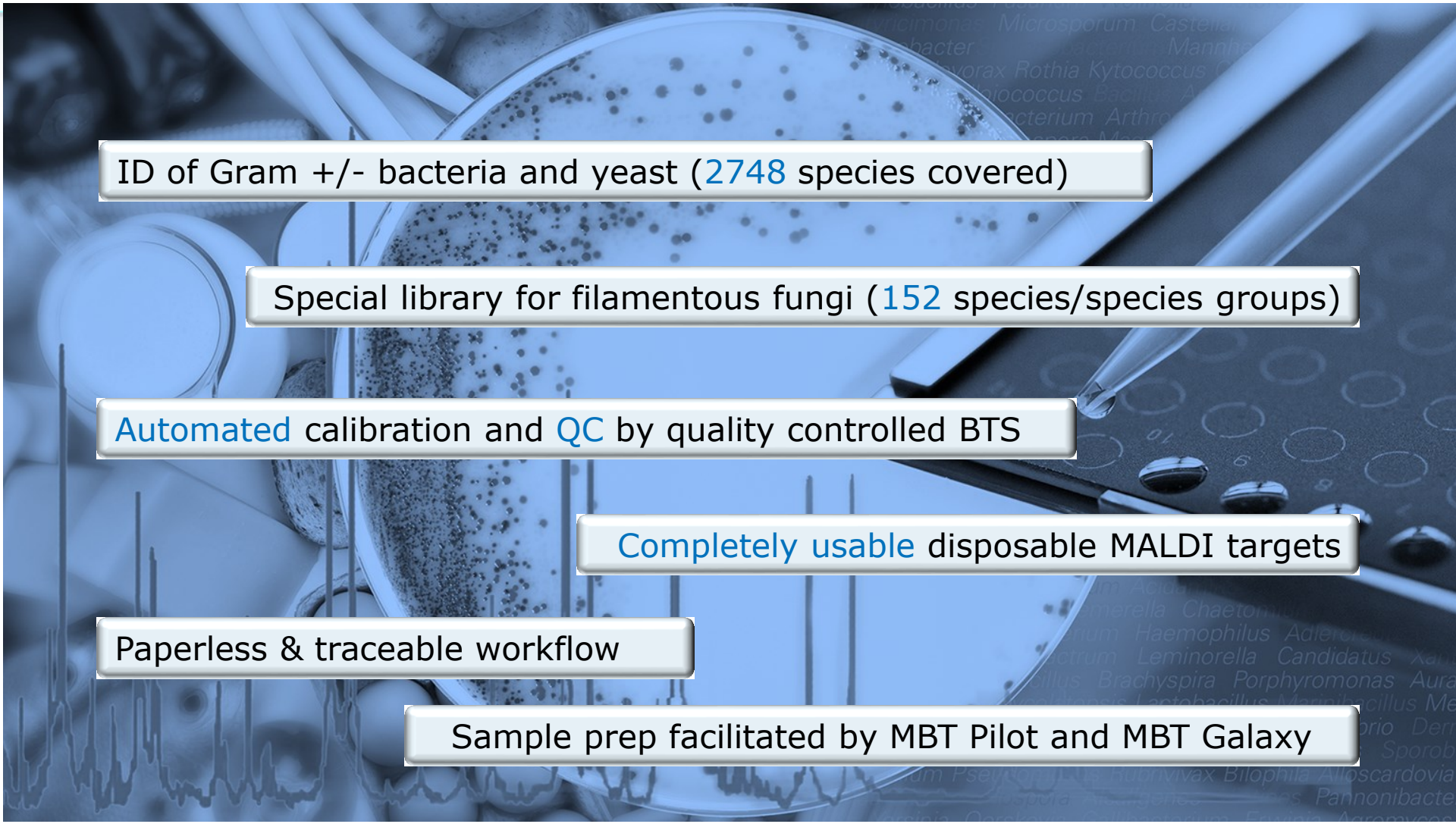
MEGBÍZHATÓ
- Kiterjedt referencia könyvtár 2748 fajból, folyamatos frissítés

ROBUSTUS és REPRODUkálható
Az üzemeltetőtől független

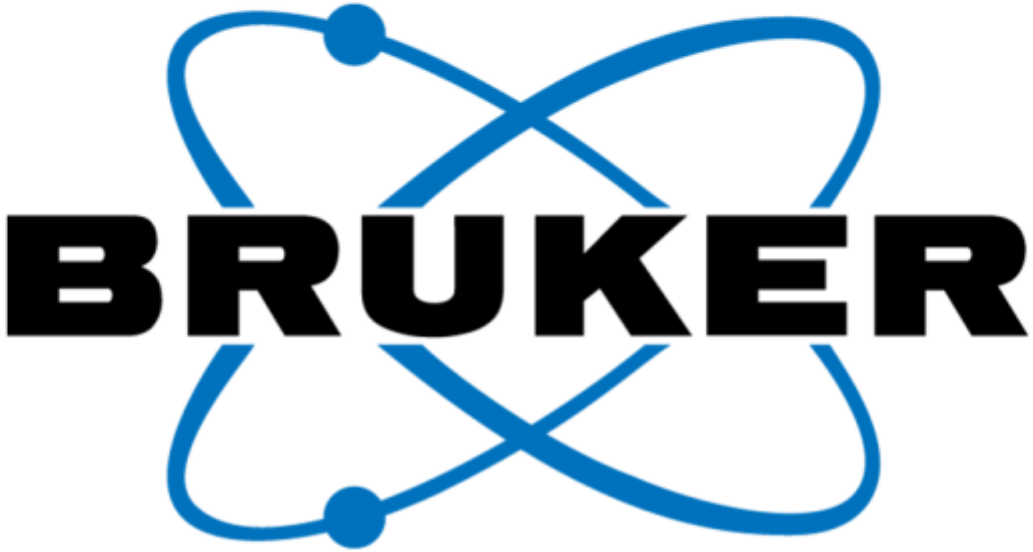
RUGALMAS
A kultúra körülményeitől független
Hozzon létre saját hivatkozási könyvtárakat

KÖLTSÉGHATÉKONY
Az alacsonyabb tesztenkénti költség a többi módszerhez képest

MALDI Biotyper
További előnyök



- ID of Gram +/- bacteria and yeast (2748 species covered)
- Special library for filamentous fungi (152 species/species groups)
- Automated calibration and QC by quality controlled BTS
- Completely usable disposable MALDI targets
- Paperless & traceable workflow
- Sample prep facilitated by MBT Pilot and MBT Galaxy



www.bruker.com