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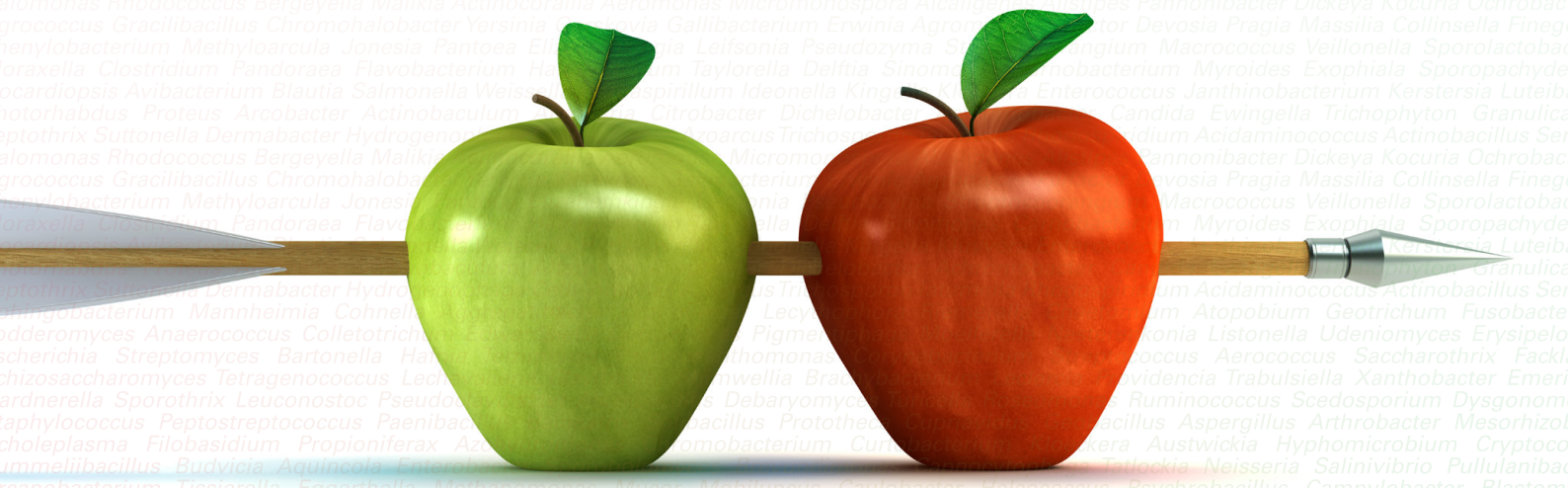


MALDI Biotyper®

MBT HT Subtyping Module

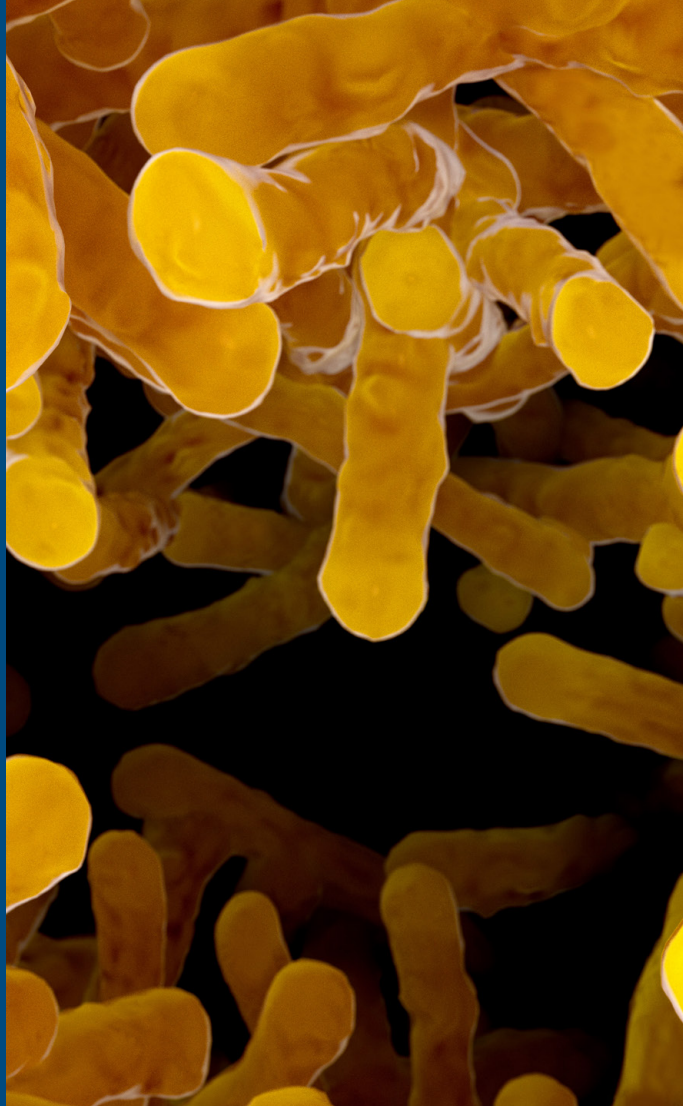
Fast microorganism identification combined with instant typing

Innovation with Integrity

RUO

Fast microorganism identification combined with instant typing

Over the past decade, the implementation of Bruker's MALDI Biotyper in many microbiology labs worldwide has entirely changed microorganism identification. Its high discriminatory power permits the identification of thousands of different species, but some genera are still difficult to differentiate. Bruker has therefore developed the MBT HT Subtyping Module, allowing for automated differentiation of some of these typically very difficult to distinguish species.
And there's more! The potential of MALDI-TOF mass spectrometry reaches beyond species identification; the MBT HT Subtyping Module combines the identification of important pathogens with subsequent detection of specific resistance markers in one automated workflow.



The principle

A prerequisite for the automated typing process is a high confidence identification of the bacterium in the MALDI Biotyper workflow.

For species differentiation, the MBT HT Subtyping Module then looks for decisive peaks in the identified mass spectrum, or triggers a sophisticated additional algorithm.

For detection of specific resistances, the software searches for peaks associated with proteins related to antibiotic resistance and, if present, reports the respective bacterium as presumptive resistant.

The applications

Facilitated species differentiation

- Accurate differentiation of *Mycobacterium chimaera* from *Mycobacterium intracellulare*
- Differentiation of *Listeria monocytogenes*
- *Elizabethkingia* species differentiation
- Differentiation of *Streptococcus pneumoniae*, *S. mitis_oralis* and *S. pseudopneumoniae*

Instant resistance marker detection as an early warning

- Detection of *cfiA* positive/negative *Bacteroides fragilis* strains
- *Staphylococcus aureus* typing for MRSA detection
- Detection of bla_{KPC} expressing *Enterobacteriaceae*

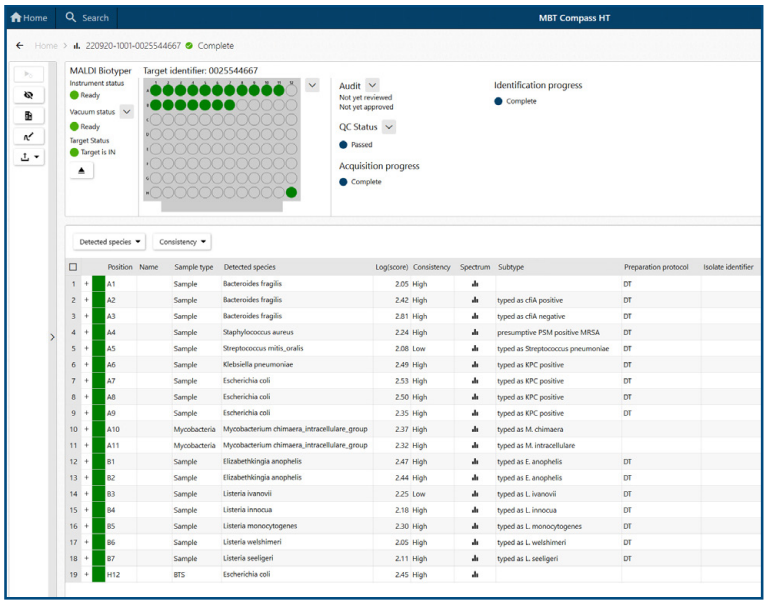
One seamless and fast workflow

No additional work | A clear report

Besides the usual sample preparation for routine microbial identification by the MALDI Biotyper, no additional work needs to be done to benefit from the MBT HT Subtyping Module, no special kits are needed. After high confidence identification of the bacterium in the MALDI Biotyper workflow, the MBT HT Subtyping Module automatically triggers additional algorithms and shows the typing results in both the MBT Compass HT software and in the report.

Seamless workflow

- Routine identification by MALDI Biotyper is performed
- Only when successful, typing is executed automatically
- No additional steps are required
- Results easily interpretable from the report



The subtyping result is shown in the Subtype column of the result table in the MBT Compass HT software.

Sample identifier (Type)	Target Pos.	Organism (best match)	log (score) (Conf.)
A3 (Sample)	A3	Bacteroides fragilis (typed as cfiA negative)	2.81 (+++)
A4 (Sample)	A4	Staphylococcus aureus (presumptive PSM positive MRSA)	2.24 (+++)
A5 (Sample)	A5	Streptococcus mitis_oralis (typed as Streptococcus pneumoniae)	2.08 (+++)
A6 (Sample)	A6	Klebsiella pneumoniae (typed as KPC positive)	2.49 (+++)
A7 (Sample)	A7	Escherichia coli (typed as KPC positive)	2.53 (+++)

The report shows the identification results with subtyping results below the species name in the Organism (best match) column.

Instant resistance marker detection

Contributing to antimicrobial stewardship

Antibiotic resistant bacteria are on the rise and are a major global public health threat. Effective prevention and control are therefore of high importance to reduce the risk of infections associated with antibiotic resistant microorganisms. The MBT HT Subtyping Module enables fast detection of specific resistances in an automated workflow.

Antimicrobial resistance is particularly troublesome in hospitals, nursing homes and other facilities where immunocompromised patients with open wounds or invasive devices are at great risk of nosocomial infections. The rise of antimicrobial resistant strains of bacteria is also observed in livestock. Surveillance and epidemiological studies are currently of major concern in food animal production, particularly in dairy cattle.

Detection of KPC-producing *Enterobacteriaceae*

In many countries a significant increase of carbapenem resistant *K. pneumoniae* (CRKP) is observed, which is a major concern as infections result in high rates of morbidity and mortality.
The most important mechanism of resistance by CRKP is the production of the *K. pneumoniae* carbapenemase enzyme (KPC), encoded by the bla_{KPC} gene. This plasmid encoded resistance can easily be exchanged between bacteria by horizontal gene transfer, making it even more dangerous in healthcare settings.
This mechanism and the fact that KPC resistance could spread rapidly between patients – if not detected in time - demand efficient identification methods for laboratory testing, active surveillance and screening of patients.

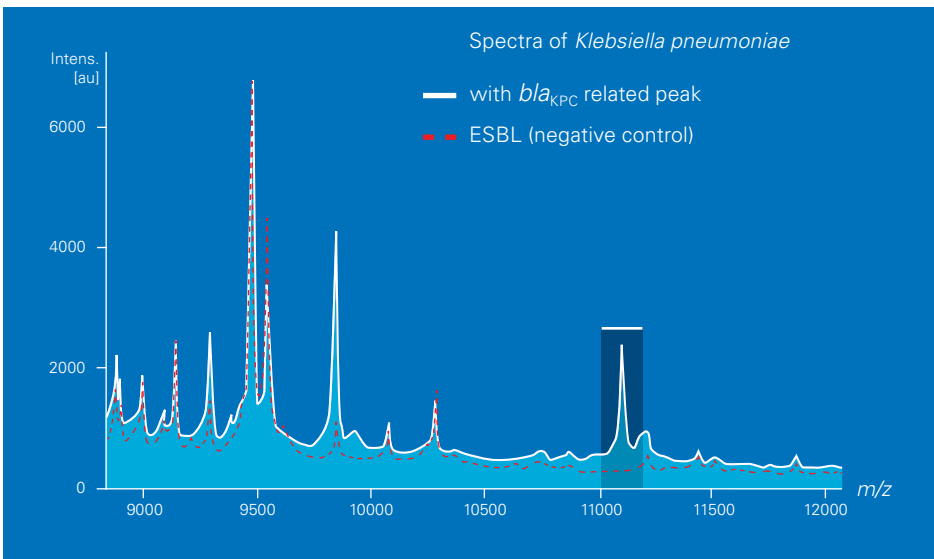
Lau *et al.* (2014) discovered a peak in MALDI-TOF mass spectra of KPC-producing *K. pneumoniae*, related to the plasmid carrying bla_{KPC}. This specific peak at 11,109 m/z is clearly detectable in bacterial MALDI-TOF mass spectra.

Prerequisite for the automated detection process with the MALDI Biotyper is the successful identification of the bacterium, i.e. the log(score) ID value must be ≥ 2.0. The MBT HT Subtyping Module then looks for the bla_{KPC} related peak in the sample spectrum and, if present, the software will report this sample as a KPC positive one. If no characteristic peak has been detected, nothing is mentioned.

The automated detection of the bla_{KPC} related peak for presumptive presence of KPC is available for the following species:

Citrobacter freundii
Enterobacter aerogenes
Enterobacter asburiae
Enterobacter cloacae
Enterobacter kobei
Enterobacter ludwigii
Escherichia coli
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae
Klebsiella variicola
Serratia marcescens

Anna F. Lau *et al.*, Journal of Clinical Microbiology 2014:52(8):2804-2812. https://doi.org/10.1128/JCM.00694-14
Yung-Ho Youn et al., Journal of Clinical Microbiology 2016:54(1):35-42. https://doi.org/10.1128/JCM.01643-15



Bacteroides fragilis cfiA subtyping

B. fragilis is the most frequently isolated anaerobic pathogen. Carbapenem resistance in *B. fragilis* is often associated with presence of the *cfiA* gene encoding for a metallo-beta-lactamase conferring resistance to nearly all beta-lactam antibiotics. As a result, infections with *B. fragilis cfiA* positive strains are difficult to treat.

After successful identification, the MBT HT Subtyping Module looks for specific peaks associated with the protein expressed by *B. fragilis cfiA* positive strains. If present, the best match for the respective sample will be reported as a presumptive *cfiA* positive strain.

MRSA subtyping

MRSA (Methicillin-Resistant *Staphylococcus aureus*) strains are genetically distinct from other strains of *Staphylococcus aureus*, and show multi-drug resistance to beta-lactam antibiotics.

As with the *Enterobacteriaceae*, successful identification of *S. aureus* is the prerequisite for the subsequent resistance subtyping process, in which the module looks for identification of the PSM-mec peak. PSM-mec is a surface peptide and the proportion of MRSA with PSM-mec is variable, depending on regional epidemiology. Absence of this peak does not mean that the respective strain is not an MRSA, but, if detected, it can be reliably considered as an MRSA positivity alert.

Only the direct transfer or extended direct transfer method should be used for the MRSA resistance marker detection application. When using preparation procedures including extraction/washing steps, there's a risk that the surface bound peptides/proteins get removed.

An early warning system

The MBT HT Subtyping Module quickly detects bla_{KPC} expressing *Enterobacteriaceae*, PSM-mec carrying MRSA and *cfiA* positive *B. fragilis* strains, hence giving an alert to the microbiologist about potential resistance issues.
Please note that negative results do not necessarily mean that these strains are susceptible but will require additional confirmation methods.

Tackling the challenging species by accurate differentiation

Elizabethkingia species differentiation

The genus *Elizabethkingia* covers mainly three very difficult to distinguish species: *E. meningoseptica, E. miricola* and *E. anophelis*, the latter has been first described in 2011 only. *E. anophelis* is an emerging pathogen for which outbreaks have been reported over the last years. As the antimicrobial susceptibility of *Elizabethkingia* may vary depending on the species, identification to species level is desirable.

In addition to improvements of the MALDI Biotyper reference library, the differentiation of the three known *Elizabethkingia* species has been further strengthened by another application of the MBT HT Subtyping Module. After successful identification of one of the *Elizabethkingia* species, the MBT HT Subtyping Module is automatically activated to start searching for characteristic mass spectrum peaks and will generate a species output.

Confident differentiation of *Mycobacterium chimaera* from *Mycobacterium intracellulare*

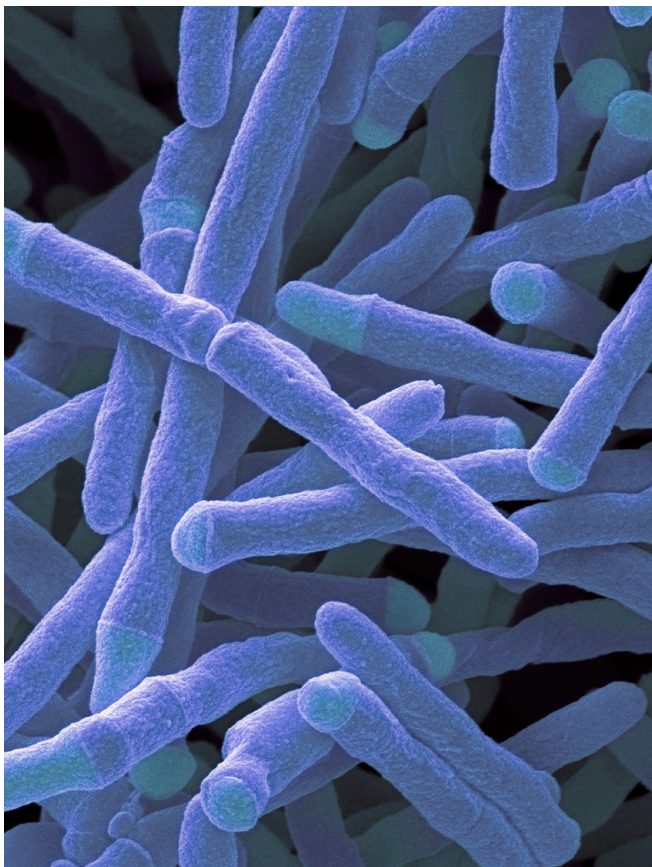
Mycobacterium chimaera very rarely causes infections in humans, but there have been recent reports about contamination of medical equipment used in heart surgery. The reports outlined the risk that heater-cooler units used in open-heart surgery could be contaminated with *M. chimaera* and that exposure of patients to these units in the operating theatre may lead to infections that can appear months to years after surgery. Most reported infections were those of prosthetic valves or vascular grafts.

Routine identification using MALDI-TOF mass spectrometry is restricted to the identification of the *M. chimaera / intracellulare* complex because mass spectra of both species are very similar. Conventional identification methods suffer from the same limitation.

After successful identification of the *M. chimaera / intracellulare* complex by the MALDI Biotyper, application of the MBT HT Subtyping Module allows - for the first time - fast and accurate differentiation of both species by thorough comparison of characteristic mass spectrum peaks as described by Pranada *et al.* (2017). This differentiation will support further insights into the pathogenic role of *M. chimaera* and can contribute to epidemiological studies which might improve infection control in the future.

Mycobacterium avium / intracellulare complex includes several species and only *M. intracellulare* and *M. chimaera* can be subtyped. Direct analysis of samples, for example sputum, without any cultivation step does not form part of the intended purpose; a cultivation step is required.

Note: Prerequisite for successful identification and differentiation of Mycobacteria is an installed MBT HT Mycobacteria Module.

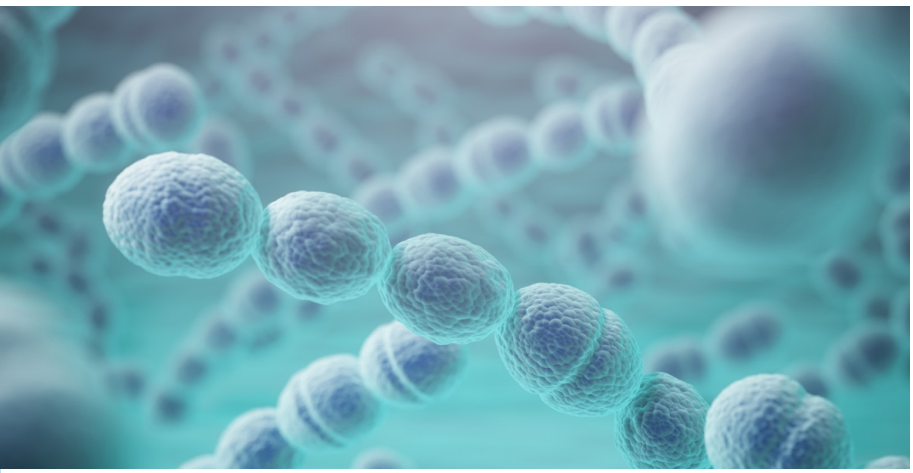


Arthur B. Pranada *et al.*, J Med Microbiol 2017:66:670-677. https://dx.doi.org/10.1099/jmm.0.000469

Species confirmation of *Streptococcus pneumoniae, S. pseudopneumoniae* and *S. mitis_oralis*

Unambiguous species differentiation of "mitis-group streptococci (MGS)" has always been a challenge, even when using state-of-the-art MALDI-TOF mass spectrometry, due to close phenotypic and genotypic similarities within this group. The majority of MGS (*S. mitis* and *S. oralis*) are part of the normal flora and rarely cause severe diseases. On the contrary, *Streptococcus pneumoniae*, also belonging to the MGS, is one of the prominent global pathogens, requiring unequivocal identification. The phenotypically closely related *S. pseudopneumoniae* is regarded as an emerging pathogen causing lower-respiratory-tract infections, while being linked with high rates of antimicrobial resistance, hence its clear-cut confirmation was included as well in the MBT HT Subtyping Module.

Inka Harju *et al.* I, Journal of Clinical Microbiology 2017:55:914–922. https://doi.org/10.1128/JCM.01990-16
Geneviève Garriss *et al.*, MBio 10:e01286-19. https://doi.org/10.1128/mBio.01286-19
Christian Salgård Jensen *et al.*, Diagnostic Microbiology and Infectious Disease 2021:101:115487.
https://doi.org/10.1016/j.diagmicrobio.2021.115487



This new application of the MBT HT Subtyping Module is based on a novel algorithm enabling species confirmation or correction of *S. pneumoniae* and *S. mitis* species group streptococci. After being identified by the MALDI Biotyper as one of the MGS species, a weighed ranking list interpretation is applied to differentiate these species more reliably. Additionally, the species *S. mitis* and *S. oralis* are grouped together in the new reference library version 2022, to the combination "*Streptococcus mitis_oralis*".

As a result, for *Streptococcus pneumoniae*, *S. pseudopneumoniae* and *S. mitis_oralis*, the initial species identification based on log(scores) can be confirmed or corrected for a more accurate species identification.

Facilitating *Listeria monocytogenes* differentiation

Worldwide, *Listeria monocytogenes* is a very important foodborne pathogen causing potentially fatal listeriosis. Particularly pregnant women and their newborns, adults aged 65 or older, and people with weakened immune systems are affected by this life-threatening infection.

Listeria spp. are widespread in the environment. Clear differentiation of the pathogenic *L. monocytogenes* from other *Listeria* species is of great importance for food business operators and risk managers.

Listeria identification is challenging because of close species relationships on genetic and proteomic levels. Even so, differentiation becomes a no-brainer by implementing the MBT HT Subtyping Module into the identification workflow.
Comparison of the sample mass spectrum with the *Listeria* reference spectra is performed by sophisticated bioinformatics, leading to reliable species differentiation.

The powerful *Listeria monocytogenes* differentiation requires only the fast and easy direct sample transfer method, enabling e.g., food QC laboratories to implement testing on *L. monocytogenes* in the daily routine workflow, directly from culture with minimal effort.

In food safety, the MBT HT Subtyping Module is an essential part of the bioinformatics of

- The AOAC-OMA #2017.10 method for the confirmation and identification of *Listeria monocytogenes* and *Listeria* spp., and other gram-positive organisms
- The ISO 16140-6 method validated by MicroVal for the confirmation of *Listeria* spp. and *Listeria monocytogenes* from various agar plates (Certificate 2017LR75)

ORDER INFORMATION

Part-No. 1889527
MBT HT Subtyping Module

The MBT HT Subtyping Module enables the automated detection of strain specific characteristics.
MBT Compass HT software is a prerequisite for the use of the MBT HT Subtyping Module. Differentiation of *Mycobacterium chimaera* from *M. intracellulare* needs an installed MBT HT Mycobacteria Module.

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