RUO

# Expert Insights

- MALDI Biotyper® advances the development of clinical microbiology

MALDI-TOF

Innovation with Integrity

# MALDI Biotyper® advances the development of clinical microbiology

Researchers at the Beijing Tongren Hospital's Department of Laboratory Medicine are pioneering medical advances with the support of the MALDI Biotyper

*"statement"*

Affiliated with Capital Medical University, Beijing Tongren Hospital is a large-scale, comprehensive tertiary hospital, specializing in ophthalmology and otorhinolaryngology. As Capital Medical University's Fourth Clinical School, Tongren Hospital also takes on the training of bachelors, masters and doctoral students in medicine.

As part of the Department of Laboratory Medicine, the Microbiology Laboratory has established an organizational structure that is jointly developed by physicians and laboratory technicians to improve clinical service capabilities.

The primary research conducted in the Microbiology Laboratory focuses on the detection of typical microbes, pathogenic molecules and infectious biomarkers and participation in clinical consultations, as well as undertaking a number of national-level research projects including pathogen diagnosis, new technologies, pathogenic epidemiological studies, and hospital infection epidemiology research.

## About the Author

Dr. Xinxin Lu is Director of the Department of Laboratory Medicine and is Deputy Director of the Department of Experimental Diagnostics for Capital Medical University. Dr. Lu specializes in clinical diagnosis and pathogenic microbial testing and has been involved with research and teaching work for more than 40 years.

Dr. Xinxin Lu

## Investment in the Latest Technology

Clinical microbiology plays a vital role in the diagnosis of infectious diseases, drug guidance, hospital infection control and antimicrobial drug management. Traditional biochemical identification methods are relatively complex and cumbersome and cannot fully meet the requirements of turnover time, sample diversity, and identification accuracy. The hospital laboratory has an ongoing commitment to excellence in scientific research and, as part of that commitment, was seeking alternatives to traditional culturing methods for pathogen diagnosis.

In 2010, after extensive research, the Beijing Tongren Hospital became of one the first organizations in China to invest in matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) technology for microbial identification, with the purchase of the Bruker autoflex III. In 2015, Dr. Lu purchased a benchtop MALDI-TOF MS system, Bruker's MALDI Biotyper (microflex LT/SH model). Today, mass spectrometry technology has become an indispensable laboratory identification tool.

The benefits of the MALDI Biotyper:

- **Fast:** The MALDI Biotyper helps cut the time to result by 24 hours.
- **Accurate:** The difficult pathogens that cannot be identified using routine biochemical methodologies are identified by the MALDI Biotyper. The database covers all types of bacteria and fungi, with a clinically common pathogen identification accuracy of more than 98%.
- **Low cost:** the cost of reagent supplies is low, nearly 10 times lower than that of biochemical methods.

*"Rapid identification helps to determine whether drug susceptibility testing is required, and helps to distinguish between pathogenic, conditional pathogenic and contaminating bacteria, which reduces blind diagnosis, especially in critical cases,"* comments Dr. Lu.

Unlike traditional biochemical methods, the MALDI Biotyper uses MS to determine the protein profile of microbial high-abundance proteins (mostly ribosome proteins) and compare their characteristic spectra with proprietary libraries holding reference spectra. Most microorganisms can be accurately identified at species level.

Species-conserved protein sequencing, extensive library references, and strict library quality control processes ensure the accuracy of MALDI Biotyper identification. A single sample preparation takes only 20 seconds, and about 100 samples can be analyzed in 60 minutes, greatly improving the speed of identification.

For critically ill patients, obtaining the results 24 hours sooner is important for rapid targeted antibiotic treatment. The cost advantages of the MALDI Biotyper are also significantly changing clinical workflows. Dr. Lu comments:

*"The cost of biochemical identification typically prohibits the analysis of every potentially contaminating bacterium, which means that some significant microbes may be missed. MS breaks down this barrier."*

After 10 years of using MALDI-TOF MS technology, the most significant change in the Microbiology Laboratory of Beijing Tongren Hospital is to improve the identification of difficult pathogens, Dr. Lu added:

*"MALDI-TOF MS is used to identify microorganisms by their protein profile spectrum and, unlike conventional methods, it has high identification accuracy, particularly for anaerobic bacteria with very low biochemical identification rates. These laboratory identification problems are no longer an issue with the introduction of MALDI-TOF MS."*

The initial reasoning behind implementing the MS system in the Microbiology Laboratory of Beijing Tongren Hospital was to identify challenging pathogens. Unlike common bacteria and yeast, these pathogens are difficult to culture, slow in metabolism, diverse in growth period, and have complex cell wall structures. They are difficult to identify by conventional methods.

## Challenging Pathogens

Take filamentous fungi as an example. The existing identification methods are around cultures, microscopic examination, or molecular sequencing. These processes are cumbersome. The identification is highly dependent on the technical capability and experience of laboratory technicians. As a national key research center of ophthalmology and otorhinolaryngology, Beijing Tongren Hospital treats approximately 400 cases of fungal rhinosinusitis (FRS) each year. Misuse of antibiotics and environmental pollution are both significant contributors to the severity of FRS. Misdiagnosis of FRS, particularly in the case of acute invasive FRS, may lead to treatment failure and can be fatal. Studies show that 80% of FRS is caused by common pathogenic fungi Aspergillus spp. In addition, *Fusarium* spp., *Mucor* spp., *Rhizopus* spp., *Rhizomucor* spp., *Penicillium* spp. and *Alternaria* spp. could be causative agents.

Conventional FRS diagnostic methods include clinical observation, endoscopic examination, imaging, pathology, and fungal morphology identification.

FRS pathogens are particularly challenging to identify in clinical laboratories, due to the wide variety of causative species with phenotypically similar characteristics, despite their genetic differences. The identification relies heavily on the experience of laboratory technicians. Identifying FRS pathogens with conventional methods often incurs high costs, results in long turnaround times, low throughput and low selectivity, and cannot exclude colonization. Conventional methods are therefore not suitable for the identification of FRS pathogens.

The introduction of MALDI Biotyper and the MBT Filamentous Fungi Library, the latter currently for research use only (RUO) and not for clinical diagnostic procedures, may bring new possibilities for understanding and treating FRS. Dr. Lu comments:

*"Common pathogenic fungi causing FRS include Aspergillus spp., Fusarium spp., Mucor spp., Rhizopus spp., Rhizomucor spp., Penicillium spp. and Alternaria spp. MALDI-TOF MS can identify the individual species using direct transfer to the target or extraction methods, and shortens the identification time of fungi species."*

In 2017, the Microbiology Laboratory of Beijing Tongren Hospital used the MALDI Biotyper to conduct a study to evaluate the performance of MALDI-TOF MS in identifying 153 different FRS filamentous fungi isolates [1]. The results showed 151 out of 153 FRS isolates had correct species identification (98.7%) (Table 1). The rate was significantly higher than that of the traditional phenotypic methods (93.5%). MALDI-TOF MS was capable of identifying very closely related species including *Aspergillus versicolor* (1), *Aspergillus fumigatus* (3), *Aspergillus fumigatus* (2) and *Aspergillus terreus* (1) that were indistinguishable with conventional phenotypic methods. The MALDI-TOF MS method has proven to be more accurate and quicker than phenotypic methods for the identification of filamentous fungi in clinical microbiology laboratories.

In addition to filamentous fungi, the problem of identifying other challenging clinical pathogens such as anaerobic bacteria and mycobacteria can be solved with the help of the MALDI Biotyper.

Table 1: Comparison of the identification of the 153 FRS isolates obtained by conventional phenotyping and the MALDI Biotyper (Microflex LT/SH system), Bruker Daltonics

| Definitive identification | No. of total isolates | Conventional phenotypic method | | | Bruker MALDI Biotyper® | | |
|---|---|---|---|---|---|---|---|
| | | Correct identification to the level of | | Mis ID | Correct identification to the level of | | Mis ID |
| | | Species | Genus | | Species (score) | Genus | |
| ***Aspergillus fumigatus*** | 30 | 28 | 0 | 2 | 30 (2.300-2.600) | 0 | 0 |
| ***Aspergillus flavus*** | 20 | 17 | 0 | 3 | 18 (2.300-2.500) | 0 | 2* |
| ***Aspergillus terreus*** | 20 | 19 | 0 | 1 | 20 (2.300-2.400) | 0 | 0 |
| ***Aspergillus nidulans*** | 15 | 15 | 0 | 0 | 15 (2.00-2.370) | 0 | 0 |
| ***Aspergillus versicolor*** | 10 | 9 | 0 | 1 | 10 (2.000-2.356) | 0 | 0 |
| ***Aspergillus niger*** | 15 | 15 | 0 | 0 | 15 (2.300-2.473) | 0 | 0 |
| ***Alternaria alternata*** | 10 | 10 | 0 | 0 | 10 (2.200-2.401) | 0 | 0 |
| ***Scedosporium apiospermum*** | 10 | 10 | 0 | 0 | 10 (2.200-2.389) | 0 | 0 |
| ***Schizophyllum commune*** | 10 | 10 | 0 | 0 | 10 (2.200-2.375) | 0 | 0 |
| ***Rhizopus oryzae*** | 2 | 0 | 2 | 0 | 2 (2.200-2.400) | 0 | 0 |
| ***Rhizopus stolonifer*** | 1 | 0 | 1 | 0 | 1 (2.317) | 0 | 0 |
| ***Fusarium moniliforme*** | 5 | 5 | 0 | 0 | 5 (2.200-2.400) | 0 | 0 |
| ***Fusarium solani*** | 5 | 5 | 0 | 0 | 5 (2.200-2.401) | 0 | 0 |
| **Total** | 153 | 143 (93.5%) | 3 (1.9%) | 7 (4.6%) | 151 (98.7%) | 0 | 2 (1.3%) |

## Scientific Exploration by Using Mass Spectrometry

In addition to routine laboratory testing, the Microbiology Laboratory of Beijing Tongren Hospital is also responsible for teaching and scientific research. The introduction of the MALDI Biotyper MS system has played an important role in this process. Dr. Lu said:

*"We have published more than 30 scientific articles related to mass spectrometry in Chinese or English. Initially, we explored the clinical value of mass spectrometry-based identification. After it was proven valuable, we then focused on identification and pre-treatment methods for challenging microorganisms. In addition, mass spectrometry bacterial resistance analysis and research were carried out. Now, mass spectrometry is an essential tool for our laboratory research and is used to explore scientific applications."*

Based on the standard application recommended by Bruker, Beijing Tongren Hospital continuously optimizes the MS application according to clinical situations encountered, to further improve the efficiency of laboratory testing. Based on the MALDI Biotyper platform, the laboratory developed a method of direct identification from positive blood culture specimens and urine samples, as well as the "Horizontal Oscillation" method for filamentous fungi sample pre-treatment (Figure 1) and the rapid direct identification method for liquid cultured mycobacteria [2].

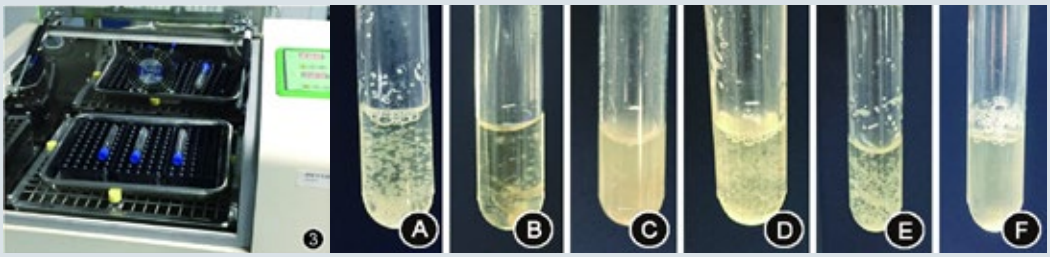
The efficiency of the MALDI Biotyper in terms of speed and accuracy is improved. MALDI Biotyper is an indispensable part of the laboratory testing process.

Beijing Tongren Hospital is also exploring wider applications for MS, such as carbapenem resistance detection. As early as 2013, Tongren Hospital carried out research on the detection of carbapenemases by using the Bruker MALDI-TOF MS system. The hospital used MALDI-TOF MS, combined with Bruker ClinProTools biomarker identification software, to identify challenging bacteria – VIM-2-producing *Pseudomonas aeruginosa,* AmpC- and KPC-producing carbapenem resistant *Enterobacteriaceae* and OXA-23-producing *Acinetobacter baumannii* [3]. The detection accuracy rate of MALDI-TOF MS for enzyme-producing and non-enzyme-producing strains is up to 100%, which proves that MALDI-TOF MS has great advantages over other existing phenotypic methods on carbapenemase detection.

The national average resistance rate for carbapenem-resistant *Klebsiella pneumonia* has reached 10.1% (according to 2018 National Bacterial Resistance Detection Network) [4] since it was first reported in Zhejiang Province in China in 2007. The mortality of nosocomial infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) is as high as 33.5%. Bloodstream infections are as high as 43.1% [5].

Because it is easy to spread, enzyme-producing strains accounted for more than 90% of CRE. Rapid detection of enzyme-producing strains is very important for timely and effective clinical treatment. It is also beneficial to promote the rational application of antibiotics and ease the severe situation of antibiotic resistance.

Figure 1: Filamentous fungi cultivation – 2 days after culture with the Horizontal Oscillation method developed by Tongren Hospital Microbiology Laboratory. This method can shorten the filamentous fungi culture time to 2 days. The mycelium is evenly distributed, and the identification score is higher.




A: *Aspergillus flavus*; B: *Scedosporium apiospermum*; C: *Rhizopus oryzae*; D: *Trichoderma koningii*; E: *Penicillium chrysogenum*; F: *Fusarium solani*

## Growth with Bruker

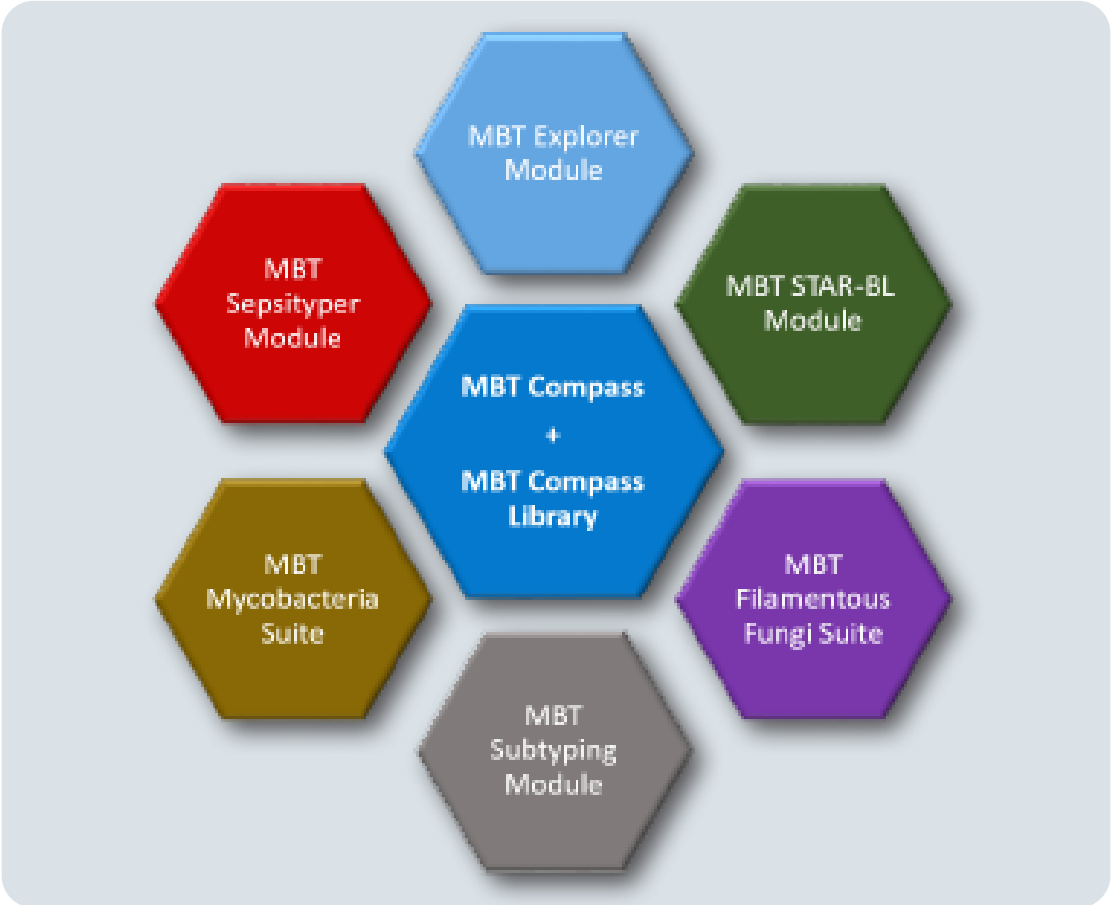
As of July 2020, there are approx. 4000 MALDI Biotypers in the field, with more than 100 papers published annually which are included in the Science Citation Index. Like the Tongren Hospital Microbiology Laboratory, users willing to explore MS technology are our growth drive. Bruker dedicates its work to understanding the clinical users' needs, and improves hardware and software systems continuously:

Dr. Lu said: *"Mass spectrometry is a new technology in the field of microbiology, and good interaction between manufacturers and users has driven the application and development of this technology."*

In response to the needs of different users, Bruker has introduced a new MBT Compass software system (NMPA certification in November 2019):

- For challenging microorganisms such as mycobacteria and filamentous fungi, Bruker has set up special identification modules, equipped with specific algorithms and a dedicated reference library, to improve the accuracy of the identification.
- With regards to MALDI-TOF testing on drug resistance, which is of great clinical concern, Bruker provides the MBT STAR-BL beta-lactamase activity detection module and the MBT Subtyping module for rapid detection of certain CRE, MRSA and other resistant strains.
- For direct positive blood culture (PBC) sample testing, the MBT Sepsityper module, combined with the Sepsityper kit, enables rapid extraction and identification of PBC samples, making it easy for laboratories to deliver fast reporting and targeted treatment.

Figure 2: The Research Use Only MALDI Biotyper MBT Compass Software Modules




The different functional MBT Compass modules are independent, and users can select what they want according to real laboratory situations. It is easy to add additional modules, and the platform supports the expansion and development of each individual module.

As one of the first clinical users to use MBT Compass software in China, Dr. Lu also recognized the new features and application potential of the software:

*"The new software has the ability to give us insight on bacterial resistance during the identification process, which is our current focus. We have already used the software to test MRSA strains - the results are highly specific. We will continue to explore this approach on other pathogens."*

## Looking Forward to the Next Decade

As an emerging rapid detection technology, MALDI-TOF MS has become a routine clinical identification tool. The technology has undergone rapid development in clinical microbiological testing in China over the past ten years, and we are looking forward to further advancements over the next decade.

Dr. Lu concludes: *"Bacterial resistance detection, bacterial typing and homologues analysis, pathogen antigens, serum antibodies, toxicity detection and pathogen identification through fluorescence are the areas that could be expanded beyond microbial identification."*

Undoubtedly, these areas will be the key focus for both Bruker and its customers over the next decade. It has always been our common goal to continue to develop more clinical microbial solutions and promote the development of the clinical microbiology industry.

## References


1. Huang et al., Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for rapid identification of fungal rhinosinusitis pathogens. *Journal of Medical Microbiology* 2017;66:328–333DOI 10.1099 / jmm. 0.000435

2. Huang Yanfei,, filamentous fungi mass spectrometry pre-processing methods and applications. *Chinese Medical Journal*, August 8, 2017, Volume 97, No. 30.

3. Lijun Wang et al., MALDI-TOF MS applied to indirect carbapenemase detection: a validated procedure to clearly distinguish between carbapenemase-positive and carbapenemase-negative bacterial strains. *Anal Bioanal Chem* (2013) 405:5259–5266 DOI 10.1007/s00216-013-6913.

4. http://www.carss.cn/Report/Details?ald=648

5. Yawei Zhang et al., Epidemiology of Carbapenem-Resistant Enterobacteriaceae Infections: Report from the China CRE Network. *Antimicrobial Agents and Chemotherapy*. February 2018 Volume 62 Issue 2 e01882-17.


Figure 3: Microbiology Unit of the Inspection Department at Tongren Hospital using the MALDI Biotyper for microbiological identification

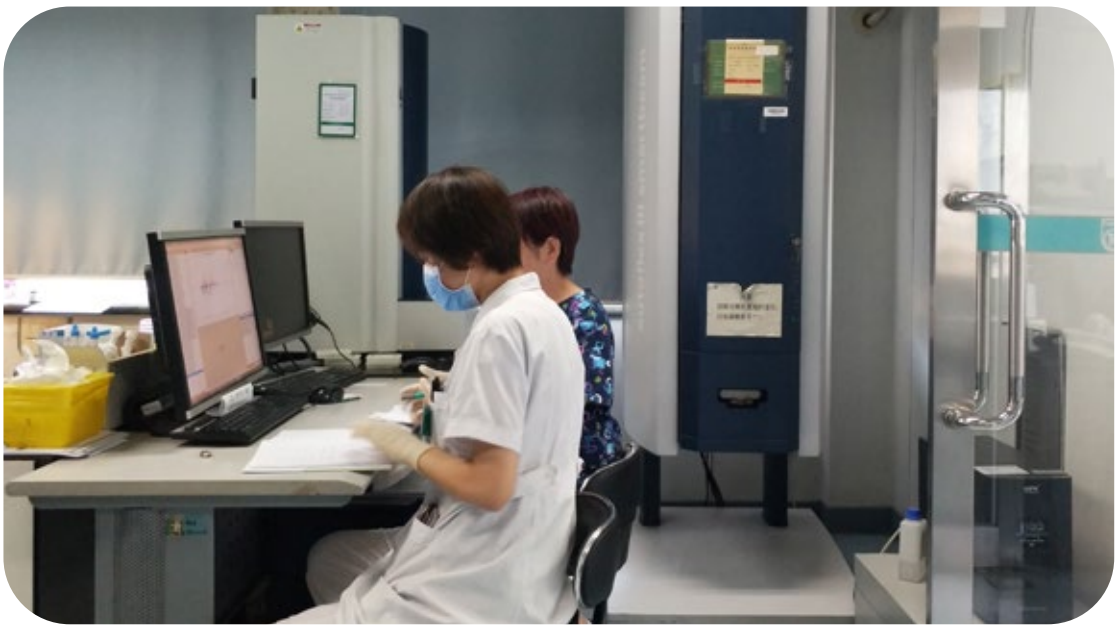


Figure 4: Tongren Hospital, Beijing

# Expert Insights

## About Beijing Tongren Hospital

CFounded in 1886, Beijing Tongren Hospital is affiliated with the Capital Medical University and is a large-scale, comprehensive tertiary hospital, specializing in ophthalmology and otorhinolaryngology. It is National Center for The Prevention and Control of Allergic Reactions (Ear, Nose and Throat Allergies) and Diabetes, as well as the national and World Health Organization (WHO) blindness prevention cooperation center.

The hospital has more than 3,600 employees. There are 64 clinical and medical technology departments in the hospital, with about 2000 beds. Annual outpatient and emergency treatment volume is 2,754 million people. As Capital Medical University Fourth Clinical School, Tongren Hospital also takes on the training tasks of bachelors, masters and doctoral students in medicine. The Beijing Academy of Ophthalmology and the Academy of Otolaryngology of Capital Medical University are located in the hospital.

The Microbiology Laboratory is an important medical and technical department of Tongren Hospital. Recognized by ISO15189 CNAS Medical Laboratory in 2007, the department currently employs 78 people, including 8 PhDs, 23 masters and 17 undergraduates, with four testing sub-specialties - cell biology, microbiology, biochemistry and immunology. The main research focus of the microbiological laboratory is the application of the new technique on pathogen diagnosis and the epidemiological study of pathogens.

Microbiology Laboratory, The Department of Laboratory Medicine, Beijing Tongren Hospital has validated all non-IVD methods, software, kits and workflows described in the article in-house for clinical use in the Microbiology Laboratory, The Department of Laboratory Medicine, Beijing Tongren Hospital.

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