

IVD



## Expert Insights

- Tackling Antibiotic Resistance:  
Using Antimicrobial Susceptibility Testing  
to Improve Patient Care

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# Tackling Antibiotic Resistance: Using Antimicrobial Susceptibility Testing to Improve Patient Care

Clinical Scientists at Santa Maria Misericordia University Hospital, Udine and Angelo Hospital, Mestre, introduce MICRONAUT AST with Bruker's MALDI Biotyper in laboratory routine, for improved antibiotic treatment of patients.



Working with Bruker

Dr. Claudio Scarparo, Microbiology Manager at Santa Maria Misericordia University Hospital, Udine and Angelo Hospital, Mestre, adopted MERLIN's MICRONAUT system in 2016 in order to measure true minimum inhibitory concentration (MIC) values in the labs, to better inform clinical decision makers. He comments on its use:

*"MICRONAUT system determines the true/real MIC for the correct quantification of an antibiotic dosage in vivo, allowing clinicians to prescribe targeted therapy according to the pharmacokinetic/pharmacodynamic parameters."*

## Synopsis

The worldwide emergence and spread of antibiotic resistance among human pathogens is a serious problem for human health, particularly for critically ill patients. Infectious diseases caused by multidrug resistant bacteria are especially problematic, as there are often only limited treatment options. Therefore, reliable and meaningful antimicrobial susceptibility testing (AST) in microbiological laboratories is highly important for rapid patient treatment, with the appropriate dosage of active drugs.

Current methods for AST provide accurate phenotypic detection of common antimicrobial resistance mechanisms [1]. However, there is a need for diagnostic systems that can be updated quickly if new antibiotic resistance mechanisms emerge, breakpoint values of common drugs change, or new antibiotics become available. The combination of the MERLIN MICRONAUT system with the Bruker (matrix-assisted laser desorption/ionization) MALDI Biotyper (MBT) for identification of microorganisms, leads to an overall reduction in time for AST results. This allows clinicians to start the correct antibiotic

therapy much earlier. Another advantage of the MICRONAUT system is that microbiological laboratories can either choose standard plates or compose their own customized test plates for susceptibility testing. The latter enables laboratories to account for the local epidemiology of pathogens or occurrence of resistance mechanisms.

This case study highlights the work of a specialized collaborative laboratory in Italy, to provide diagnostic tests for infectious diseases in clinical practice set against the backdrop of a country with the highest level of antimicrobial resistance across EU member states [2].

## Laboratory work

The Operative Units of Microbiology and Virology in Udine and in Mestre work in a multidisciplinary way, as explained by Dr. Scarparo:

*"We carry out bacteriology tests at the Mestre and Udine facilities."*

*These can include urine culture, blood cultures, culture of stool samples, diagnosis of respiratory infections, research of parasites and fungi, identifications and antibiograms (the overall profile of AST results), investigations of serology, and infectious molecular biology.*

*We have over 40 staff members at the Udine facility and over 20 staff members on-site in Mestre." Every week, the Mestre laboratory completes 6,600 tests within bacteriology, immunology, serology and infectious molecular biology, while at the Udine facility, the team completes 6,800 tests. The Udine and Mestre institutes complete approximately 23,000 identifications/20,000 antibiograms per year and 14,000 identifications/12,000 antibiograms per year, respectively.*

## **The emerging threat of antibiotic resistance**

Antibiotic resistance is a prevalent and growing threat to patients worldwide. It has an impact on sensitive areas of modern medicine including organ transplants, chemotherapy, and routine surgical procedures in which antibiotic treatment plays a major role. Millions of infectious diseases worldwide are caused by antibiotic resistant bacteria, often leading to prolonged hospital stays for patients.

In the US, it is estimated that eight million extra days of hospitalization, 23,000 deaths and over \$20 billion in additional healthcare costs result from bacterial resistance to key antibiotics [3]. In a report published by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), multi-drug resistant bacteria have been identified as one of the major threats to public health, due to limitations in effective treatment options or treatment failure [4].

## **About the Departments of Microbiology at Santa Maria Misericordia University Hospital, Udine, and Angelo Hospital, Mestre**

Dr. Claudio Scarparo has worked as Microbiology Manager at the Santa Maria Misericordia University Hospital for 10 years and has been at the Angelo Hospital of Mestre since August 2017. Dr. Scarparo coordinates the diagnostic activity and oversees all staff in the department of microbiology. The two sites are specialized centers of laboratory medicine, providing diagnosis, prevention and treatment of infectious diseases, laboratory assistance activities, out-patient and consultancy services for the local area. The key benefits of the hub and spoke model, with eight hospitals (spokes) sending their samples for diagnostic testing to the two specialist laboratories (hubs), are reduced costs and greater access to state-of-the-art laboratory instruments, resulting in increased diagnostic speed and quality. With the introduction of the new 'Rapid' MBT Sepsityper IVD kit a turn-around-time of 15-20 minutes is achievable from a positive blood culture alert.

## **The advantage of AST by broth microdilution: Determining true MIC values**

Broth microdilution is a standardized and globally accepted AST reference method for the determination of the minimum inhibitory concentration (MIC) of an antibiotic agent. Traditional breakpoint AST methods only give information on the interpretive category (whether the bacterium is susceptible, intermediate or resistant to a respective test drug), whereas multiple concentration AST covers a broad range of drug concentrations and MIC is additionally determined for each antibiotic agent tested. The latter provides more precise information about the resistance of bacteria and is helpful in antibiotic dosage finding.

Broth microdilution plates can be freshly prepared by the user or are commercially available as microtitration plates, including the antibiotic agents in a vacuum-dried form. At least one well on a microtitration plate does not include an antibiotic agent and serves as growth control for the respective test bacterium. For susceptibility testing, a suspension with a defined cell count of the test strain is prepared in the test medium. In the next step, the microtitration plates are inoculated with the cell suspension. Inoculated plates are sealed and incubated for a defined time period and temperature.

## MICRONAUT

It is important to respond rapidly to current and newly emerging antibiotic resistant threats by carefully tracking the spread of antibiotic-resistant bacteria in humans as well as in animals. Benefits of the MICRONAUT include:

- AST is based on the standard broth microdilution method, resulting in true MIC determination.
- The system provides a tool for phenotypic detection of clinically relevant resistance mechanisms in aerobic and anaerobic pathogens.
- It is one of the few systems on the market that is completely customizable. More than 200 antibiotics and antibiotic combinations are available for customers to design their individual antibiogram.
- It enables laboratories to test up to four patient samples on the same plate, reducing costs and speeding up time to correct diagnosis.
- Broth microdilution (BMD) is so far the only valid method recommended by EUCAST and CLSI for Colistin MIC determination.

Additionally, plates for antifungal agents MIC testing with up to 9 antimycotics in different concentrations are available.

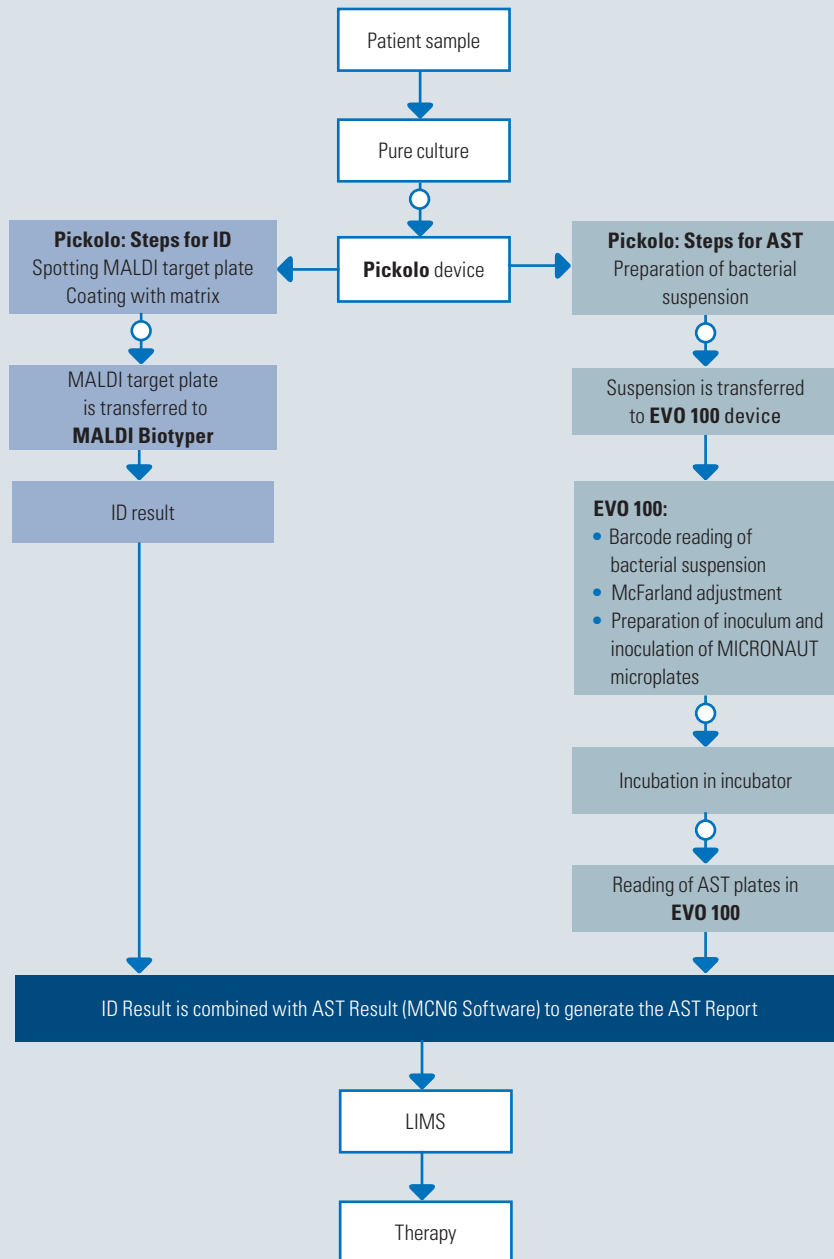
After incubation, the MIC, representing the lowest concentration of an antibiotic agent which prevents visible growth of a bacterium, is read visually or by semi- or fully automated systems. MIC results are interpreted according to European (i.e. European Committee on Antimicrobial Susceptibility Testing [EUCAST]) or international standards (i.e. Clinical and Laboratory Standards Institute [CLSI]) by comparing the evaluated MIC value with breakpoints listed in the respective documents [5,6]. This comparison is necessary to categorize test bacteria as susceptible, intermediate or resistant to an antibacterial agent.

However, many microbiological laboratories still determine antimicrobial susceptibility by the agar diffusion method. Here, a defined cell count of the test bacterium is spread out onto an agar plate. After drying of the agar surface, paper disks loaded with a specific concentration of a respective antibiotic agent are placed onto the agar. After a defined incubation period the diameter of the growth inhibition zone is visually detected and manually measured. The zone diameter around every antibiotic disk indicates the effectiveness of the respective antibiotic agent against the test strain. At first sight, the agar diffusion method seems to be an alternative to the broth microdilution, but there are some crucial differences. Dr. Scarparo highlights the benefit of the broth microdilution technique:

*“The agar disk-diffusion method provides qualitative results by categorizing bacteria as susceptible, intermediate or resistant, but it’s not appropriate for determining the MIC. Antimicrobial gradient methods combine the principle of dilution methods with that of diffusion methods in order to determine the MIC, but written data often overestimates the MIC value. Broth microdilution provides true MIC values and is also recognized by the EUCAST as the unique recommended method for highly effective backup drugs like tigecycline and colistin. Colistin is of great importance for the treatment of multidrug resistant, carbapenemase-producing Gram-negative bacteria. Broth microdilution allows a good correlation between the in vitro data and the in vivo evolution.*”

## ID/ AST Semi-Automated Sample Workflow

Combining Bruker's MALDI Biotyper & MICRONAUT Technology enables confident ID/ AST/ True MIC Results



○ Manual steps (e.g. Transfer of agar plates, MBT target or microplates from one device to another)

Note: The Tecan Freedom EVO® 75 with Pickolo™ Colony Picker is abbreviated as "Pickolo" while "EVO 100" stands for the Tecan Freedom EVO® 100 Micromic.

FIG. 1: Semi-automated workflow for identification and antimicrobial susceptibility testing



Fig 2: The MICRONAUT system offers customized plates for Antimicrobial Susceptibility Testing (AST) of Multi-Drug Resistant microorganisms.

*In this era of emerging multi-drug resistant bacteria, which have become an important clinical threat, antimicrobial susceptibility testing based on true MIC values is important to provide clinicians with detailed information for an optimal therapeutic approach and a better clinical outcome."*

True MIC is fundamental to a correct pharmacological approach to patient care and to support effective Antibiotic Stewardship Management. The aim of Dr. Scarparo and his team is to determine true MIC values to allow clinicians to determine the optimal therapy for infectious diseases.

In 2016, Dr. Scarparo implemented the M.I.B. (Modular Innovation in Bacteriology) system (from Biomedical Service s.r.l.), a system integrating Bacteriology Assistant Sharing Epidemiology (BASE) and LIMS software, MALDI technology and any other microbiological system connectable to BASE (e.g. Molecular Biology, Blood Culture System). The M.I.B. system was evaluated for inclusion

into the laboratory workflow. The system enables each laboratory to select the level of automation it requires and connects the modules through the BASE software. Subsequently, the system was used in both microbiology laboratories due to the speed and accuracy of analysis as well as up-to-date information, leading to better interpretation of patient needs and the resulting care.

## **The MICRONAUT system enters the laboratory**

In 2014, the MICRONAUT system was introduced in the M.I.B. system as part of the laboratory workflow alongside the MBT (for bacterial identification), to enable accurate susceptibility testing and fast MIC interpretation. The MICRONAUT system offered the laboratories a true MIC value on an easy-to-use system that could be customized to meet specific requirements of the Udine and Mestre laboratories. Due to the increasing antibiotic resistance complexity becoming more prevalent in the Italian market, the larger automated systems frequently do not provide the accuracy and breadth of antibiotics needed to determine at what point susceptibility occurs and at which concentrations. The MICRONAUT system offered a wide range of bug/drug concentrations which provided the level of data needed for the physicians to assess dosage requirements for patient treatment decisions.

The MICRONAUT system is based on the broth microdilution method for phenotypic MIC determination. It allows the user to choose between standard test plates or customized test plates. The latter enables laboratories to design a test plate and to include susceptibility interpretation according to EUCAST or CLSI. The resulting plates are simple to read and the MIC results are easy to evaluate by the laboratory staff.

Dr. Scarparo uses a set of MICRONAUT plates, customized in partnership with other microbiologists, infectious disease managers and pharmacologists. The layout design of the microtitration plates resulted from an evaluation of the different laboratory needs regarding the bacteria that should be tested, the choice of antibiotics, and the number of different antibiotic concentrations.

Dr. Scarparo explains the key benefits for the implementation of the MICRONAUT system:

*“The new system allows us to test the sensitivity of a bacterium to different antibiotic agents in vitro with accuracy, precision and reproducibility.”*

*The true MIC result is a key element for targeted therapy, especially in the era of multi-drug resistant bacteria.*

*Plates have been prepared for different groups of microorganisms (Gram-negative, Gram-positive, fastidious bacteria, streptococci, urinary pathogens). We have also observed increased standardization of the inoculation (via M.I.B.), traceability of the process (via BASE) and automated reading and interpretation of the antibiogram, all of which results in a faster and more accurate process.”*

Dr. Scarparo’s previous systems also contained an extensive list of test antibiotics and relative concentrations, but one of the systems (Sensititre) presented the major problem of bacterial inoculation below the international recommendations of EUCAST or CLSI [7]. The other previous method (Vitek 2) had reduced accuracy and reliability due to the fact that the MIC was ‘calculated’, and not the true MIC. In addition, users could not previously visualize microbial growth on the cards.



Fig. 3: The SUNRISE Reader allows standardized reading of the MICRONAUT antibiogram and gives proof of adjusted McFarland dilution.

Users are increasingly wanting to see their results visually, thus giving more confidence in their results. The involvement of the MICRONAUT system in the M.I.B. manages the whole susceptibility testing process, from colony selection, inoculum preparation over dispensing microtitration plates to the reading of the antibiograms by the SUNRISE reader. The McFarland (McF) adjustment according to the recommendations of the CLSI [7], for example, is performed by automated pipetting and dilution and is checked by a spectrophotometer inside one of the two robots. Dr. Scarparo adds:

*“The results obtained from the MICRONAUT system and automation are excellent, resulting in greater microbiological and clinical satisfaction.”*

## Integration with the MALDI Biotyper

Additionally, the MICRONAUT system can interface with the MBT platform. The MBT, which can identify more than 2,600 microbial species, together with the MICRONAUT system (for determination and interpretation of true MIC values), leads to more detailed information, enabling clinicians to de-escalate from an empiric therapy with

broad-spectrum antibiotics to targeted therapy. The MBT is connected to the BASE middleware to allow complete traceability of the isolates / samples entering the identification process.

The enhanced workflow, by combining robotic technology and the MICRONAUT system, has been a key aspect in the success for Dr. Scarparo and his team. The automation results in increased standardization and accuracy, decreased risk of human error and complete traceability of the entire process.



Fig. 4: The Micromic (TECAN FREEDOM EVO 100 platform) device combines barcode reading of the bacterial suspension, McFarland adjustment, inoculum preparation as well as inoculation and reading of MICRONAUT AST plates.



Dr. Scarparo explains the true impact of this:

*“With the MICRONAUT system at the heart of our workflow, we have been able to increase automation, remove the need for additional staff, and increase our efficiency and accuracy, leading to more clinically useful information.*”

*This is all set against a backdrop of an increase in workload due to the centralization of our microbiological lab service. Moreover, this type of organization and automation enables us to visually monitor all the processes performed in bacteriology and to validate both the routine and the urgent results.”*

### **Benefits to the patient**

Beside benefits in the laboratory, based on the implementation of the MICRONAUT system, Dr. Scarparo also sees benefits for patients:

*“Thanks to the MICRONAUT system, the quality of our results has improved. We can see the benefits for patients in a more targeted pharmacological therapy, which improves the patient’s clinical outcome, and reduces hospitalization time and associated costs. The hospital pharmacy has also been able to modify the list of antibiotics used.”*

### **The personalized medicine approach – the future?**

The MICRONAUT system is aligned with the current clinical trend for personalized medicine. As Dr. Scarparo describes:

*“Our system has been developed through extensive collaborations between our pharmacists and infectious disease managers. The resulting custom layouts are helping us to develop a precision medicine approach, delivering relevant therapies to patients much faster.*”

*This reduces costs by reducing both hospital stays and the initial use of therapies that are not effective. Targeted therapy improves clinical outcomes of patients – a desired outcome for all patients concerned.”*



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For more information on the MICRONAUT system, please visit <http://www.merlin-diagnostika.de/en/products/micronaut-special-plates/index.html>

For more information on Bruker's MALDI Biotyper please visit <https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-biotyper-systems.html>

For more information about the Microbiology department at Santa Maria Misericordia University Hospital, please visit <https://asuiud.sanita.fvg.it/distretti/dipartimenti-ospedali/osped-ale-smm/medicina-laboratorio/microbiologia>

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# Expert Insights

About the Microbiology Departments at Santa Maria Misericordia University Hospital, Udine and Angelo Hospital, Mestre

## Udine

The Department of Microbiology of the Hospital Santa Maria della Misericordia of Udine, located in Piazzale Santa Maria della Misericordia 15, is headed by Dr. Claudio Scarparo. The department is located on the ground floor of Pavilion 20 (Centro Servizi Laboratori) and provides a specialist consultancy service, in particular the most suitable tests to settle the diagnostic questions, the interpretation of the microbiological tests and antibiotic therapy. Udine performs organic material sampling, biological samples, and health education activities. The service has a small waiting room from which you can directly access the sampling reception clinic and the secretary desk and the acceptance activity takes place in two outpatient clinics reserved for the different types of collection.

## Mestre

Dr. Claudio Scarparo has been entrusted with the role of Chief for the Ospedale dell'Angelo, the Complex Operating Unit of Microbiology. In this role he also oversees a specialized structure of Laboratory Medicine that deals with diagnosis, prevention and treatment of infection diseases, through laboratory assistance activities, outpatient and consultancy in the local district of the ULSS 3 Serenissima.

The Ospedale dell'Angelo has been equipped with a Complex Operating Unit that will deal in a highly specialized way in infectious disease diagnostics, aligning itself with the main hospitals of the Region and Italy.

## About Bruker Corporation (NASDAQ: BRKR)

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