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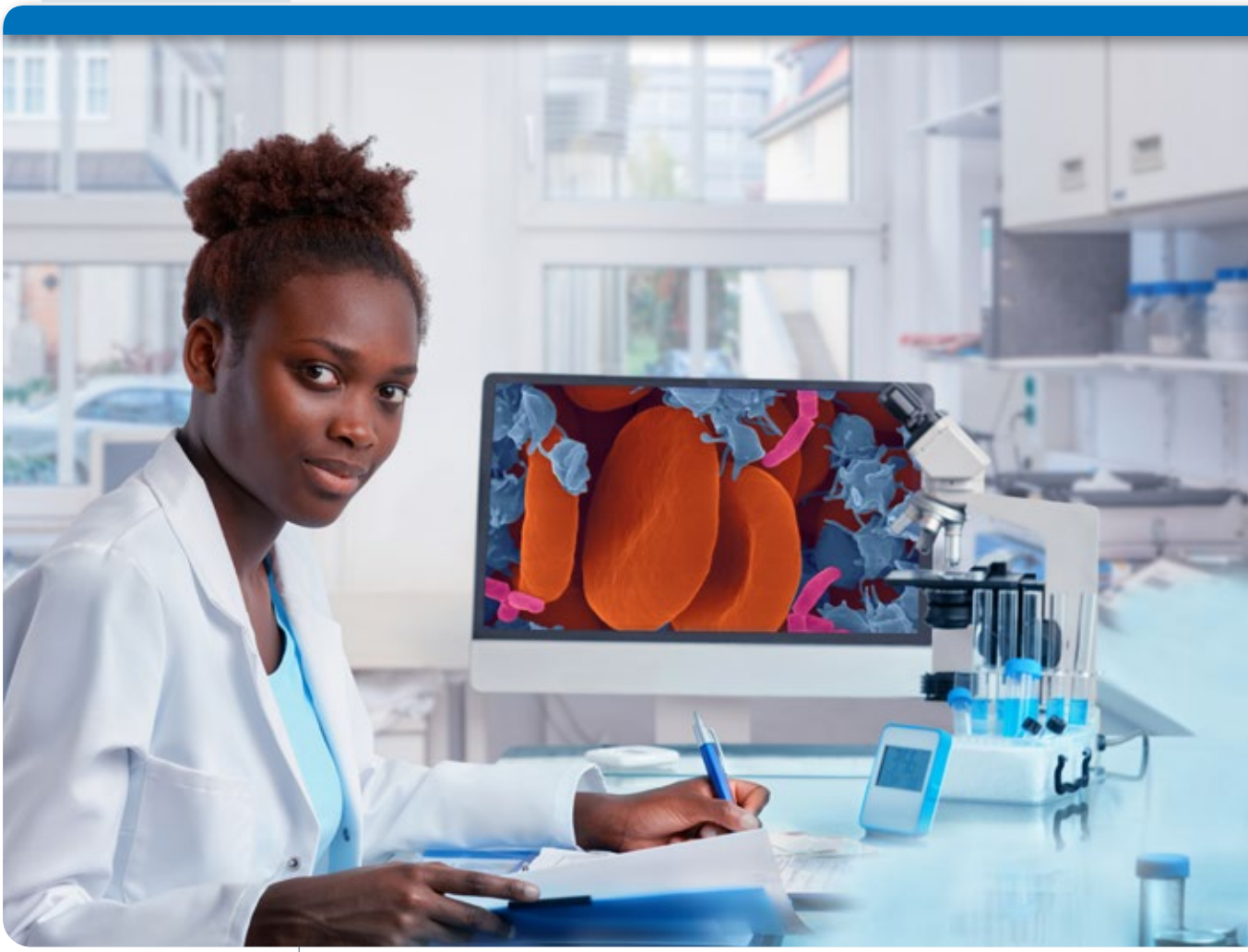

Expert Insights

- Advancing Infectious Disease Testing with MALDI-TOF Mass Spectrometry

MALDI-TOF

Innovation with Integrity

Advancing Infectious Disease Testing with MALDI-TOF Mass Spectrometry

Clinical scientists are speeding up turnaround time for pathogen identification in sepsis cases

Working with Bruker

Ike Northern, Director of Infectious Disease Testing and Immunology at CompuNet, provides hospitals with faster organism identification directly from positive blood cultures using Bruker's MALDI Biotyper® technology in combination with the MALDI Sepsityper® Kit.

"From what we've seen, the MALDI-TOF MS method is the most accurate and reliable way to identify microorganisms."

CompuNet, a full-service clinical reference laboratory located in Dayton, Ohio, serves physicians, hospitals, and healthcare industry organizations throughout Southwest Ohio. Founded in 1986, the company gives healthcare providers access to vast resources, including complex and specialized reference laboratory testing, and management support.

CompuNet has a menu of over 2,000 tests, ranging from routine blood screens to complex cancer diagnostics. It also provides advanced diagnostic services including anatomic pathology, hematopathology, immunohematology, coagulation, chemistry/toxicology, electrophoresis, infectious disease testing and immunology.

The infectious disease testing alone accounts for over 60,000 tests per month and includes microbiology (bacteriology), parasitology, mycology, molecular diagnostics, virology, serology, and mutation testing. These tests are performed in three different departments, within the Department of Immunology and Infectious Disease, and overseen by Director Ike Northern. He is also System Director for the five hospitals that are associated with CompuNet and manages all microbiology testing in these facilities, which is a core business component at CompuNet.

About the Author

Ike Northern has been employed at CompuNet Clinical Laboratories in Dayton, Ohio since 1989 and serves as the Director of Infectious Disease Testing. He has also held the position of Microbiology System Specialist for Premier Health System for the past 4 years. He has a BS in Medical Technology from the University of Cincinnati and a MS in Microbiology & Immunology from Wright State University. Ike has been active in training Clinical Lab Science students both in the classroom and the lab environment. He has also been a member of the American Society for Microbiology and the South-Central Association for Clinical Microbiology, where he has held several board positions. His duties include overseeing the Microbiology, Serology, and Molecular Departments at CompuNet. In addition, he is charged with standardization of microbiology testing across the Premier Health System in Dayton, Ohio and serving on the Antibiotic Stewardship and Infection Control Committees.

The microbiology laboratory at CompuNet performs approximately 315,000 tests per year, all subspecialties including bacteriology, parasitology, mycology, and mycobacteriology and is staffed 24/7. The specimen workflow is divided into benches based upon the type of sample or culture. For example, on day shift the laboratory has two urine benches, two wounds/body fluid benches, two anaerobic benches, a respiratory bench, a blood bench, a stool bench, a matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) bench, a susceptibility bench, a specimen preparation bench, an acid-fast bacilli (AFB)/mycobacteria bench, and three tech assistant benches that put away supplies, stain trichromes, and set up antimicrobial susceptibility tests (AST). The laboratory currently uses the MicroScan for AST testing, the BACTEC™ FX for blood culture monitoring, and the Bruker MALDI Biotyper® System for most microorganism identifications.

The department changed from a phenotypic identification technology to using MALDI-TOF mass spectrometry (MS) for organism identification in 2015. Ike explains the impact of this transition:

"All our studies for every type of organism have so far shown MALDI-TOF MS to be an excellent way to obtain identifications.

We've sent samples out for sequencing to get the 'gold standard' test for comparison, as well as traditional biochemical testing, and the MALDI Biotyper has always given us the best results, even on the Research Use Only (RUO) side. Once we started using the Bruker MALDI Biotyper, our physicians became used to obtaining the identification faster and recognized its positive impact on patient care. One of the areas of biggest impact was implementing the MALDI Sepsityper® Kit 50 for rapid microorganism identification from positive blood culture."

"The MALDI Sepsityper® Kit 50 data has allowed us to adjust antibiotic therapy according to our local antibiogram. This, at times, has allowed us to narrow or stop certain antibiotic treatments while in other cases, it has allowed us to escalate antibiotic coverage pending sensitivity data. It has become a crucial tool for our Antimicrobial Stewardship team."

Professor
Steven D. Burdette,
MD, FIDSA, FACP.

Dr. Burdette is Professor of Medicine, Wright State University, Boonshoft School of Medicine Infectious Diseases Fellowship Director; Medical Director, Infection Prevention, Miami Valley Hospital (Main, North, and South); and Medical Director, Antimicrobial Stewardship, Miami Valley Hospital and Premier Health (2019 IDSA Center of Excellence).

Sepsis: The Silent Killer

Rapidly identifying pathogens from patient samples is particularly crucial in cases of sepsis. Each year, at least 1.7 million adults in America develop sepsis, nearly 270,000 Americans die as a result of sepsis, and 1 in 3 patients who die in a hospital have sepsis. [1]

Although commonly referred to as blood poisoning or septicemia, sepsis is the result of the body's immune response damaging tissues and

organs when attempting to fight an infection. Sepsis is most commonly caused by bacterial infection, but viral and fungal infections can also trigger the disease. The seriousness of sepsis can vary from mild cases to severe sepsis and septic shock. Vital organs become damaged as the condition progresses and the entire body can be affected. Some patients may not deteriorate until the sepsis has progressed significantly, whereas other cases can rapidly decline and become fatal within a few hours.

Early recognition and treatment of sepsis is therefore vital for positive clinical outcomes. Rapid and appropriate antimicrobial therapy as a result of timely diagnosis can significantly improve chances of survival – for every hour that correct treatment is delayed, survival decreases by 7.6%.[2] The standard procedure for treating sepsis is to begin with broad-spectrum antimicrobials that target a range of microorganisms. While this may help to bring the infection under control, without knowing the identity of the microorganism, the effectiveness of broad-spectrum treatment is limited, and potentially exposes the patient to adverse health effects. Following correct identification of the microorganism, patients can be switched to targeted treatment, which often includes a combination of two or three drugs.

In addition to minimizing prolonged exposure to potentially aggressive treatment, another advantage of de-escalating from broad-spectrum antimicrobials sooner is to reduce the risk of microorganisms developing multidrug resistance. Changing to a more targeted therapy also aligns with the World Health Organization (WHO) Global Action Plan on Antimicrobial Resistance.[3] CompuNet is part of the Public Health System in Ohio, which is a partnership of healthcare professionals working together for the benefit of the public, tackling the increasing number of emerging organisms such as Cyclospora and Cryptosporidium (both gastrointestinal parasites), Hanta virus and Ebola. Additionally, many diseases that were once easily treated with novel antibiotics are re-emerging as resistant, leading to an ongoing public health threat. CompuNet plays a key role in reporting communicable disease results, not only to the healthcare provider that ordered the test, but also to the local health department as required by the Ohio Department of Health (ODH).

Premier Health, the health system CompuNet belongs to, has a number of programs it is involved in, such as the Antimicrobial Stewardship Program (ASP). Dr. Steven Burdette, the physician leading the ASP for the Health System gives talks to the Premier Health physicians (including CompuNet) about the importance of proper antimicrobial use. A key trend observed at the CompuNet laboratory is the increasing incidence of multidrug-resistant (MDR) organisms and resistance patterns, particularly carbapenem-resistant *Enterobacteriaceae* (CRE), extended-spectrum beta-lactamases (ESBL) and Methicillin-resistant *Staphylococcus aureus* (MRSA).

Implementing the Bruker MALDI Sepsityper Kit 50

"Recently, we started conducting additional testing for rapid identification and susceptibility testing from positive blood cultures (PBC) so we can get information to the physicians quicker for better patient outcomes," comments Ike.

Ike participates in the Antibiotic Stewardship Committee for the Premier Health System. There was a push from this committee to look at options for rapid identification of blood culture isolates, and studies started to show the dramatic impact that rapid identification could have on patient care. CompuNet investigated new technologies to enable this rapid identification such as fluorescence *in situ* hybridization using peptide nucleic acid probes (PNA FISH). However, this test was labor intensive and could only identify a select few organisms. The laboratory also compared a blood culture identification panel (BCID) used with multiplex polymerase chain reaction (PCR), which is simpler than PNA FISH but still only provided identification for 15-20 organisms – and was more expensive. This led the CompuNet microbiology laboratory to Bruker's MALDI Sepsityper Kit 50,

which can rapidly identify almost all clinically relevant organisms directly from PBC and is used in conjunction with the Bruker MALDI Biotyper. Bruker also covers many HAI organisms that molecular methods do not identify.

The time to identification from PBC with the MALDI Sepsityper Kit 50 is considerably shorter compared with traditional phenotypical methods. In the past, the laboratory would conduct a Gram stain from the PBC to confirm what kind of organisms were present, gram-negative or gram-positive, and this result was reported to the treating physician. The sample would then be sub-cultured and incubated for 18-24 hours. After incubation, *Staphylococcus aureus* or coagulase negative *Staphylococcus* was confirmed using a Staph Latex; providing an identification 24 hours after the blood culture became positive. A Pyrrolidonyl Aminopeptidase (PYR) test was performed for the presumptive identification of *Enterococcus* sp and a Rapid Strep Antigen Detection test for the presumptive identification of group A beta-hemolytic *Streptococci*. All other gram-negative and gram-positive isolates were set up on a traditional biochemical panel and incubated an additional 18-24 hours. In summary, all gram-negatives and all gram-positive isolates, except *Staphylococcus* sp., *Enterococcus* sp. and Beta *Streptococcus*, took approximately 48 hours from the time the blood culture flagged positive for identification confirmation. Ike comments on the speed of the new MALDI Biotyper with MALDI Sepsityper Kit 50 workflow:

"Now, we are getting results back within 3-4 hours from PBC. Physicians have become so used to this faster turn-around time that if we ever have maintenance on the equipment, they call us asking where the identification is."

CompuNet carried out a validation study of the MALDI Biotyper using the MALDI Sepsityper Kit 50 RUO (Research Use Only) Kit in May 2017, to verify the rapid identification ability of the system from PBC bottles. The laboratory tested 106 PBC bottles that were sent for routine testing at CompuNet microbiology laboratory.

Once the bottle flagged positive on the blood culture instrument, an identification was performed using the MALDI Sepsityper Kit 50 and an identification using MALDI-TOF and conventional biochemical ID from solid media was performed in parallel.

The Sepsityper software module was used to determine the confidence level for each identification obtained when a sample was prepared using the MALDI Sepsityper Kit 50. A log(score) between 1.8 – 3.0 was reported to the species level and a log(score) between 1.6 – 1.79 was reported to the genus level.

An accurate identification was obtained in 87/106 (82%) of the PBCs submitted to the study (Table 1). Further, out of the 87 accurate identifications obtained, 74/106 PBCs (70%) were identified to the species level, and 13/106 (12%) were identified to the genus level.

No identification was obtained for the remaining 19 PBCs (3 no peaks/16 low log(score)) and this was considered preferable over producing a wrong identification (Table 2).

CompuNet will be further investigating the isolates that resulted in no identification to achieve a better understanding of what variables can contribute to this result. For example, sample preparation technique, unique patient blood composition such as polycythemia, and length of time to process the PBC after removal from the blood culture monitoring instrument are only a few of the variables that can influence the probability of acquiring an identification from the MALDI Sepsityper Kit 50. All *Escherichia coli* were identified as *E.coli/Shigella*, as MALDI-TOF cannot differentiate these two organisms.

Table 1: CompuNet MALDI Sepsityper Kit 50 Validation Identified Organisms

Total Identified Organisms – 87/106 (82%)

Species Level 74/106 = 70%						**Genus Level 13/106 = 12%**	
S. aureus	17	*E. faecium*	2	*M. morganella*	1	Coagulase-Neg Staph	3
S. capitis	2	*S. anginosis*	1	*P. aeruginosa*	3	Alpha streptococcus	1
S. epidermidis	8	Group A Streptococcus	1	*P. vulgaris*	1	Beta streptococcus	1
S. haemolyticus	2	Group B Streptococcus	1	*S. marcescens*	2	*Bacillus* sp.	3
S. hominis	2	*E. cloacae*	1	*C. albicans*	1	*Corynebacterium* sp.	3
M. luteus	3	*E. coli*	18			*Enterobacter* sp.	1
S. faecalis	4	*K. pneumoniae*	5			*Bacteroides* sp.	1

The study demonstrated the MALDI Sepsityper Kit 50, utilizing MALDI-TOF technology, is a suitable method for rapid identification from PBC bottles. The validation resulted in an identification for 82% of the samples and allowed physicians to optimize patient treatment up to 48 hours sooner than before implementing the MALDI Biotyper and MALDI Sepsityper Kit 50. Ike comments on the clinical impact of this:

"If we get gram-negative rods and it is Pseudomonas *vs.* E. coli*, the treatment will be very different. Knowing the causative species allows physicians to rapidly administer the precise treatment for that pathogen.*

Table 2: CompuNet MALDI Sepsityper Kit 50 Validation Unidentified Organisms

Unidentified Organisms – (3 no peaks/16 log(score) ≤ 1.7)

No Identification 19/106 = 18%			
Coagulase-Neg Staph	7	*Lactobacillus* sp.	1
B. fragilis	1	*Moraxella osloensis*	1
E. faecalis	1	*Propionibacterium* sp.	1
E. coli	2	*S. aureus*	1
Fusobacterium sp.	2	*S. pneumoniae*	2

For S. aureus*, we have been using some other molecular methods so that once we get an identification on the MALDI Biotyper with MALDI Sepsityper Kit 50, we can go directly to a molec-*

ular method to inform physicians whether it is an MRSA isolate. Some labs use this method already, if they have gram-positive cocci in clusters, they will go directly to that test. But these tests are expensive, so if we can screen out the S. aureus *isolates with MALDI-TOF first, we can minimize molecular testing to these samples and reduce costs."*

As well as reducing costs for CompuNet, the MALDI Biotyper in conjunction with the MALDI Sepsityper Kit 50 workflow, saves healthcare providers money. The ability of physicians to de-escalate therapy sooner, from broad-spectrum to narrow-spectrum antimicrobials, ensures patients receive the most appropriate drug to fight the infecting organism, therefore leading to improved clinical outcomes. This can include shorter bed stays, which is a significant source of cost savings for hospitals, improves patient wellbeing and, ultimately, can reduce the likelihood of nosocomial infections and re-admission, as well as further complications such as post-sepsis syndrome (PSS).

In the US, many hospitals are paid by a disease related group (DRG) for inpatients. DRGs are payment categories that are used to classify patients, especially Medicare patients, for the purpose of reimbursing hospitals for each case in a given category with a fixed fee regardless of the actual costs incurred. For example, if a patient arrives in the ER and is admitted with sepsis as a diagnosis, the hospital is paid a specified sum to treat them. If the hospital can treat the patient and discharge quicker, the costs incurred will be less than the amount paid out, improving finances while providing better patient care. A majority of all MALDI Sepsityper Kit 50 charges in a traditional hospital setting would fall under a DRG because most individuals with sepsis would be an inpatient. This is not the case for CompuNet. Since CompuNet is a large reference testing site and performs testing for offsite institutions, the requests for the MALDI Sepsityper Kit 50 tests are paid using a Current Procedural Terminology (CPT). A CPT code set is a medical code set maintained by the American Medical Association (AMA) through the CPT Editorial Panel. The AMA provides a standard language and numerical coding system to accurately communicate to all stakeholders the medical and diagnostic services rendered by qualified healthcare providers. (CPT Professional 2020 (CPT / Current Procedural Terminology (Professional Edition)), American Medical Association, September 2019).

Table 3: CPT Codes Used for MALDI Biotyper Microbial ID and MALDI Sepsityper Kit 50 (Source: CPT Plus)

HCPCS Code	CPT Description
87015	Concentration
87076	Anaerobic identification
87077	Aerobic identification
87106	Organism identification, yeast
87107	Organism identification, mold
87118	Organism identification, mycobacteria

CompuNet simply charges the ordering institution for (2) CPT codes if a MALDI Sepsityper Kit 50 test is performed, one for the MALDI TOF ID and one for the concentration of the sample (Table 3).

The MALDI Sepsityper Kit 50, as a frontline application for the rapid identification from PBC, captures 82% of identifications without further testing. The MALDI Sepsityper Kit 50 provides a lower cost per test solution for rapid ID from PBC than molecular methods (Table 4).

"I think a lot of laboratories are realizing that they need to use MALDI-TOF MS technology for microbial identification,"

comments Ike. *"Many are now making this investment when they recognize the long-term cost benefits. The MALDI Sepsityper Kit 50 will be the next step for a lot of clinical microbiology laboratories. Many are currently using multiplex PCR tests but once you have the MALDI Biotyper instrument, it is more cost-effective to use the MALDI Sepsityper Kit 50 than large PCR panels."*

Clinical Impact on Sepsis Outcomes when Combining MALDI-TOF Technology with Sepsityper Kit in Sepsis: Case Studies

Early recognition, timely treatment and reassessment of antibiotic therapy are important actions to decrease the incidence of sepsis and improve patient outcomes. Using the Sepsityper Solution identification workflow reduces the turnaround time provided by traditional positive blood culture identification workflows, and identification results can therefore be reported significantly quicker to critical care providers. The impact of using the Sepsityper Solution:

- Efficient and faster result reporting to treating physicians – can save up to 48 hours compared to classical methods
- Earlier de-escalation and/or administration of targeted antimicrobial treatment, possible earlier change from intravenous (IV) to oral
- Improved patient management
- Reduced length of hospital stay and associated costs
- Early detection of blood culture contaminants, eliminating unnecessary AST testing.

Case 1

A 29-year-old male with a history of intravenous heroin drug abuse was admitted for fever and right arm pain. The patient was started

Table 4: Compunet Clinical Laboratories Comparing Cost Per Test using MALDI Sepsityper Kit 50 and Molecular Method

- 60,000 Blood Cultures a year (culture = set with aerobic/anaerobic bottle)
- 11% overall positivity rate (60,000 x 11% = 6,600)
- MALDI Sepsityper Kit 50 performed on approximately half of total PBC's (Sepsityper performed on only one set out of two)

11% Positivity Rate	60,000 Blood Cultures Annually (sets)	Annual Cost to Perform Test
Total Positive Blood Cultures	6,600	
# of Sepsityper Kit Samples Performed	3,300	
# of MultiPlex PCR ($105.00 Cost Per Test)	3,300	$346,500
MALDI Sepsityper Kit 50 ($9.50)	3,300	$31.350
Add Tech Time for MALDI Sepsityper Kit 50		$38,000
Annual Savings Using MALDI Sepsityper Kit 50		$277,150.00

empirically on vancomycin and piperacillin-tazobactam after blood cultures were obtained. A computed tomography (CT) scan of the arm revealed cellulitis. 18 hours after admission blood cultures were positive for gram-positive cocci based on Gram stain, after which the admitting hospitalist assumed endocarditis due to the patient's social history, and ordered a Trans-esophageal Echocardiogram (TEE) to further evaluate. Approximately one hour after the Gram stain results, the MALDI Biotyper, in conjunction with the MALDI Sepsityper Kit 50 identified *Streptococcus pyogenes*. The results of the blood culture were sent to the Antimicrobial Stewardship team, which recommended that the antibiotics be narrowed to ampicillin and a consult with an Infectious Disease (ID) physician was recommended. The Infectious Diseases service cancelled the TEE as they were not concerned about infectious endocarditis, but an ultrasound of the arm was performed which revealed an extensive thrombosis of the axillary and subclavian vein. Vascular surgery was consulted but no intervention was required. The patient was treated with six weeks of IV ampicillin with good clinical response.

Case 2

A 77-year-old female was admitted for weakness and failure to thrive. Blood cultures were obtained in the emergency department and vancomycin and piperacillin-tazobactam were started empirically. On the first day of hospitalization the blood cultures were positive for gram-positive cocci and the MALDI Sepsityper Kit 50 results demonstrated *Enterococcus faecalis* in two out of two sets, which was reported to the Antimicrobial Stewardship team. Infectious Disease was consulted, which immediately stopped the vancomycin and piperacillin-tazobactam after 24 hours. Based on the local antibiogram, ampicillin and gentamicin were initiated, and a TEE was ordered. On the second day of hospitalization, the TEE was performed which revealed a large vegetation on the mitral valve. The patient was evaluated by the cardiothoracic surgery service on the day of the TEE, which recommended medical management only. The patient was given six weeks of ampicillin and gentamicin without issue.

Case 3

A 41-year-old male with a complicated and prolonged hospitalization following abdominal surgery developed fever and an elevated white blood cell count on hospital day 21. His peripherally inserted central catheter (PICC) line had been present since admission. Blood cultures were collected, and vancomycin, ceftriaxone and fluconazole were started empirically. On hospital day 22, blood cultures were positive for gram-negative rods and the MALDI Sepsityper Kit 50 demonstrated *Enterobacter cloacae*. The Antimicrobial Stewardship team was notified, and fluconazole and vancomycin were discontinued based on the Gram stain results. The Antimicrobial Stewardship team noticed that three months prior, *Enterobacter cloacae* was found in a urine culture of the patient, so recommended changing the ceftriaxone to meropenem while sensitivity results were pending. MicroScan results available on hospital day 24 confirmed that the *Enterobacter* was a ceftriaxone-resistant strain and therapy with meropenem was continued for 10 days. His PICC line was exchanged and the patient responded well to therapy.

Working with Bruker

Two or three years before purchasing the MALDI Biotyper, Ike had begun learning of the benefits of MALDI-TOF MS technology for rapid micro-organism identification. As part of the research process, Ike visited two different laboratories to compare the instruments available on the market and to talk to current users to judge the best fit for CompuNet. One of the specifications Ike preferred over the competitor system was the target plates used on the MALDI Biotyper. The sample wells were slightly larger and easier for technologists to use. The footprint of the Bruker system, being a benchtop instrument, is considerably smaller than many competitive systems and is a significant advantage for most laboratories where space is often limited.

"All of this led us to the purchase of the MALDI Biotyper, and we couldn't be happier,"

comments Ike.

"Bruker came in and carried out extensive training with our key users, including running the instrument and troubleshooting issues. When it came to training our technologists, it was really quite straightforward. We started off running samples in duplicate, but very quickly eliminated this step and used one spot position on the plate.

The uptime for the MALDI Biotyper is another benefit – I don't think we've had any major unplanned downtime."

Continuing success into the future

Ike is hoping to obtain a second MALDI Biotyper to increase CompuNet's identification capacity:

"The current system is in use almost all day long, so a second system would really help our capacity.

We would be able to conduct many other tests, such as identifying more wound and urine isolates, to make an impact in other areas beyond sepsis."

Ike describes the technological developments he would like to see in the future:

"We do a lot of testing. When we see something that we think would be an improvement on the system we are pretty open about it.

One of the things I would like to see would be some more resistance markers.

I know Bruker has released a new MBT Subtyping Module, which contains markers for Klebsiella pneumonia *carbapenemase enzyme (KPC)-producing* Enterobacteriaceae*, MRSA,* Bacteroides fragilis cfiA *positive strains,* Elizabethkingia *species, and* Mycobacterium chimaera*. We're really excited about this, because these markers will be really important for the future fight against MDR organisms."*

For more information on CompuNet, please visit https://www.compunetlab.com/

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

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Expert Insights

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About CompuNet

CompuNet is a clinical laboratory serving Southwest Ohio and is part of the Premier Healthcare System. Founded in 1986, CompuNet is a clinical diagnostic laboratory offering over 2,000 accessible, responsive, and reliable lab tests for physicians, hospitals, patients, and employers in our communities. CompuNet provides a customized partnership with healthcare providers tailored to their unique needs.

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Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology.

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