

Automation allows simultaneous antimicrobial susceptibility testing and MALDI-TOF sample preparation for bacterial identification

A Freedom EVO®-based platform for automated antimicrobial susceptibility testing and MALDI-TOF sample preparation is proving beneficial to the Medical Laboratory Bremen, offering enhanced reproducibility compared to manual processes and greater accuracy of results.



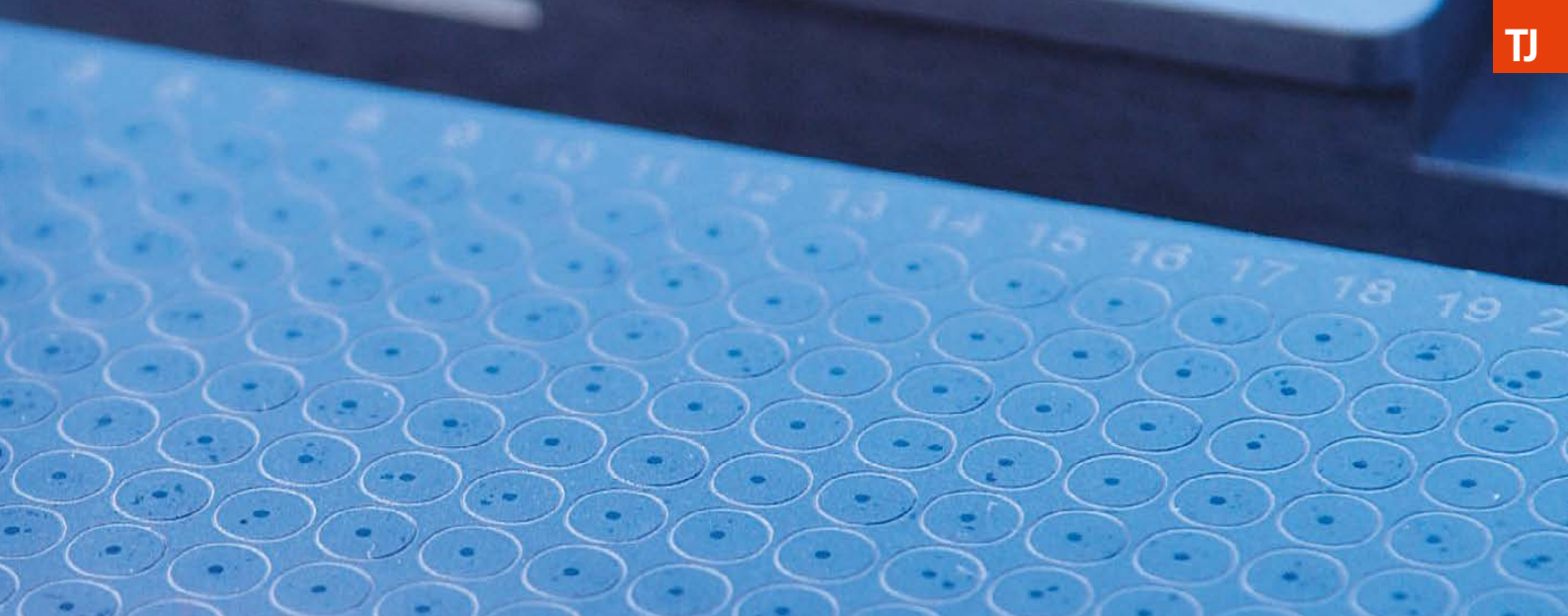
The Medical Laboratory Bremen in Germany is a private laboratory offering specialist analytical services across all areas of laboratory medicine and medical microbiology to clients throughout the country, as well as the rest of Europe. The laboratory is renowned for employing the most advanced analytical technology available, and has worked extensively with microbiological diagnostic products manufacturer MERLIN Diagnostika to tailor the Freedom EVO-based MICRONAUT ASTroID workstation to meet its particular needs. This automated platform combines sample preparation for microbial identification by MALDI-TOF

(matrix-assisted laser desorption ionization - time of flight) mass spectrometry (MS) with antimicrobial susceptibility testing (AST) by microbroth dilution. Dr Johann-Wolfgang Wittke, the laboratory's specialist in microbiology and infectious disease epidemiology, explained: "Typically, we receive around 450 medical microbiology analysis requests a day from the state of Bremen and neighboring Niedersachsen, performing measurements to determine the antimicrobial resistance of *E. coli* and other enterobacteria, *Pseudomonas* and other non-fermenters, oxidase-positive fermenters, many different gram-positive rods and cocci, as well as some yeasts. We needed to

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Mariola Richter and Dr Johann-Wolfgang Wittke perform automated antimicrobial susceptibility testing and MALDI-TOF sample preparation on the Freedom EVO-based MICRONAUT ASTroID workstation



automate this process; it was impractical to continue using manual procedures for AST and bacterial identification sample preparation. We acquired our current ASTroID system in January 2011, and this was the start of a collaboration with MERLIN to optimize the platform and implement it in our daily routine testing procedures.”

While agar dilution is considered the gold standard for AST of fast-growing bacteria, it places considerable demands on laboratory resources and, for a routine testing laboratory, the microdilution technique employed by the ASTroID system offers a cost-effective alternative, delivering accurate, precise results with minimal hands-on time. The ASTroID workstation is based on a Freedom EVO platform equipped with a four-channel Liquid Handling Arm, a PosID™ barcode scanner, a temperature-controlled adapter for MALDI-TOF target plates, plus carriers for up to nine MICRONAUT AST plates and one 96-well dilution plate. After barcode identification of the microplates and sample tubes, the ASTroID takes aliquots of each sample for preparation of the AST inoculum, or spotting onto a MALDI-TOF target plate. The inoculation, incubation, measurement and evaluation of the AST plates are performed on the workstation, according to the MICRONAUT standard operating procedure. For MALDI-TOF MS, the system adds a matrix solution to the dried sample on the target plate, which is then transferred to the mass spectrometer. After MALDI-TOF analysis, the identification result is transferred to the MICRONAUT 6 software via direct online connection and linked to the corresponding AST pattern. The integrated expert system checks the plausibility of the results, determining resistance mechanisms



and providing clinical interpretation. Dr Wittke continued: “We perform antimicrobial resistance testing in 96-well microplates, mainly using the breakpoint method to determine whether an organism is sensitive, intermediate or resistant, and always include a growth control. Depending on how many wells are needed for each organism, we can analyze as many as four micro-organisms per plate. In special circumstances, for example multi-resistant organisms, determination of minimum inhibitory concentration is required; this uses an entire plate for one bacterium.”

“Automation offers several advantages over manual testing, in particular the reduction in hands-on time required and

improved reproducibility, making the process much less labor intensive and time consuming than manual pipetting of numerous microplates. Hands-on time is reduced by 30 to 40 % and, because there is a significant reduction in variation, reliability and precision are much better; far fewer tests need to be repeated. When the analysis is performed manually, around 10 to 15 % of tests are likely to be repeated. Using automation, this falls to 3 % or less. In addition, we find that while this method may take slightly longer than the commonly used agar diffusion technique, greater accuracy is achieved for beta-lactamases (including extended-spectrum), plasmid-encoded AmpC and carbapenemases, enabling us to provide more reliable results, allowing the patient to receive the most appropriate treatment earlier.”

“Our collaboration with MERLIN has been very successful. The Freedom EVO-based ASTroID workstation is fully validated and has been in routine operation in our laboratory for around three years, giving us the capability to simultaneously perform resistance testing and MALDI-TOF sample preparation. The system is now well-established, and works very reliably, providing precise, accurate results,” concluded Dr Wittke.

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