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MALDI-TOF MS in the Microbiology Laboratory: Current Trends

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Abstract
Within less than a decade matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a gold standard for microbial identification in clinical microbiology laboratories. Besides identification of microorganisms the typing of single strains as well as the antibiotic and antimycotic resistance testing has come into focus in order to speed up the microbiological diagnostic. However, the full potential of MALDI-TOF MS has not been tapped yet and future technological advancements will certainly expedite this method towards novel applications and enhancement of current practice. So, the following chapter shall be rather a brainstorming and forecast of how MALDI-TOF MS will develop to influence clinical diagnostics and microbial research in the future. It shall open up the stage for further discussions and does not claim for overall validity.

Introduction
Within less than a decade MALDI-TOF MS has entered the microbiological diagnostic laboratories around the world providing a fast, cheap and reliable tool for identification of bacteria and fungi cultivated on agar plates or in liquid media (Kostrzewa and Schubert, 2016; Schubert and Kostrzewa, 2016). First steps have been made to introduce MALDI-TOF MS for antibiotic and antimycotic resistance testing as well as for some typing applications. The future development of MALDI-TOF MS for clinical diagnostics will likely be driven by technical advances, on the machine and software side as well as regarding sample preparation, and integration of MALDI-TOF MS in fully automated workflows pushing forward already established application. On the other hand, new fields of application for MALDI-TOF MS may be envisaged extending the use on imaging and maybe even *in vivo* applications. This development should bring together different laboratory disciplines like pathologists, medical microbiologist and pharmacologists.

Improvement of current MALDI-TOF MS technologies
One of the foreseeable main developments for microbiological laboratories in the next future will be the implementation of automated processes in the lab workflow, in particular in diagnostic laboratories. Several diagnostic companies, e.g. BD Kiestra (Drachten, The Netherlands) and Copan (Brescia, Italy) have developed fully automated workflows for the culture based microbiological laboratories (Matthews and Deutekom, 2011). Here, the allocation and barcode labelling of agar plates, streaking of the samples onto the plates and inoculation of liquid culture media as well as the transfer of culture media to specialized incubators is managed by a fully automated robotic system. Moreover, growth on the plates can be monitored and documented by digital imaging at defined time points. The complete implementation of MALDI-TOF MS in these workflows, however, is still in its infancy as several steps in the 'from-the sample-to-result' workflow are still done manually. Today, the identification using MALDI-TOF MS is performed on demand initiated by lab personnel at different time points. The selection of respective colonies for further identification is essentially based on the skills and experience of the staff. In future, a fully automated, seamless MALDI-TOF MS workflow for identification could be triggered by software assessing the images of the agar plate for putatively different colonies. These colonies then could be prepared automatically for the MALDI-TOF measurement. Subsequently, sample targets might be transferred to and introduced into the mass

spectrometer in an automated manner, without human interaction. Depending on the identification results microbouillons could be inoculated by robotics to initiate antibiotic resistance testing. From this, the system could be adjusted to perform further tests like resistance testing only from previously defined bacterial species in case specimen from mucosal surfaces are investigated. In samples from primarily sterile body-sites all growing micro-organisms would be selected. The new generation of MALDI-TOF MS equipped with high-performance lasers (up to 10.000 laser shots per second versus 200 Hz operating in the fastest commercial microbial ID MALDI-TOF MS available today) gives a glimpse of how powerful the future technical development of MALDI-TOF MS will be. As soon as rapid MALDI-TOF MS based resistance testing and integrated automated typing of resistance or virulence markers will enter the stage, a further significant step towards speeding up the diagnostic workflows in microbiological laboratories will be in our grasps.

Immuno-MALDI and imaging mass spectrometry: potential for further value of MALDI-TOF MS in microbiology

The application of MALDI-TOF MS as a standard analysis method in clinical routine diagnostics beyond microbial identification has not yet been established. On the other hand, in view of a deeper understanding of physiological processes and the discovery of new biomarkers, the requirements for sophisticated analysis methods are growing. Additionally, modern disease management demands for better diagnostic tools to improve early diagnostics and monitoring of therapeutic intervention. Established immunological methods for biomarker detection and quantification, i.e. ELISA (enzyme-linked immunosorbent assay) or RIA (radio immune assay) rely on the availability of two antibodies, one to capture the target molecule and the second for signal generation, and use a single principle of detection for both selective steps. Thereby, these conventional detection methods do not reveal any further molecular information. The combination of immunoaffinity and MALDI-TOF MS, Immuno-MALDI, may provide much better specificity because the antibody is only used for antigen capturing whereas the MALDI-TOF analysis determines an intrinsic characteristic of the respective molecule of interest, i.e. its molecular weight. Thus, the specificity of analysis can be significantly improved by using a capture antibody combined with specific mass identification by MALDI-TOF (Sparbier *et al*., 2009). This can be of particular advantage in case small proteins and peptides are in the focus, which hardly offer an epitope for a second antibody. This holds also true if secondary modified molecules have to be differentiated, e.g. glycosylated or phosphorylated molecules. Quantification can be achieved by spiking and co-capture of stable-isotope labelled, heavier peptides of exactly the same composition. For these labelled peptides a calibration curve has to be established. Development of methods to quantify hypertension markers has already been described (Reid *et al*., 2010; Camenzind *et al*., 2013). Applications of this technology to solve microbiology-related problems, e.g. toxin quantification, still have to be demonstrated.

Another MALDI-TOF MS based technology, imaging mass spectrometry (IMS), has been recently developed and might be applicable in diagnostic laboratories in the near future. This imaging technique allows molecular mapping of different kind of biomolecules in their natural environment (Walch *et al*., 2008; Neubert and Walch, 2013; Aichler and Walch, 2015). IMS has already entered the field of tissue-based research providing unique advantages for analysing tissue specimen in an unprecedented detail. As a mass spectrometry-based technology combined with the two-dimensional mapping of molecules it enables the direct correlation of tissue histology and proteomic, metabolomic or lipidomic information. IMS allows for a label-free analysis of numerous analytes of multiple types. Most encouragingly for clinical purposes, this technology keeps the tissue intact, thereby allowing investigation of tissue morphology by traditional clinical standard procedures, in parallel (Neubert and Walch, 2013). IMS can analyse a multitude of analytes ranging from proteins, peptides, protein modifications to small molecules, drugs and their metabolites as well as pharmaceutical components, endogenous cell metabolites, lipids, and other analytes in situ, without any labelling. While current research and development in this area is mainly focused on cancer diagnostics and inflammatory diseases like inflammatory bowel disease (M'Koma, 2014; Kriegsmann *et al*., 2015), IMS might also have a future role in microbiology research and even diagnostic. Direct profiling of molecules from tissue samples by MALDI-TOF MS provides a means to study the pathogen-host interaction and to discover potential markers of infection (Moore *et al*., 2014a,b). Further, IMS is able to investigate metabolic exchange factors of intraspecies, interspecies, and polymicrobial interactions (Yang *et*

al., 2012). Although still in its infancy, the application of imaging mass spectrometry in microbiology therefore might allow for fundamentally new insights into microbial communities.

The future will show in which way MALDI-TOF MS is going to further revolutionize microbiology diagnostics.

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