news digest #017

# Negative ion mode: A sirius feature unlocking new opportunities

## Lipids: A vital component in the biological world

Lipids hold a pivotal role as functional and structural building blocks within cells, participating in a multitude of critical functions in biological systems. They contribute to the creation of cellular membranes, facilitate cell signaling, store energy, and enable cell recognition.[1,2,3] To fulfill this wide array of functions, cells contain an extensive variety of lipid molecules, differing in terms of fatty acid count, backbone nature, head group type, fatty acid chain length, and double bond count.

Given their remarkable diversity, lipids, together with proteins, emerge as valuable potential biomarkers for microbial identification. As a result, we have seen numerous studies trying to validate large-scale, cost-effective lipid profiling for microbial identification purposes.

Among various methods, MALDI-TOF MS in negative ion mode has proven to be a powerful analytical tool for lipid profiling. In order to make this research field more accessible to microbiologists, a dedicated software module, the MBT HT LipidART Module, has been developed for MALDI-TOF MS analysis in the negative ion mode on Bruker's MALDI Biotyper® sirius System.

Presently, lipid profile analysis using the MALDI Biotyper® sirius System has found research applications in microbial identification, the detection of antibiotic resistance, and food science research.

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## Lipid profiling for bacterial identification: *Shigella species* and *Escherichia coli*

While the differences in lipid profiles between Gram-positive and Gram-negative bacteria are well established, it's important to note that lipid composition can also vary among species within the same Gram category.[4] Consequently, lipid profiling has emerged as a complementary approach for bacterial identification when protein-based methods fall short.

For example, distinguishing between *E. coli* and *Shigella*, two closely related species, is particularly challenging due to their high genetic similarity. Research by Brenner et al. indicates that the nucleotide similarity between *Shigella* and *E. coli* falls within the range of 80-90%, while other *Escherichia* species are more genetically distant.[5,6] Despite this genetic proximity, *Shigella* species and *E. coli* exhibit distinct phenotypes in terms of epidemiology and pathogenicity, underlining the need for efficient discrimination methods.

Because of the genetic similarity, traditional methods based on ribosomal protein or 16S rRNA sequence comparisons have proven ineffective in distinguishing between *E. coli* and *Shigella*. As a result, more expensive methods such as duplex real-time PCR and double locus variants (DLV), utilizing conserved housekeeping gene (e.g., *rpoB* and *mdh*) sequence data, as well as whole-genome sequencing (WGS) analyzing k-mers and SNPs have been widely used to differentiate these two species so far.

Recent developments however indicate that MALDI-TOF MS analysis of lipids, in negative ion mode, can offer a more cost-effective, rapid, and reproducible method for distinguishing between the closely related species *E. coli* and *Shigella* spp.[7] Compounds like lipid A and Lipopolysaccharides (LPS) in *Shigella* species exhibit differences, for example in acylation numbers or the presence of phosphoethanolamine groups, in comparison to *E. coli* lipids.[8] Research by Pizzato and colleagues has demonstrated that routine MALDI-TOF MS analysis in negative ion mode can serve as a reliable tool for rapid pathogen identification. This method addresses the unmet needs in clinical microbiology diagnostics and assists in determining effective treatment options.[7]

## Lipid profiling for rapid identification of antimicrobial resistance

Antibiotic resistance is a pressing global concern and a major public health threat.[9] The limited availability of new antimicrobials and the spreading of multidrug-resistant (MDR) organisms have made us increasingly reliant on a few state-of-the-art antibiotics for the treatment of MDR Gram-negative bacteria. At the forefront of these latest generation agents, we find compounds such as polymyxin, polymyxin B, and colistin.[10,11] Unfortunately, the elevated use of colistin for MDR bacteria has led to the emergence of colistin resistance.

Detecting resistance to these antibiotics quickly is crucial for improving the treatment of patients infected with MDR bacteria. However, the current standard method for detecting polymyxin resistance is time-consuming (taking 24 hours) and challenging to perform in clinical and veterinary laboratories.[12,13] Additionally, conventional methods like PCR-based testing for colistin resistance are complicated due to the extensive range of chromosomal mutations that can lead to colistin resistance.[12]

Given that resistance to this antibiotic family in most Gram-negative bacteria arises from modifications to the lipid A portion of their LPS, researchers have focused their efforts on exploring lipid profiles as a means to detect colistin resistance.[14,15]

Recent publications have shown that lipid profile analysis using the MALDIxin assay, adapted for the MALDI Biotyper® sirius, offers an unbiased, rapid, reliable, cost-effective, and high-throughput approach to detect colistin resistance in clinical isolates of various Gram-negative bacteria.[13,16,17]

## Lipid profiling for food fraud detection

In addition to playing a crucial role in the human diet, lipids serve various functions, and their lipid profile defines the identity and quality of our food. Lipid profiling offers a promising solution for authenticating food products and uncovering mislabeling and food fraud. Hence, it's imperative to employ robust analytical methods to characterize and detect potential alterations in food lipid profiles, even at trace levels.

MS-based analysis has emerged as a rapidly growing and widely used method for lipid analysis in food, providing an alternative to traditional methods. For instance, the laboratory of Dr. Gerald Larrouy-Maumus at Imperial College London has developed a workflow for discriminating between bovine milk and non-dairy milk by analyzing the lipid fingerprint using the Bruker MALDI Biotyper®. This research has also demonstrated the capability of lipid profiling to detect milk adulteration.[18]

One significant advantage of this technology is its ability to provide a comprehensive spectrum of lipid content in a single step. The lipid fingerprint is obtained simply by applying a sample dilution with added matrix onto the MALDI target, which is then placed into the instrument.

## Conclusion

Lipids, a diverse group of molecules, serve a multitude of crucial biological functions in nature, such as providing structural support in cell membranes, acting as signaling molecules, and serving as energy reserves. The wide diversity of lipids suggests their potential as biomarkers for microbial identification and the study of antibiotic resistance. Furthermore, lipid profiling can be a valuable tool for assessing the identity and quality of food.[19]

It's clear that clinical, veterinary, and agri-food laboratories require access to technology that is not only swift and cost-effective but also guarantees reproducibility. Among the available methods, MALDI-TOF MS with negative ion mode has clearly established itself as a powerful tool for lipid profile analysis.

Nonetheless, it's important to acknowledge that despite recent progress, challenges remain to be addressed before the analysis of lipid profiles by MS can be established as the standard for validation and quality control. The availability of benchtop MALDI-TOF systems offers promise in overcoming some of these challenges, marking a significant step forward in the field.

One of the pioneering benchtop systems with the capability to directly analyze lipid profiles is the MALDI Biotyper® sirius System by Bruker. The MBT HT LipidART Module, specifically dedicated to the analysis of lipid spectra, has proven to be a game-changer. Some studies have already demonstrated that this system is adept at detecting strains resistant to colistin and distinguishing between *E. coli* and *Shigella*. Additionally, it has been shown that the MALDI Biotyper® sirius holds the potential to directly analyze the lipid profiles of various food items, including milk.

These capabilities make the MALDI Biotyper® sirius a promising candidate to become a standard analytical instrument for lipid profiling in laboratories in the future. It has the potential to significantly propel the field of lipid analysis and its applications across various domains, ultimately aiding in microbial identification, antibiotic resistance assessment, and food quality control.

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