L-24
ANALYSIS OF BACTERIAL LIPOPOLYSACCHARIDES ("SEROTYPING") BY MALDI-TOF MASS SPECTROMETRY: MISSION IMPOSSIBLE?

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In the last decade, the use of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) revolutionary changed clinical microbiology. The technology allowed significant shortening of turnaround time needed for taxonomical identification of bacteria and fungi, as well as rapid identification of microbes from blood cultures and other clinical specimens, e.g., urine (1, 2, 3). Similarly, applications for antibiotic resistance determination have been also developed and validated for the use in clinical diagnostics (4). Among them, the routinely used is beta-lactamase activity determination by a detection of the changes of molecular mass of indicator beta-lactams, or detection of polymyxin resistance using analysis of lipid A of lipopolysaccharides (5).

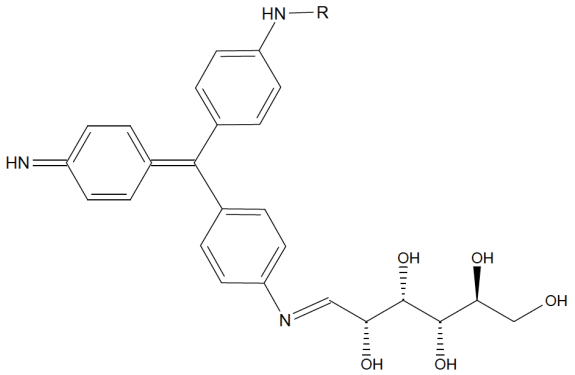
As MALDI-TOF MS provides efficient and rapid species identification, there is a key issue whether the method can be used for epidemiological typing directly from obtained spectra. So far, no general typing algorithm was proposed but only specific peaks representing significant epidemiological markers have been identified in some species. Very recently, artificial intelligence for spectra analysis has been described as a promising tool for prediction of antibiotic resistance and epidemiological typing (6).

Despite the use of artificial intelligence methods for analysis of big data, MALDI-TOF MS should be considered as a biochemical tool allowing precise analysis of molecules based on their molecular weight and fragmentation characteristics. Thus, we believe that scientific community should not resign ourselves to exact identification of detected molecules/peaks. For such an analysis, it is usually insufficient to analyze crude bacterial extract without further processing, i.e., specific extraction and enhancement of MALDI-TOF MS-based ionization (1).

Recently, MALDI-TOF MS-based analysis using cell-wall lipid fingerprinting was developed not only for the detection of colistin resistance, but also for identification and typing of some bacteria with the cell-walls rich for lipids, i.e., *Mycobacterium* spp. Similarly, periplasmic proteins, e.g., beta-lactamases, can be specifically isolated and detected via MALDI-TOF MS (7).

Surface structures of bacterial cell wall play an important role in antibiotic resistance, typing, and in vaccination strategy as most of them are common targets of immune response to the infection. The most important surface structures are lipopolysaccharides (e.g., *Enterobacterales* including *Escherichia coli* and *Salmonella* spp., *Pseudomonas aeruginosa*), polysaccharides (e.g., *Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus* spp., and *Streptococcus* spp.), and proteins including flagella and outer membrane proteins (e.g., *E. coli*, *Neisseria meningitidis*, *Streptococcus pyogenes*) (8). Analysis of polysaccharides is hindered by a poor ionization ability, especially in comparison to detection of proteins or lipids where there are many protocols available. On the contrary, in the field of polysaccharide detection there is a need for innovative approaches.

We present here a novel approach for saccharide derivatization and polysaccharide fingerprinting that allows detection of those structures by MALDI-TOF MS as well as LC/MS. The method can be used not only for bacterial typing but also for identification of bacteria and fungi directly from clinical specimens.



Scheme 1. **Glucose derivatization for MALDI-TOF MS measurement.**

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REFERENCES

1. Clark A. E., Kaleta E. J., Arora A., Wolk D. M: Clin. Microbiol. Rev. *26*, 547 (2013).
2. Buchan B. W., Ledeboer N. A: Clin. Microbiol. Rev. *27*, 783 (2014).
3. Kostrzewa M: Expert Rev. Proteom. *15*, 193 (2018).
4. Hrabák J., Chudáčková E., Walková R: Clin. Microbiol. Rev. *26*, 103 (2013).
5. Pizzato J., Tang W., Bernabeu S., Bonnin R. A., Bille E., Farfour E., Guillard T., Barraud O., Cattoir V., Plouzeau C., Corvec S., Shahrezaei V., Dortet L., Larrouy-Maumus: MicrobiologyOpen *11*, e1313 (2022).
6. Weis C., Cuénod A., Rieck B., Dubuis O., Graf S., Lang C., Oberle M., Brackmann M., Søgaard K. K., Osthoff M., Borgwardt K., Egli A: Nature Med. *28*, 164 (2022).
7. Solntceva V., Kostrzewa M., Larrouy-Maumus G: Front. Cell. Infect. Microbiol. *10*, 621452 (2021).
8. Frasch C. E: Methods Enzymol. *235*, 159 (1994).