MILESTONE 16

Signatures of disease

Treating a disease effectively depends on making the right diagnosis. For a large part of the twentieth century, medical laboratory diagnostics relied on often laborious and occasionally imprecise staining procedures or biochemical assays. A fast, sensitive and reliable technique to detect pathogens such as bacteria or biomarkers of disease in tissue or bodily fluids was badly needed, but had remained tantalizingly out of grasp.

Mass spectrometry, with its power to accurately quantify and identify miniscule amounts of a dazzling array of biomolecules, had just the right mix of capabilities for analyzing bacteria but was hindered in the 1970s by the harsh conditions required to prepare samples. To render such samples suitable for mass spectrometry analysis, they had to first be decomposed by heating at high temperature. Invariably, this process of pyrolysis led to the almost total degradation of the material, therefore making mass spectrometry of little use for diagnostic purposes.

In 1975, John Anhalt and Catherine Fenselau showed that gentler pyrolysis conditions could be modified to allow retention of larger and more complex biomolecules, generating mass spectra unique to specific microorganisms. This team demonstrated that lyophilized bacterial samples could be loaded directly onto the mass spectrometer for ionization. Indeed, when applying this approach to different bacterial species, they observed highly characteristic mass spectra derived largely from the pyrolysis of ubiquinones and phospholipids. Even closely related bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* could be readily distinguished on the basis of their mass spectra. Subsequent work has revolutionized microbial diagnostics further through the introduction of soft ionization approaches such as the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) technique (**Milestone 18**), which has been successfully applied to identify a broad range of microorganisms from a variety of tissue sources. MALDI-TOF results in little molecular fragmentation and allows rapid, highly accurate identification of microorganisms right down to the species level.

Mass spectrometry–based approaches are used today to screen for metabolic disease in newborns.

Challenges remain in using mass spectrometry for clinical microbial diagnostics, such as reliably distinguishing between highly related microbes and analyzing spore-forming bacteria. However, modern approaches that combine mass spectrometry with techniques such as the polymerase chain reaction have started to address these issues and even have the potential to rapidly identify the presence of antibiotic resistance.

Mass spectrometry has proven to be a game-changer in medicine by also enabling the diagnosis of inherited metabolic disorders. Because these conditions result in the production of abnormal acylcarnitine metabolites that can be cytotoxic, their prompt diagnosis in newborns is critical. Yet chromatographic technologies do not permit the detection of these metabolites in blood samples, in part because acylcarnitines are small, low in abundance and structurally similar to normal blood plasma analytes. An additional challenge was the forbidding complexity of blood plasma—a morass of thousands of biomolecules that stymied the straightforward detection of any disease signature.

That is, until 1990, when D.S. Millington and colleagues showed that it was possible to use tandem mass spectrometry (MS/MS; **Milestone 13**) to accurately profile acylcarnitines from dried blood spots taken during routine newborn heel-pricks. MS/MS was especially useful for such metabolic screening because it allows multiple molecules to be analyzed simultaneously. This not only increases the throughput but also allows relative levels of analytes to be compared, which provides a better diagnostic of metabolic disease than absolute levels of a single biomarker. This work charted a path to using MS/MS routinely in newborn blood-spot diagnosis. Indeed, MS/MS is now well-integrated into standard newborn screening and is used for the detection of dozens of inherited metabolic disorders.

Building on these landmark medical mass spectrometry studies is the search for new biomarkers and approaches to improve diagnosis and monitor disease. In particular, the generation of larger and more accurate libraries of pathogen mass spectra should increase the number of microorganisms that can be identified and the depth at which they can be analyzed. Future advances in mass spectrometry technology should enable the diagnosis of potentially any condition that leads to derangement of body chemistry, including cancer, atherosclerosis and diabetes.

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ORIGINAL RESEARCH PAPERS Anhalt, J.P. & Fenselau, C. Identification of bacteria using mass spectrometry. *Anal. Chem.* **47**, 219–225 (1975) | Millington, D.S., Kodo, N., Norwood, D.L. & Roe, C.R. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. *J. Inher. Metab. Dis.* **13**, 321–324 (1990)

FURTHER READING Wilcken, B., Wiley, V., Hammond, J. & Carpenter, C. Screening newborns for inborn errors of metabolism by tandem mass spectrometry. *N. Engl. J. Med.* **348**, 2304–2312 (2003) | Rifai, N., Gillette, M.A. & Carr, S.A. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat. Biotech.* **24**, 971–983 (2006)