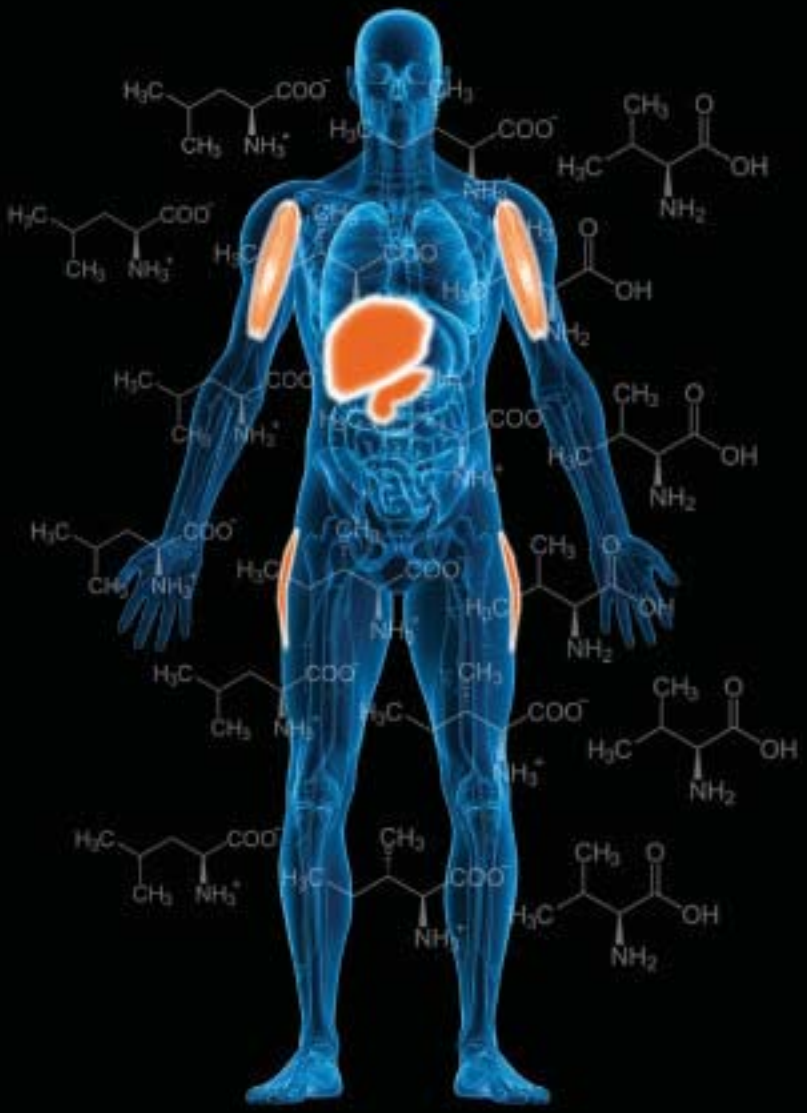

Prospects for Metabolomics in the Development of *In Vitro* Diagnostics

By Ralph Zahn and Guido Dallmann at Biocrates Life Sciences AG

Metabolomics enables the simultaneous analysis of hundreds of biochemical parameters, paving the way for the development of panel tests that quantify more than one parameter in a single sample.

Keywords

Metabolomics
In vitro diagnostics (IVDs)
Mass spectrometry
Personalised medicine
Biomarkers

Metabolomics is the non-biased identification and quantification of metabolites in a biological system; it is the latest -omics technology and is the closest to expressing the phenotype of a given organism (1). Metabolomics allows the systematic study of all the biochemical processes and pathways in cellular processes, thus providing 'fingerprints' of specific cellular events.

The common methods used in metabolomics are mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy – both techniques allowing the handling of complex samples with high sensitivity, selectivity and throughput (2,3). A recent approach includes the combination of MS and NMR data analysis, with the goal of identifying unknown metabolites and their assignment within the human metabolome database (4).

Image: Biocrates Life Sciences AG

The application of metabolomics to diagnostics, drug research and nutrition might become integral to improve health and personalised medicine – that is, providing the right treatment at the right time to the right patient in the right dose.

The Vision of Introducing Metabolomics into Clinical Diagnostics

In vitro diagnostics (IVDs) are essential for the improvement of therapeutic treatment and, in particular, for personalised medicine – that is, for the early detection of disease and/or the subsequent monitoring of drug efficacy (5). Currently IVD tests basically rely on single parameter tests; however, the trend is towards point-of-care testing, at least for nucleic acid- and immunoassay-based assays. These assays are limited by the prerequisites of simple operation, robust use and clear results.

The diagnostic benefit of panel tests is that they quantify more than one parameter in a single sample and, as such, are likely to surpass single parameter products in the near future. By this means, metabolomics allows the simultaneous analysis of hundreds of biochemical parameters, enabling the identification of metabolic pathway-dependent biomarker panels.

Such multi-marker panels will improve routine diagnostics by the introduction of biomarkers for early disease detection. Moreover, these biomarkers can also be used in companion diagnostics and in pharmacogenetics.

There is no doubt that metabolomics will evolve as a diagnostic technique as soon as both diagnostic and pharmaceutical companies start to make use of these technologies (5).

Multi-Parametric Sample and Assay Technologies

When measuring multiple biomarkers, the choice of appropriate technologies requires special attention. Mass spectrometry, coupled with chromatography, in particular liquid chromatography – tandem mass spectrometry (LC-MS/MS), is a valuable multiplexing tool for small molecule analysis in biological samples. Modern analytical instruments allow the simultaneous detection of hundreds of endogenous metabolites. However, measuring this huge number of compounds is a challenge because of their diverse chemical structures and properties (6). In 'targeted metabolomics' the focus is on a subset of metabolites representative of key pathways (such as glycolysis) and/or certain classes of substance (for example, amino acids and steroids).

The focus on a specified subset in combination with tandem MS and stable isotope labelled internal standards enables highly sensitive and accurate assays. Development and validation of a multiplex LC-MS/MS method is quite demanding, and establishing proper sample preparation is one of the most important steps. Sample preparation is inevitable – but it is also a lengthy and labour-intensive process that is an important source of uncertainty. The introduction and implementation of automated 96-well extraction has brought about faster sample-preparation procedures, improved precision and more cost-effective analysis.

Standardised and Validated MS Assays

At Biocrates Life Sciences AG, we have developed and established a targeted metabolomics technology platform that allows the systematic quantification of numerous metabolite classes of biologically relevant metabolites in body fluids, tissue and cells. The technology includes an automated sample preparation, a method for analysis of the latest and most sensitive mass spectrometer, and an integrated software solution (Met*IDQ*™) that controls sample management, data collection, validation and evaluation. Using this platform, more than 600 metabolite concentrations can be determined in human plasma.

An essential prerequisite for the introduction of MS technology into clinical diagnostics is the standardisation of analytical methods. In recent years, innovative methods have been developed that are delivered to customers in 'kit format' and include standardised equipment.

The kits are based on the targeted metabolomics approach, that is, the targeted identification and quantification of a multiplicity of known metabolites. The advantage of this concept is that quantitative or semi-quantitative information can be obtained. Furthermore, targeted metabolomics methods are applicable for high-throughput and routine applications.

The bottleneck for the use of metabolomics in routine clinical diagnostics is the availability of standardised procedures and analytical methods that allow accurate, high-throughput measurements with prompt turn-around time and low costs. In addition to innovative multi-parametric assays in kit format, highly sensitive mass spectrometers will be needed that are robust and suitable for routine use. In this case, it will be only a matter of time until metabolomics, capable of the simultaneous quantification of a large number of metabolites, complements or even replaces current single-parameter assays.

Metabolomic Biomarkers for Personalised Medicine

At present, the most successful use of targeted metabolomics in clinical diagnosis is the area of

newborn screening. Newborn screening programmes have been established worldwide to detect inborn errors of metabolism such as amino acidopathies, organic acidopathies or fatty acid oxidation disorders. Phenylketonuria (PKU) is one of the most common inborn errors of metabolism leading to severe mental development disorders if not treated. Affected patients have elevated levels of phenylalanine and the ratio of phenylalanine to tyrosine increases. Advances in analytical techniques are enabling a wider analyte panel, so that today a larger number of disorders can be detected in a single test.

The diagnosis of endocrine disorders by steroid analysis is another important application. Typically, (radio-) immunoassays are used for this purpose; however, they have significant weaknesses because the corresponding methods suffer from a lack of specificity, limited dynamic range and matrix effects. The superiority of LC-MS/MS based assays has been demonstrated several times and acceptance of these methods in clinical diagnostics is increasing (7). The possibility of measuring a steroid panel enables the monitoring of multiple steroid pathways; this is often necessary for clinical diagnosis as in the case of congenital adrenal hyperplasia (CAH). CAH refers to a group of autosomal recessive disorders of steroid biosynthesis caused by deficiencies of different enzymes with similar characteristics making a differential diagnosis of CAH difficult. Measuring a steroid panel – including, for example, androstenedione, cortisol, deoxycorticosterone, 17-hydroxyprogesterone and progesterone – has improved the diagnostic accuracy (8). These are both examples of multiplex, disease-oriented assays that have already found their way into clinical diagnostics.

Other areas where an application of targeted metabolomics appears sensible are metabolic diseases and cancer. Diabetes mellitus is a group of complex metabolic disorders with the common feature of increased blood glucose concentration (9). Several deregulated metabolites have been identified – including sugar metabolites (1,5-anhydroglucoitol), ketone bodies (3-hydroxybutyrate) and branched chain amino acids – that have the potential to detect diabetes-related complications under sub-clinical conditions (10). The development of reliable tests to detect patients at risk of diabetes, and bringing them to the market, is one of the great challenges that metabolomics companies have to face.

Ralph Zahn, PhD, is Chief Technology Officer of Biocrates Life Sciences AG (Innsbruck, Austria). He has a wealth of experience in biological sciences from his research work at international institutions, as well as in the biotech industry. He is a biotech founder and has deep expertise in executive life science management. As an Assistant Professor at the Eidgenössische Technische Hochschule (ETH) (Zürich, Switzerland), he worked on the three-dimensional structure determination of proteins using multidimensional NMR spectroscopy. In his current position, he is responsible for the development and production of multi-parametric mass spectrometry assays for research and clinical diagnostics. Email: ralph.zahn@biocrates.com

Guido Dallmann, PhD, is Director for Customised Method Development, Biomarker Development and Funding at Biocrates Life Sciences AG. A trained analytical chemist, he graduated in Biochemistry from the University of Wuppertal (Germany) and is experienced in developing MS-based bioanalytical methods, with a background in targeted metabolomics. He joined Biocrates in 2009 and in his current position is responsible for the coordination of research projects for the identification and validation of metabolomic biomarker panels in selected diagnostic and medical areas. His special expertise lies in the development of multi-parametric analytical mass spectrometry techniques. Email: guido.dallmann@biocrates.com

References

1. Nicholson JK and Lindon JC, Systems biology: Metabolomics, *Nature* 455: pp1,054-1,056, 2008
2. Baraldi E, Carraro S, Giordano G, *et al*, Metabolomics: moving towards personalized medicine, *Italian Journal of Pediatrics* 35: p30, 2009
3. Coen M, Holmes E, Lindon CJ and Nicholson JK, NMR-based metabolic profiling and metabolic approaches to problems in molecular toxicology, *Chem Res Tox* 21, pp9-27, 2008
4. Pan Z, Raftery D, Comparing and combining NMR spectroscopy and masspectrometry in metabolomics, *Anal Bioanal Chem* 387, pp525-527, 2007
5. Dallmann G, Zahn R, IVDs: Delivering on the promise of personalized medicine, *Innovations in Pharmaceutical Technology* 40, pp28-30, 2012
6. Purvis D, Thomas J, IVDs: Transforming the prospects for personalised medicine, *Innovations in Pharmaceutical Technology* 40, pp32-34, 2012
7. Soldin SJ, Soldin OP, Steroid hormone analysis by tandem mass spectrometry, *Clin Chem* 55(6), pp1,061-1,066, 2009
8. Minutti CZ, Lacey JM, Magera MJ *et al*, Steroid profiling by tandem mass spectrometry improves the positive predictive value of newborn screening for congenital adrenal hyperplasia, *J Clin Endocrinol Metab* 89: pp3,687-3,693, 2004
9. American Diabetes Association, Diagnosis and classification of diabetes mellitus, *Diabetes Care* 201(33)(Suppl): pp62-96, 2010
10. Suhre K, Meisinger C, Döring A *et al*, Metabolic Footprint of Diabetes: A Multiplatform Metabolomics Study in an Epidemiological Setting, *PLoS ONE* 5(11): e13,953., doi:10.1371/journal.pone.0013953, 2010