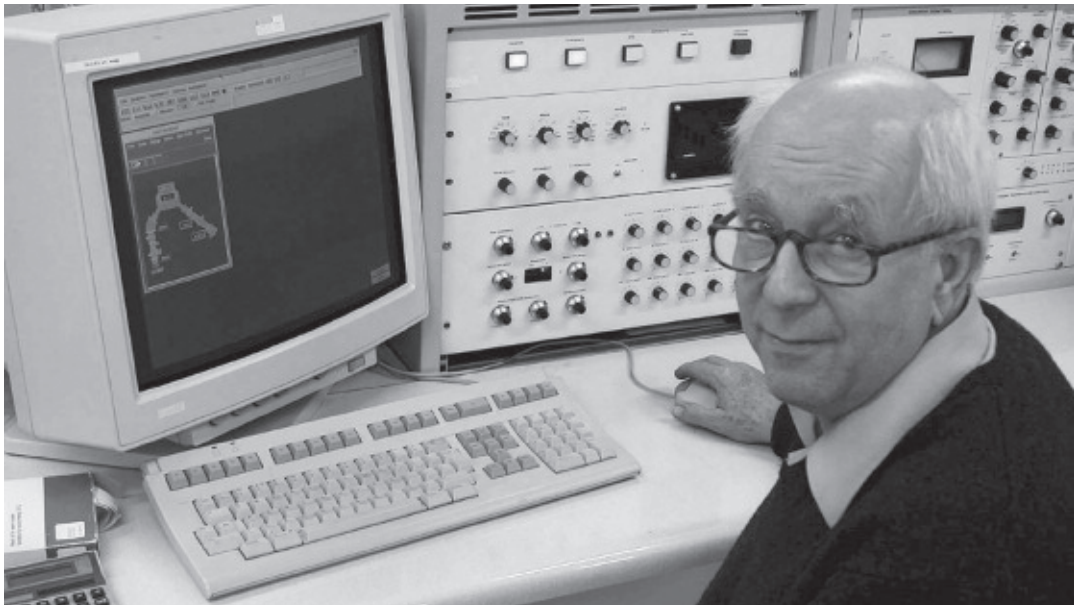
Clinical Mass Spectrometry 1977 to 2017



Professor Anthony Mallet studied Chemistry at the University of Oxford, going on to do his PhD in natural product chemistry. He lectured at the Royal University of Malta for 10 years and then spent 25 years in Clinical Pharmacology at St John's Institute of Dermatology at St Thomas' Hospital London. From 1998 until retirement Professor Mallet has been based at the University of Greenwich, being responsible for the mass spectrometry teaching and research laboratory.

In the late 1970s I fell into the hands of London based clinicians who were beginning to explore the power of mass spectrometry both for the characterisation of endogenous compounds as well as to perform assays of markers of pathologies. GC-MS was a well-established technique, still a little fiddly to perform but with good but fragile interfaces between the end of the GC column and the EI/CI ion sources available at that time. The concept of stable isotope dilution quantitative assays was already in use in several laboratories in the UK, Europe and the USA, but there was little ability to perform large scale fast routine clinical studies. The major clinical analytical method at this time was, and still is, automated immunoassay. Invented in the early 1970s and improved with enzyme labelled ELISA, fluorogenic and chemiluminescent labels and recently a DNA probe, these provide the day-to-day reliable clinical assay systems.

Mass spectrometry has to try to claim a niche from this methodology by both matching the sensitivity and automation and improving on the specificity of those samples which contain immune system cross-reacting components. The other area where MS can be employed is in assays for a family of analytes which are markers of an equal number of pathologies, most notably in paediatric medicine.
In the late 1970s, a few simple improvements appeared to simplify the operation of GC-MS systems. These were the invention of the capillary-fused quartz silica column with its protective imide coating and, flexibility permitting, direct admittance of the stream of gas from a GC column into the EI/CI source. O! the joy of ditching the 'separator' added to the end of the GC column! Another useful improvement was the O-ring seal for vacuum flanges. This permitted rapid dismantling and repairs to the instrument. Most important was the small light quadrupole analyser. Magnetic instruments still played an important role both for higher resolving power and for the nascent tandem mass spectrometry then being reported, but LKB and Finnigan quadrupole GC-MS systems were popular for being small enough to put in ordinary laboratories. The Paul ion trap was also introduced at this time and with linear triple quadrupole, the tandem MS analysis arrived.

In the early 1980s, progress in the analysis of clinically relevant compounds was still hampered by their reluctance to separate through GC columns. A raft of clever ideas were promoted including the Belt Interface, Thermospray and Fast Atom Bombardment (FAB) which is still occasionally in use but which in spite of much work was not an ideal LC to MS system. FAB however permitted Millington and colleagues to develop the first multi-sample Dried Blood Spot (DBS) analyses for detecting in-born errors of metabolism such as MCAD deficiency and amino acid disorders. This process has now developed into a world-wide method, analysing a million or more samples with nearly all neonates being sampled with a heel prick blood sample stored on a DBS card.

The thermospray method introduced those of us working at that time to the idea of spraying the eluent from an HPLC column, pumping away the solvent and hoping a small fraction of the analyte could be bled into a mass spectrometer source. Finally in 1984, John Fenn published the first account of electrospray ionisation for mass spectrometry, since when a number of alternative spray methods have been implemented. This had the great advantage of taking place at atmospheric pressure of transmitting a near solvent-free analyte into the analyser and removing most of the solvents, but also one which could simultaneously ionise it and do this for large as well as small molecules. The ionisation chemistry could not have been more accommodating in that it suited solvent requirements of the recently developed range of HPLC columns. The take up by the Clinical, Pharmaceutical and Biological communities was instantaneous and LC-MS became a method of choice for quantitative assays of small endogenous disease markers as well as characterisation of large biomolecules.

Any suggestion that mass spectrometry should supplant immune-assays is unnecessary; the two techniques are complementary. However the common comparison of mass spectrometry assays

with immuno-assays has led to a number of critical surveys in the past 15 years. In 2008 Vogeser and Seger *(doi: 10.1016/j.clinbiochem.2008.02.017)* suggested that while progress had been made there were still limits on the automation and compatibility of the instrumentation. In January 2016 the Journal Clinical Chemistry *(see free on-line)* published an issue dedicated to the same postulate. This is much more optimistic about the role mass spectrometry is, and will play, in this area and should be essential reading for anyone entering this field.

The current strengths of mass spectrometry for clinical applications lie in structural analysis of biological compounds, in providing so-called gold standard assays against which specificity suspect immuno-assays can be checked, for surveys of collections of molecular species and those for which no antibody yet exists or can be validated and, most recently, in the area of point-of care testing (POCT) and real-time diagnostics. A recent development illustrates some of the immuno-assay problems. Thyroglobulin is a middle-size protein, the analysis of which is essential in the management of thyroid cancer. Many patients however develop antibodies themselves and these interfere with commercial assays. A recent assay for this marker has been developed using a classical stable isotope dilution procedure. Developing methods for the quantitative assay of peptides and proteins is also a rapidly developing area with most of the efforts being designed to obtain satisfactory stable isotope labelled internal standards.

Another useful approach to the problem of routine multi-sample analytes has been the employment of multiplexing. For some years this has meant the attachment of multiple HPLC systems onto one mass spectrometer and running the injections in an offset manner making full use of the spectrometer operating time. More recently, the analyte molecules themselves have been multiplexed. This has been achieved by having a derivative molecule attached to the analyte which has a number of different small groups attached and therefore is detected at a number of different m/z values. Each of these can flag different classes of samples and be reported by the data system in a different set of channels. If five variants are present all five can be injected at the same time considerably improving the sample throughput.

The most recent revolution in the application of ionising techniques has been the realisation that ionisation of molecules is not always difficult or a high energy process but that it can be achieved by simple methods in the course of evaporation of analyte solutions sometimes with little or no heat or high voltage. A whole raft of techniques, often referred to as ambient ionisation mass spectrometry, now exist which can be used in the open room to provide fast and easy analysis of samples on surfaces, dried blood spot samples, molecular imaging of solid samples such as tissues and cell cultures. The most recent development has been the discovery and subsequent use by surgeons of the 'iKnife', a device which uses a high frequency electric current to evaporate and remove live tissue in a surgical procedure and leads to a cloud of lipid ions in the evaporate. These can be transmitted over a significant distance to a mass spectrometer where, in real time, a signal can be detected which will distinguish the pattern of ions between diseased or normal tissue. The current need in mass spectrometry is for fast high-powered data systems which can be programmed to present the analytical results in a form easily understood by a clinician. Ideally we need integrated chromatography, mass spectrometry and data systems which preferably all come from one commercial source which can fully understand their implementations and which have intelligent fail safe methods which quickly flag up when the current 'black box' is putting out erroneous data.

Tony Mallet
Greenwich, 2017

aimallet@waitrose.com

Supporting our young scientists:

As part of BMSS's dedication to educating our young scientists, we have funded over £200,000 worth of travel grants in the last 20 years, and over £80,000 of studentships in the last 10!

The BMSS John Beynon Travel and Conference Fund

So far in 2017 we have sponsored 35 members of BMSS to travel about in the name of mass spectrometry. There is still a small amount of money left in this pot so if you are thinking of venturing out, get your applications in pronto! For more details on the application process and eligibility requirements please visit www.bmss.org.uk/travel These awards are named in honour of John Beynon, a highly notable British mass spectrometrist. You can find out more about John Beynon and many other key figures at www.bmss.org.uk/historyPeople