75TH ANNIVERSARY ARTICLE

Advances in biochemistry over the past 25 years

Sujata Hemmady

There have been several technological and scientific developments of great importance in the field of biochemistry (chemical pathology) over the last 25 years that have enhanced the capabilities of the laboratory in providing necessary support to clinicians to improve patient diagnosis and treatment. I have chosen to highlight three areas of innovation and development for the significant impact they have had on routine biochemistry in New Zealand: automation, population-based screening programmes, and liquid chromatography with tandem mass spectrometry.

Automation in biochemistry

The modern laboratory is a complex integration of robotics, liquid handling, and numerous other technologies with the fundamental purpose of saving time and improving performance.

Automated testing in biochemistry over the last 25 years has grown in leaps and bounds with the emphasis being on efficiency and low operating cost to achieve high quality samples, improve turnaround times and reduce errors.

Twenty-five years back, the biochemistry department was semi-automated with several assays requiring a degree of manual intervention at the pre-analytical stage, samples were physically transported from specimen reception to be manually loaded on biochemistry analysers and some laboratories in New Zealand maintained hard copies of printed reports. We have come a long way: today, in the age of total laboratory automation, most biochemistry departments in New Zealand include pre-analytical and post analytical functions combined with analysers that are directly interfaced with an automated system. Several manual processes in specimen reception like centrifugation, aliquoting, storage, etc., have been replaced by automation, leading to a reduction in pre-analytical errors that are the largest source of error in the laboratory (1). Such systems accept and sort specimens, de-cap tubes, transport to a centrifuge, aliquot, analyse, and recap and deliver specimen for storage in refrigerators. Systems now have configurations that allow bidirectional control of the automated line that make retrospective testing possible without requiring to retrieve specimen manually. Embedded computers have created practices that permit loading of analysers and input modules for an automated line by staff in central reception.

This kind of functionality has enabled large multi-disciplinary hospital laboratories to evolve from a more discipline-based approach to a core laboratory that is an amalgamation of at least two to three disciplines with automated haematology, biochemistry and coagulation instrumentation. Results are validated by staff of the particular disciplines which retain intellectual ownership of the results. The range of analytical work is extensive with core laboratories offering an extensive test menu, with the ability to perform in excess of 100 different analyses on over 5,000 samples per day.

Staff numbers in core laboratories vary according to the magnitude of the workload but automation does allow laboratories to cope with high workloads. Most laboratories in New Zealand have experienced an increasing volume of work in the last few years with no additional resources in the core lab. In an era where laboratory turnaround time is considered one of the most important indicators of work efficiency, automation in biochemistry has certainly contributed quite significantly.

Screening programmes in New Zealand

A lot of work has been done recently in New Zealand to screen asymptomatic individuals for particular diseases, or conditions to reduce future mortality or morbidity.

In 2007, the National Screening Unit (NSU) identified the practice of using maternal age and/or nuchal translucency (NT) without biochemical markers to be unsafe and decided to discontinue that practice. In its place, a national antenatal Down syndrome screening programme was established that was funded by the Ministry of Health (MOH) (2). In 2009, two laboratories were appointed by the NSU to perform antenatal screening – LabPlus (ADHB) in the North Island, and Canterbury Health Laboratories (CDHB) in the South Island.

Antenatal screening for Down syndrome and other conditions provides a risk estimate for Down syndrome (trisomy 21), trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome), and other rare genetic disorders. This screening divides women into two groups based on risk, either increased risk or low risk. The screening test relies on accurate and full information being provided by the health practitioner and includes details of gestation, IVF, weight, smoking status, ethnicity, and relevant family history. First Trimester combined screening is available for women who are less than 14 weeks pregnant; measurement of serum PAPP-A and free β-hCG is combined with Nuchal translucency (ultra-sonography) to produce a risk that the pregnancy is affected by Down syndrome, or a number of other conditions. The Second Trimester screening is available for women who are between 14 and 20 weeks pregnant; measurement of serum AFP, free β-hCG, unconjugated estriol, and Inhibin A to produce a risk factor that the pregnancy is affected by Down Syndrome or a number of other conditions.

While nasal bone measurement was included in 2011 to improve the screening test's ability to detect Down syndrome, an under and over-reporting of absent nasal bone, or a poorly performed nasal bone assessment can give inaccurate risk estimates (falsely high or falsely low) because of the high predictive nature of the measurement. Therefore, in 2018, the nasal bone measurement was removed from the risk algorithm for Down syndrome and other conditions.

Results for antenatal screening are given as the value compared to the population median value for that analyte at that gestational age (MoM, multiple of the median). Hence 1.0 MoM is on the median value, 2.0 MoM is high at twice the median value and 0.5 MoM low at half the median value. In New Zealand, the screening cut-off is 1:300; based on international data, antenatal screening finds approximately 85% of babies with Down syndrome.

New-born screening, popularly known as the 'Heel Prick Test' or 'Guthrie Test', was first introduced as a national programme in New Zealand in 1969. The programme has been managed and coordinated by the biochemistry department of LabPlus in partnership with the NSU and has evolved considerably over time. Seven small spots of blood are punched out of the Guthrie card, one of these spots is used to test for the 17 amino acid and fatty acid oxidation disorders using the tandem mass spectrometer. The other punched spots are used to test for six other disorders. The programme currently screens for 23 metabolic disorders that includes congenital hypothyroidism, cystic fibrosis, congenital adrenal hyperplasia, galactosaemia, biotinidase deficiency, and severe combined immunodeficiency (SCID).

From time-to-time new disorders are considered for inclusion in the screening programme and current disorders considered for removal. The NSU decided to stop screening for a group of rare carboxylase deficiencies in 2015 when new evidence showed screening for the disorder to have no clinical benefit for the child. Similarly, in 2017, screening for carnitine transport defect was ceased due to the high number of false positive results and lack of true cases detected. A year later, testing for severe combined immune deficiency (SCID) was included as part of the programme. As part of metabolic screening, about 1% of samples are currently tested for a mutation associated with cystic fibrosis (3).

Almost all babies born in New Zealand have new-born metabolic screening (about 64,000). 2019 marked 50 years of new-born metabolic screening – it has gone on to become one of the most successful screening programmes in New Zealand and the world.

Bowel cancer, or colorectal cancer, is the second highest cause of cancer death in New Zealand. Around 3000 New Zealanders are diagnosed with bowel cancer every year and more than 1200 die from it. In 2011, MOH implemented the Bowel Screening Pilot and contracted laboratory services for faecal immunochemical testing (FIT) for people of Waitemata DHB aged 60 to 74 to detect bowel cancer early, when it can often be successfully treated. The sample is collected by participants at home and the collection kit is sent to the laboratory for analysis. Samples are analysed for the presence of haemoglobin in faeces, indicating bleedings associated to pathologies of the gastrointestinal track such as polyps, adenomas, colorectal carcinoma, ulcerative colitis, and Crohn's disease. A positive screening test would be followed up by a colonoscopy and intervention.

In 2017, the success of the six-year pilot programme prompted the Ministry to allocate approximately $197 million to get the programme running and to cover the cost of establishing our National Coordination Centre and developing the Bowel Screening Register (the IT system to support the national programme). Since then, bowel screening has been offered to the eligible population every two years; they receive an invitation letter, a consent form, and a free bowel screening kit that the participant is required to collect and send to the biochemistry department of LabPlus for analysis. DHBs are responsible for delivering colonoscopies and general practitioners manage positive test results with their patients.

The National Bowel Screening Programme (NBSP) is being rolled out gradually across the country, sixteen DHBs currently offer bowel screening. The roll-out of the programme is expected to be completed by the end of November 2021. Since the introduction of the NBSP, 909 bowel cancers have been detected to date, out of which 35% are Stage I where the cancer has not spread past the muscle wall. Previously, nearly half of bowel cancers were diagnosed in later stages where the cancer had spread beyond the bowel - 9 in 10 would not survive five years post diagnosis (4).

Liquid chromatography with tandem mass spectrometry

The enzyme-linked immunosorbent assay (ELISA) is an immunological assay commonly used to measure antibodies, antigens, proteins, glycoproteins, and drug compounds in biological samples. However, although ELISA screens are quick and simple, they do not always *quantify* lower levels of analytes in a patient sample and gas chromatography with mass spectrometry (GC/MS) was used for many years to provide a quantitative result for such samples. In the last couple of decades, these traditional technologies are being replaced by high-pressure liquid chromatography/ultra-high-pressure liquid chromatography (HPLC/UHPLC) column technology, and ultra-sensitive liquid chromatography with tandem mass spectrometry (LC-MS-MS). This powerful analytical technique combines the separating power of liquid chromatography with the highly sensitive and selective mass analysis capability of triple quadrupole mass spectrometry, opening up a wealth of technical opportunities for biochemistry laboratories.

Driven by the need for improved accuracy, speed, and cost-efficiency in routine medical laboratories, the last few years have seen a considerable emphasis on the development of robust, reliable, accurate, and sensitive LC-MS-based assays. LC is much more amenable to the separation of thermolabile and biologically active molecules than GC, and it gives scientists the flexibility to analyse a diverse range of biological matrices, including blood, plasma, serum, urine, etc. From therapeutic drug monitoring and clinical toxicology assays to the analysis of hormones and steroids, MS has established itself as a versatile tool for an expanding range of clinical applications in New Zealand hospital laboratories.

Despite these advances in technology, new analogues of a deadly synthetic cannabinoid that surfaced in 2017/18 in New Zealand made identification of the drug difficult on most LC-MS-MS. High resolution mass spectrometry (HRMS) instruments that had the ability to accurately measure the mass of unexpected compounds present in a sample were required. Many of the HRMS instruments found in clinical laboratories use time of flight (TOF) mass analysers that use ion flight time to measure mass. Single stage TOF instruments measure the accurate mass of all ions entering the flight tube. Compounds are identified by a combination of their mass, the measured isotope pattern, and chromatographic retention time. Addition of a quadrupole and a collision cell to a TOF forms a QTOF, a more expensive instrument that is able to isolate and fragment specific ions, as well as measure accurate mass. Molecular fragmentation is largely reproducible, thus the pattern of product ions that is produced in the collision cell can be used as an additional identifying characteristic of the precursor ion and aid in identification.

Challenges faced in the medical laboratory over the last few years required innovative approaches to the development and implementation of new technologies. Automating the biochemistry laboratory has ensured a range of assays are analysed rapidly and at low costs. Implementation of screening programmes in New Zealand has led to the development of clinically specific and sensitive biochemical markers that complement evidence-based intervention. State-of-the-art technologies, like the LC-MS-MS and the QTOF, have helped in measuring and identifying previously unknown molecules. Without question, the value of these platforms will become even greater in the future as they are refined through adoption and use.

AUTHOR INFORMATION

Sujata Hemmady, BSc MSc MNZIMLS, Section Leader

Department of Specialist Chemical Pathology, LabPlus, Auckland Hospital, Auckland.

Correspondence: sujatah@adhb.govt.nz

REFERENCES

1. Berman, R. Designing and Implementing Laboratory Automation for Improved Patient Safety. *Lab Med* 2004; 35: 52–57.
2. A Report of the Antenatal Down Syndrome Screening Advisory Group to the National Screening Unit, 2007. https://www.moh.govt.nz.
3. Newborn metabolic screening | Ministry of Health NZ. https://www.health.govt.nz/your-health/pregnancy-and-kids.
4. The cost effectiveness of bowel cancer screening in New Zealand: a cost-utility analysis. (health.govt.nz). https:// www.health.govt.nz/system/files/documents/publications. n.d.