By combining techniques from the microchip industry with advances in biomedicine, British engineers have devised biological microchips (biochips) that can provide rapid and inexpensive diagnosis of a growing number of medical conditions. A UK-based company Randox Laboratories Ltd has pioneered the biochip to automate blood tests. Recently approved by the US Food & Drug Administration, Randox’s Evidence® technology is set to change the face of medical diagnosis.

Growing expectations, new medical knowledge and the ability to treat a broader range of diseases impose a growing financial burden on the National Health Service (NHS). Early detection and prevention of diseases could help us to tackle the growing costs of healthcare. However, despite the potential for diagnostic tests to reduce healthcare costs, the NHS currently allocates far less to this activity than to its drugs budget. The British In Vitro Diagnostics Association (BIVDA) highlights this position in its 2002 annual review where it states that ‘Currently only five per cent of the NHS budget is spent on diagnostics, which provides 60 to 70 per cent of the information on the electronic patient record.’

Treatment decisions based on limited test results are a false economy. They can lead to missed diagnoses and costly mistakes. For example, many patients are discharged from hospital emergency units due to misdiagnosis of heart attack, often with fatal consequences. Others are kept in hospital when they do not have heart problems, taking up beds that would otherwise be available for genuine patients.

A revolutionary new diagnostic system combines expertise from engineering, biochemistry, physics and other disciplines to deliver more information, in a shorter time, for a diverse range of conditions. For example, this new approach can detect a range of different proteins in blood that indicate that a patient has suffered from a heart attack.

The new technology can also provide better, cheaper and more thorough detection of illegal drugs in urine samples. Indeed, researchers are already working on a whole series of ‘test panels’ that could, with automated testing of blood samples, improve the detection and diagnosis of a growing range of diseases. These test panels identify proteins in the blood that are indicators of specific conditions. Hence, there is a panel of protein tests for cardiac markers, another for tumour markers and further panels for various hormone conditions.

Today’s technology

Diagnostic tests can detect and measure components in a blood sample that may indicate a particular disease. Many of these tests use immunoassay techniques that rely on biological components, such as antibodies, to capture specific analytes.
(proteins) in a blood sample from a patient. The antibodies and the analytes form chemical complexes. A detection system can then identify and measure these complexes.

Today’s immunoassay systems utilise an array of reaction wells. Each well contains a test for a particular analyte. A detector then inspects the reaction wells one at a time.

Commercial systems automate immunoassays to deliver higher throughput and ease of use. When a patient’s sample has to go through multiple tests, the analyser must repeatedly perform the same operation, using a different test well for each analyte. The requirement for multiple tests leads to an overall reduction in the throughput of samples. It also takes longer to process each sample and requires a larger volume of each patient sample for the range of tests that are required.

Randox has introduced a novel approach to immunoassay testing. In its approach, the company attaches multiple antibodies to a single ‘biochip’. The result is a solid chip containing an array of discrete reaction sites on the surface. Each reaction site has a specific antibody that will capture one specific protein in the sample. After the patient sample is added to the biochip, a complex forms simultaneously at each test site. The system then uses a charge-coupled device (CCD) camera to interrogate the whole chip and to measure the signal from each discrete test region. This process enables simultaneous detection of multiple complexes formed on the biochip’s surface, and the measurement of multiple analytes in the patient sample, within the time taken to perform a single test using conventional technologies.

Biochip production
At first, the biochip’s surface is chemically inert. Silanation, which chemically activates the biochip’s surface, makes the surface hydrophobic and provides a substrate onto which linker molecules can be attached. The linker molecules act as ‘anchors’ that become attached to the capture molecule which can be an antibody, an antigen or a nucleic acid. Essentially the linker molecules allow covalent attachment of the capture molecules to the biochip’s surface. This is a robust attachment that ensures that the capture molecules are not washed off during the subsequent wash steps involved in the testing process.

Immobilisation of the biological ligand requires accurate and specific placement at discrete test sites on the biochip’s surface. The number of test sites per biochip depends on the test panel.

As part of the development of the biochip, Randox created a novel microfluidic dispensing system. This
automates the simultaneous dispensing of multiple ligands – as 10-nanolitre droplets – onto the surface at defined positions.

After attachment, curing stabilises the biological ligand. This process involves drying the chip’s surface under pre-defined conditions and holding under optimal temperature and humidity to extend the chip’s storage capabilities. At this stage, the result is a biologically functional biochip that is ready for use.

The world’s first commercial protein biochip manufacturing facility is now operational at Randox’s headquarters in Crumlin, Co. Antrim. This can produce around 20 million biochips a year.

Light on diagnosis

The biochip is of little value unless we have some way to interrogate the device. We need to be able to detect which test sites on a chip have responded to particular components in a blood sample. Detection of positive results uses chemiluminescence – the production of light via a chemical reaction.

Chemiluminescence is the basis of many immunoassays for detection and quantification of analytes. In Randox’s biochip-array technology, an enhanced chemiluminescent substrate magnifies the light production enabling more sensitive detection. A novel imaging system detects and quantifies light emission from multiple test sites on the biochip’s surface.

Many immunoassay analysers utilise detection systems that are limited to quantification of a single source of light. The challenge for the biochip array system is the simultaneous detection and spatial differentiation of light output from multiple reactions sites on the same biochip. In the development of the Randox system, the company adopted and modified imaging techniques used by astronomers to map the solar system. These allow the quantification of a multitude of light emissions within seconds. This technology uses a specialised camera with a CCD to capture an image of the light from the biochip’s surface.

The sensitive camera contains photosites arranged in a grid format that is directly comparable to the biochip surface. This enables direct mapping of light signals to specific locations on the biochip and signal quantification at each discrete test site. The charge pattern across the CCD corresponds to the pattern of incident light and is converted to a column of pixels and digitised. The system stores images as bitmaps. These hold the image as a set of pixels corresponding to specific points in the image. Each pixel holds the x and y coordinates of the point along with the brightness data.

Image processing software manipulates the data mathematically to maximise the output from the CCD camera. The process also removes background noise from the image, resulting in a very high signal-to-noise ratio.
Automation

Applications of biochips for clinical laboratories are worth considering only if the analytical method can be fully automated to provide high throughput, excellent performance and ease of use. Randox’s Evidence® analyser achieves this starting with the selection of the test panel right through to the production of calibration curves for each test on the panel, thereby allowing accurate quantification of each analyte in the patient sample.

Biochips are presented in a 3 × 3 carrier, which facilitates transport within the analyser. The design of the analyser incorporates these operating steps into an optimum layout that combines efficiency of operation with compact physical size resulting in a system that is capable of throughputs in excess of 3600 tests per hour.

This biochip system incorporates many unique features to offer flexibility never previously seen on diagnostic analysers. In test selection, for example, there is a unique approach to performing each assay as a multi test, or on a test-panel basis, simultaneously producing an image for all the discrete test sites. To meet concerns for test redundancy and to contain costs, the software is programmed to report only results and to charge for tests selected by the user.

In addition, the Evidence® biochip system offers a facility to retrospectively report results, that have not been initially selected. After the clinicians have reviewed preliminary results, they sometimes ask laboratories to perform further assays to clarify certain issues. This can involve taking a second blood sample. Levels of the initial test analyte in the blood sample may have changed since the earlier test so creating potential uncertainties in the assessment of the results. Randox’s Evidence® system records a snapshot of the light output from each discrete test site, regardless of whether the test was requested or not. If a test is required later, the software can retrieve the data, perform the calculation and report the result.
Seal of approval

For pre-market evaluation of the Evidence® system, the drugs of abuse assay panel was selected. This panel contained the following tests:

**Drugs of abuse panel**
- Amphetamine
- Methamphetamine
- Cocaine
- Barbiturates
- Cannabinoids
- Opiates
- Methadone
- Benzodiazepines
- Phenylcyclidine

Trials took place at a major private medical company and a private testing laboratory in the US. During the trials, the results were compared with current testing systems.

It was evident from the trials that the Evidence® system reduced analytical test times and delivered savings in operator time and overall costs. Improved test throughput enabled a substantial increase in the laboratory’s output. The results of these trials led to US Food and Drug Administration to give Evidence® clearance for drug testing in December 2003. This seal of approval enables introduction of Evidence® into laboratories throughout the United States.

**Biochips in action**

An important potential use of the biochip is in early detection of heart disease. Every year, millions of patients are admitted to hospital with chest pain. About half of these patients have symptoms that are not related to a cardiac condition, one-third have less serious cardiac problems, while the remainder are diagnosed with acute myocardial infarction (AMI) or heart attack. Heart attacks involve damage to the heart muscle and can be detected by sampling appropriate biomarkers. However, almost 3 per cent of AMIs are missed and discharged from hospital.

The other side of this problem is that many non-cardiac patients are needlessly retained in hospital until the clinician is sure that the cause of the symptoms is non-cardiac. These patients occupy much needed hospital beds and use resources that could be better utilised elsewhere.

**Multi-drug testing**

Research shows that 25 per cent of patients admitted with chest pain can be correctly diagnosed on admission but the remaining 75 per cent require more accurate diagnostic procedures to confirm their clinical conditions. Additional biomarkers can contribute to more accurate patient diagnoses and can deliver substantial cost savings. For example, a recent study in Wales demonstrated an overall cost saving of nearly £22,000 over a six-month period as a direct result of implementing the new ‘troponin’ test for diagnosis of myocardial infarction. The largest percentage of the savings was attributed to reduced length of bed stays.

The new troponin test – which looks for elevated levels of the multi-protein complex troponin in blood samples – is recognised as the gold standard for diagnosing heart attack as it is cardiac specific. Troponin is, however, just one of a number of biomarkers of heart disease. Authoritative bodies now support the use of a combination of cardiac biomarkers to ensure rapid and accurate diagnosis for the diverse spectrum of coronary conditions.

The Evidence® technique offers a cardiac panel with new markers that improve the specificity and accuracy of diagnosis.

A further large market for automated blood testing is in testing for large numbers of drugs. Simultaneous multi-drug screening is now possible for up to nine drug classes. Short drug half-life and new drug formulations are pushing drug-testing to its limits.

Simultaneous testing overcomes the problems of drugs that can test negative if the analysis does not detect drug breakdown products. It also reduces the time it takes to screen thousands of samples. The ability to combine a number of tests that detect a range of drug metabolites in the
same sample also reduces false positives and eliminates expensive confirmatory testing.

Tumour markers
The recent Government document ‘Modernising Pathology Services’ embraces leading edge technologies to improve pathology services of the NHS. Biochip arrays offer flexibility for screening programmes with panels such as that for screening for prostate cancer and a tumour-monitoring panel for gastro-intestinal tumours, ovarian tumours and breast cancer.

The high throughput of biochip approaches meets the needs of the Government’s initiatives for more widespread screening of high-risk individuals for early detection of cancer. Better diagnostic information also leads to more accurate diagnosis, an improved outcome for the patient and an overall cost saving.

In conclusion
The medical community talks increasingly of ‘evidence-based diagnostics’ and of diagnostic testing more often and earlier in clinical assessment. Many studies reveal that evidence-based approaches can deliver substantial cost savings. Paradoxically, restrictions on healthcare costs have limited the use of test panels. Instead, the policy has been to conduct fewer selective and specific tests. Randox’s Evidence® technology is set to reverse the trend of fewer tests. It gives clinical laboratories a rapid and cost-effective alternative to their existing instrumentation.

Tumour marker panel
- Carinoembryonic antigen (CEA)
- AFP
- Total PSA
- Free PSA
- hCG
- Gastrointestinal tumour antigen (CA 19-9)
- Ovarian tumour antigen (CA 125)
- Breast cancer antigen (CA 15-3)

Ivan McConnell graduated from the University of Ulster in 1988 with a BSc (Hons) in Chemistry. He joined Randox immediately after graduating and is now Deputy R&D Manager. Ivan has been involved on a day-to-day basis with the development of the protein biochip array technology, particularly in the area of the surface chemistry involved in the production of the biochips and the development of a range of immunoassays for measuring traditional analytes associated with various diseases. He has broad experience in the biochip array manufacturing process and has guided the Chemistry Department in antibody conjugation techniques and the Engineering Department in the production and design of Evidence®.

Dr Peter FitzGerald, together with his parents, founded Randox Laboratories in 1982. He is currently the main shareholder and Managing Director of the company. Peter’s qualifications include BSc (Hons) in Biochemistry from the University of Strathclyde, Glasgow, PhD in Biochemistry sponsored by the Medical Research Council Scholarship at the National Institute for Medical Research, Mill Hill, London and a Doctor of Science (DSc) awarded by Queens University, Belfast in July 2000. Randox has won many accolades and industrial awards in the 22 years under Peter’s management. These include the Royal Academy Award for Innovation in Engineering in 2003 for the development of the Evidence® protein biochip array analyser.

John Lamont, who is currently Randox’s R&D Manager, has been with the company for almost 20 years. Before joining Randox he was employed as a Clinical Biochemist in the Ulster Hospital Biochemistry Laboratory. His qualifications include a BSc (Hons) in Biochemistry (Queens University Belfast) and an MSc in Clinical Biochemistry (Trinity College Dublin).

Figure 10 (Left to right) Ivan McConnell, Dr Peter FitzGerald and John Lamont