

New technologies bring Point-of-care-testing into the 21st century

Abstract

Every new technology offers the potential to effect changes in practise; many developments driven by the world-wide demand for instant results. This is never as true as in the arena of diagnostic testing. Once the sole preserve of the hospital laboratory increasingly tests are now being performed nearer to the patient in clinics and the home. New technology is driving the development of greater number of more sophisticated tests that can be applied in a wider range of clinical situations where rapid results bring benefit. These new tests rely on a spectrum of novel technologies ranging from simple, easy-to-read dip-sticks to molecular based sensing techniques.

Point-of-care testing (POCT) is the practice of performing a diagnostic or prognostic test near to the patient in order that the result can be immediately acted upon. This implies that the test has to be rapid, and easily performed without the use of large or complicated instrumentation; also a test that a minimally trained operative can perform and interpret. The POCT can be applied in many environments; in the general practitioner's surgery, hospital clinic, hospital ward, emergency room, intensive care unit and even a patient's home.

Implicit in the adoption of new POCT protocols is the need for a rapid measurement which allows management of the patient to be acted upon "on the spot". For example, in a situation where a patient has a life-threatening condition and the clinician needs a rapid result to enables the appropriate treatment to be given without delay; or the situation where a patient comes into an outpatient clinic to have a routine blood test to monitor a chronic condition, the POCT provides psychological benefit by eliminating the wait for results and the need of a second visit. In the age of the internet, patients are now more educated regarding their conditions and promises of instant results fuel their expectations of technology and leads to an ever increasing demand for POCT.

The global market for all in-vitro diagnostics is expected to exceed \$40 billion in 2010 when the total sales for POCT is predicted to be greater than \$16 billion with a predicted compound annual growth rate of 7.8%. The POCT market is growing with

the sales of all enzyme biosensors are expected to be \$5.96 billion in 2009. However, the growth in the commercial sales of other forms of biosensors (non-enzyme) is expected to account for 2.5% of the market by 2009 (\$110 million) and the predicted market for antibody based biosensors is forecast to be \$1.1 billion. The average annual growth rate for enzyme based biosensors is expected to be 2.6% whereas antibody based biosensors is expected to grow by 39% and DNA biosensors by 9% (1).

Simple tests

Historically POCT has been performed in the ward side room, based on dip-stick urine tests. These simple tests involve dipping a small square of absorbent material into a urine sample and a chemical reaction will give a colour change which can be crudely quantified by reading the colour against a chart giving the approximate concentration of the substance being measured. These tests are often used as a front line screening test and include the measurement of protein, glucose, ketones, and blood in the urine sample. In contrast to the visually read dip-sticks, blood gas measurements, using bench top instruments have been used for many years to give POC blood gas results for patients, but this has been limited to critical care facilities such as ICU and cardiac units.

Nowadays many of the tests use immunological reactions to make a measurement in just a few minutes where the traditional laboratory based test may take several hours. A new generation of “dip-stick” type tests rely on immunological interactions between an antibody and the target molecule being measured. These tests use the sample to rehydrate dried reagents which then flow through a porous membrane. The sample and the reagents are pulled through the membrane by capillary action and when they reach a line of immobilised antibody on the membrane, a coloured line becomes visible to the user. A second line acts as a control telling the user when the reaction is complete and that the test can be read. By far the most common example of this type of technology is the pregnancy test that can be bought over the counter (2). A positive result is a blue line indicating that the woman is pregnant. There are now a number of devices on the market that can simultaneously measure multiple targets such as troponin I, myoglobin and CKMB which are markers of cardiac infarction. Indeed, there is a plethora of other test devices that have been produced,

either for home use, such as ovulation prediction tests, or for use by trained personnel such as nurses but the greatest growth in lateral flow technology is for the rapid detection of infectious agents (3,4).

This immunochromatographic or lateral flow technology, as it is called, has revolutionised the way that the public use rapid test devices. In the most basic form, the lateral flow device is simple to use, robust and does not require any instrumentation to read the result. However, interpretation of the result is sometimes difficult when only a faint line develops or when being read by people with colour blindness. To overcome these problems and to remove any degree of subjectivity a number of instruments have been developed to measure the colour intensity of the line and can even present the results as a written message on a small display on the instrument.

Instrumentation

While the “dip-stick” type tests described above are rapid and robust they are used where a Yes – NO answer is required. In the case where it is important to know the concentration of the measured compound, technology has allowed the traditional laboratory test to be brought into the POCT domain. Instrumentation that is based on electrochemical measurement removes the need for any optics associated with the measurement of a colour such as the light source, filters and photo-detection technology. Instead, electrochemical measurements enable the use of inexpensive, mass-produced disposable electrodes. Miniaturisation and packaging of small volumes of reagents in disposable containers was another factor that allows traditional laboratory tests to be moved to the POC setting. A good example of this type of technology is the iSTAT system for measuring sodium, potassium, calcium, haemoglobin, blood gases and a range of other routine blood tests by electrochemical techniques.

The development of biosensors initiated a revolution in POCT (5,6). A biosensor is a device that integrates a biological molecule with a transducer that produces an electrical signal that can be processed to give the concentration value of the test compound. The biological molecule imparts selectivity to the biosensor while the

transducer and associated electronics gives the required sensitivity. The specificity of enzyme reactions has allowed a range of biosensors to be developed for measuring metabolic compounds such as glucose, lactate, ammonia, cholesterol and urea. Indeed the most successful biosensor that has been developed to date is the glucose biosensor. A glucose biosensor often uses the enzyme glucose oxidase which has been stabilised and attached to an electrode surface, which reacts with glucose in a drop of whole blood to produce an electrical current, proportional to the concentration of glucose in the blood. Glucose biosensors have no liquid reagents and the electrode is disposable. Many “glucose meters” have been developed for the glucose testing market and typifies the current state of POC technology, driven by a growing market. The market for POC glucose testing is expanding as the number of people suffering from diabetes is increasing year-on-year; the World Health Organization estimates that there will be in excess of 350 million people suffering from diabetes by 2030. Glucose testing is also an example of self-testing POC technology as many diabetics personally monitor their blood glucose and adjust their insulin dose (7).

In addition to enzymes, other biological molecules give biosensors a high degree of selectivity, in particular there are many biosensors being developed using antibodies, also called immuno-sensors (8). The advantage offered by antibodies is the significantly greater range of tests that can be developed; hundreds of potential targets have been identified. However antibodies do not possess native activity that can be exploited to generate a measurable signal. Consequently, it is necessary to identify the end point of the reaction, when the antibody has interacted with the specific target, forming a complex. This can be achieved by using a label which gives an indirect measurement of the target molecule of interest. Examples of labels used for biosensor applications include enzymes which are used to generate an electrical signal for use with electrochemical based measurements. Fluorescent labels have been used for optical based devices; one example is a capillary fill device that uses a few microlitres of whole blood to measure human chorionic gonadotrophin. A novel label, based on sub-micron magnetic particles has been developed for a number of portable systems that measure the amount of magnetic material associated with the antibody reaction (9, 10). These systems offer great sensitivity and have a very low background signal as biological fluids do not have any magnetic components, even the iron in

haemoglobin does not give significant interference. These types of device have been developed for drugs-of-abuse, cardiac markers and for infectious agents.

Future technology for POCT

The major disadvantage with using labels to indicate antibody interactions is that additional reagents have to be manufactured and integrated into the measuring device. A number of new biosensor technologies have been developed that directly respond to biological interactions on the sensor surface. These fall into two main types of device: optical systems and devices that use the piezoelectric effect. These technologies can be miniaturised and offer great promise to the POC market in the future as they do not require reagents such as a label, and can be used with whole blood. In addition, these technologies can be developed to act as the biosensing area of a lab-on-the-chip device. The optical based systems rely on the evanescent wave effect of optical waveguides, where light energy can interact with material within a few hundreds of nanometres from the waveguide surface. To form a sensor, the waveguide is treated and coated with antibody which will react with target in a sample and detected directly in real-time. Other optical systems offer enhanced sensitivity by using novel particles which allow surface enhanced raman spectroscopy to be performed (SERS) on their surface (11). The most well developed label-free optical technology for POCT employs the evanescent wave effect in Surface Plasmon Resonance (SPR) (12). In theory a SPR device could be developed that allowed a probe to be dipped into the sample to give an instant reading, but in common with most of these types of label-free technologies, there is a need for liquid handling to present the sample to the sensor surface.

Piezoelectric devices have also been developed to measure changes on a sensor surface when an antibody interacts with a target in the sample. The surface acoustic wave (SAW) is commonly used in these types of device, which measures an output frequency from the sensor (13). When binding of biological molecules occurs on the sensor surface there is a resulting change of frequency which is proportional to the concentration of target. These piezoelectric devices have similar operational characteristics as the label-free optical devices in terms of types of sample, issues with moving the sample to the sensor surface and application areas addressed.

Moving away from the analysis of blood or urine, it is possible that future POCT may involve volatile sensing which detects changes in the volatile compound produced by the body, in the breath or through the skin. Electronic nose technology has been available for the last couple of decades but is now being refined and adapted for diagnostic applications (14). Although currently biomarker discovery is being performed using mass-spectroscopy, in the future small specific sensors for volatiles of interest will be incorporated into a small portable or handheld devices for POCT. This technology has already been proven for the identification of infections and some cancers.

Future targets for POCT

For future POCT it is not only the significant developments in instrumentation that will have an impact on the practice but the target being measured will greatly change the how POCT will be applied. In the future it will be possible to detect specific sequences a DNA or RNA, associated with a known gene. Methods of integrating short sections of single stranded nucleic acid with a sensor surface have been developed to produce nucleic acid sensors. These have potential application in the rapid detection of infectious agents, screening for cancer and the detection of genetic disease in a developing foetus before birth. The sensors can use the same detection technologies described above (15); but unlike a simple blood test for glucose, for DNA testing the blood sample has to undergo a processing step to extract and prepare the DNA for presentation to the sensor before any measurement process can be applied. There may be medico-ethical issues regarding the analysis of human genetic material which could hinder the adoption of human nucleic acid sensors for POCT but the greatest growth area is in the rapid detection and identification of infectious agents such as MRSA and sexually transmitted disease at the point-of-care.

Other new approaches that may develop in to novel POCT technologies are based on direct tissue measurements to detect diseases such as cancer. The transcutaneous bilirubinometer has been used for 25 years to assess jaundice in newborn infants by measuring reflected colour. By using new techniques such as raman spectroscopy it is possible to identify cancerous tissue without the need of a biopsy.

Conclusion

POCT is a growing sector of health care, driven by the need for rapid results and new technologies. A number of traditional laboratory blood test can now be performed as a POCT. The over-the-counter pregnancy test represents the largest self test device that does not require instrumentation and glucose testing is the largest market for biosensor POCT devices. With the introduction of new biosensor technologies that utilise antibody and DNA to detect specific molecules in a blood sample there is a massive potential for future POCT particularly in the areas of diagnostics and prognostics outlined below.

- Urinalysis
- Blood gases and electrolytes
- Haemoglobin analysers
- Blood glucose
- Coagulation
- Pregnancy and fertility
- Cardiac Markers
- Drugs of Abuse
- Tumour Markers
- Infectious Diseases
- Sexually transmitted disease

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Case Studies

1. Bright future for Raman spectroscopy

Vibrational spectroscopy (Raman and FT-IR) presents a new tool for point of care applications, for example allowing discrimination of early cancers and local and systemic disease studies in body fluids. The Biophotonics Research Unit within Gloucestershire Hospitals NHS Foundation Trust has been pioneering the development of such techniques. Projects include the development of Raman probes for rapid biochemical analysis of tissue in vivo at endoscopy to aid the clinician in the targeting of early pre-cancerous changes in the oesophagus, potentially enabling immediate treatment to be delivered; also with application to other cancers. In parallel applications of spectroscopy for rapid biochemical mapping of tissue as an adjunct to histopathology are being developed. The use of drop coating deposition Raman spectroscopy of tear fluid is under investigation as tool for the diagnosis local and systemic infection for example the diagnosis of ocular infection at outpatient clinics; with potential application in primary care.

2. Smelling disease

A future where volatiles from breath, stool and urine could be readily analysed and a disease designation undertaken, would revolutionise medical diagnostics. Volatile analysis is an emerging science and offers the prospect of point of care testing, in real time and at relatively low cost. The Institute of Bio-sensing Technology, based at the University of the West of England, Bristol (UWE) has an international reputation in the field. The key aim of this Institute is to instigate new multidisciplinary research collaborations and as such the research involves a number of academic and industrial collaborations. Prof Norman Ratcliffe (UWE) and Dr Chris Probert (University of Bristol) have identified volatile biomarkers for gastro-intestinal diseases, early bladder and prostate cancer, liver conditions and cystic fibrosis amongst others. In some recent work of 312 volatile compounds emanating from human stool samples have been detected and the differences in volatiles resulting from Campylobacter food poisoning have been identified. As another example, ammonia detection in the breath

has been used to recognise *H. Pylori* (the stomach bacterium now unambiguously linked with stomach ulcer formation); the goal of this research is a handheld instrument which can be used at point of care, with a result available in minutes.

3. Revealing bug genes

Real-time PCR (polymerase chain reaction) is a common and extremely sensitive genetic technique used to identify specific and often low abundance DNA in a sample. It is recognized as the gold-standard test for biological agents in clinical, veterinary and defence laboratories throughout the world and is becoming a stipulated technology for certain applications. The Holy Grail of point-of-care molecular testing remains a low-cost, portable, rapid, fully automated, CLIA-waived system that can accommodate a range of samples (blood, swab, urine), can perform multiplexed testing, and has a minimal logistics and training burden. Enigma Diagnostics Limited, founded in 2004, specializes in developing the next generation of rapid molecular diagnostic instrument platforms for use in decentralized and point-of-care settings. This new type of PCR technology combines the speed, accuracy and sensitivity with the simplicity needed for decentralized, point-of-care tests while providing raw sample-to-result in less than 45 minutes. The potential fields of application of point of care, PCR diagnostic testing are numerous, in particular Enigma have developed tests for influenza, sexually transmitted diseases and healthcare acquired infections.

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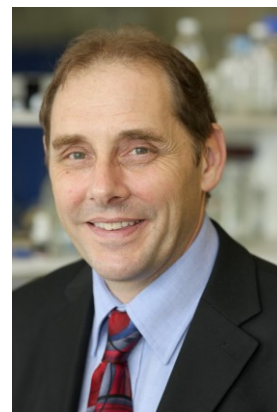
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Janice Kiely graduated from Sheffield University with a BEng (Hons) in Electronic Engineering and completed a PhD from Cardiff University. Her current research focus has been on the development of novel instrumentation. She has 10 years expertise in the development of magnetic detection systems for magneto-immunoassay systems which can be applied to biomedical diagnostics. Janice is a co-founder and co-Director of the Institute of Bio-sensing Technology and the Head of Research and Knowledge Exchange of Bristol Institute of Technology.

Pictures:



