Applications of biosensors in the clinical laboratory

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Biosensors are devices that utilise a biological recognition element in conjunction with a transducer to produce a signal in proportion to the concentration of the substance being measured. If one takes a broad definition of sensor, then sensor technologies may be responsible for up to 25% of chemistry determinations performed in clinical settings. True biosensors with the exception of glucose measurements are a small portion of this market yet the last 5 years have seen this technology mature to the extent that a number of important analytes such as cardiac markers, drugs and infectious agents are now determined using commercially available biosensors.

In the future we are likely to see biosensors that utilise the technology and clinical information emerging from the human genome project so called Gene-Chips or arrays. Despite the undoubted impact that genomics will have on disease diagnosis and management it is possible that the most important biosensor technology will continue to be glucose measurements for some considerable time in the future. The incidence of diabetes is predicted to increase significantly and the importance of regular glucose measurements has been well established.

Glucose test strips are based on so-called thick-film technology and use an enzyme as the recognition agent. Most strips used glucose oxidase and reflectance spectrophotometry for the transduction or measurement process. In recent years a new generation of glucose strips has appeared using electrochemical technology that made it possible to produce smaller meters and non-wipe strips although these features are now possible with colourimetric technology. Other refinements of these measurement systems include the use of particular mediators to act as the electron shuttle and avoid potential interference from low oxygen tensions. Most strips use glucose dehydrogenase now so as to avoid interferences from oxygen side reactions.

Thick-film, reusable sensors are an extension of the technology used for glucose strips and this type of sensor can now be used to measure other metabolites such as urea, lactate and more recently creatinine. Their manufacture involves the application of specially formulated pastes and inks to specific areas on an inert support base, often using screen-printing techniques. The result is well-defined sensor zones with electrical conduction and electrical isolation zones. Cocktails containing the recognition agent such as an enzyme or antibody are then dispensed over the sensor zone using robotic techniques after which the device is fired to a high-temperature to remove interfering binding agents in the paste and to bond the various materials to the support layer. Thick-film sensors are usually about 10-25 mm in thickness with the sensor spot approximately 1 mm in size.

This contrasts to thin-film sensors which are fabricated using wafers of thin metal oxide films and which are of submicron thickness. To manufacture thin-film sensors requires special facilities that makes them more costly to produce than thick-film sensors. In addition thick-film technology is a very robust technology that is particularly compatible with enzymatic reagents that have a stable lifetime in a sensor of between 1 – 8 weeks. The major advantage of thin-film sensors is that they can be used almost instantaneously whereas thick-film sensors need an equilibration time before being exposed to the blood sample.

Immunosensors are biological sensors where the recognition agent is an antibody that binds to the analyte. Many immunosensing devices rely upon solid phase technologies in conjunction with either flow-through, lateral-flow or immunochromatography processes. In the flow-through format, a heterogenous immunoassay takes place in a porous matrix cell that acts as the solid phase while in lateral flow, the separation stages take place as the sample passes along the porous matrix. A typical immunoassay format in a flow-through device has an antibody covalently coupled to the surface of a porous matrix. When the patient sample is added to the matrix, the analyte of interest binds to the antibody. Addition of a second labelled antibody forms a sandwich and traps the label at the position of the

first antibody. If the label is gold sol particles or coloured latex, the label can be directly visualised or quantitated by reflectance spectrophotometry in a separate reader.

In all these different formats, uniform and predictable flow of the sample through or along the solid-phase matrix is a major determinant of the reproducibility of the technique. Therefore the choice of matrix and how it interacts with the sample is of particular importance. Advances in the understanding of solid-phase technology have made a major contribution to the development of immunosensors.

Probably the most common analytes measured by immunosensing technologies are the Troponin cardiac markers but others of considerable commercial interest are D-dimer and Brain natriuretic peptide (BNP) in blood as well as various drugs of abuse such as cannabinoids and opiates in urine. A growing area is measurement of infectious disease agents such as HIV antibodies where the need to be able to give the patient the results of testing at the time of the consultation, demands an easily used biosensor device.

Biosensors are now being developed using alternatives to electrochemical transducers including optical, piezoelectric and thermometric transducers. Commercial instruments for research laboratories using surface plasmon resonance and evanescent wave transduction have been available for some time and diagnostic instruments for measurement of parameters such as cardiac markers may appear in the near future.

Advances in microfabrication technology will be a major driver in the development of Gene-Chips or arrays, devices which can rapidly screen samples for many thousands of different DNA sequences. Array devices have been developed for measuring a number of different hormones and drugs but few if any are commercialised at this point in time. Techniques borrowed from the computer industry such as the application of recognition elements through ink jetting, offer the potential to make devices with up to 10,000 sensor spots of 50 μm in size. The ability to assay many substances on one small sample means savings in time and money and will be a major driving force behind the development of this type of biosensor.