

A New Point of Care Assay Platform from Quotient Diagnostics

Worldwide Picture of Diabetes

The Global diabetes epidemic shows no sign of diminishing and the huge economic and social burden that is associated with the disease represents a major challenge for the world community in the coming decades. One of the major transformations in policy has been the recognition that the management of the disease to prevent or delay complications and to prevent the onset of Diabetes in major risk groups is a primary concern for Governments and Health Care Providers.

One of the major tools in the Physicians' arsenal in the treatment of Diabetes is the A1C test. The measurement of A1C in Patients with Diabetes is widely used and two prospective, randomised clinical trials, the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS), clearly demonstrated that A1C level gives a clear indication of the risk of development of Diabetes complications.

Haemoglobin A1C has historically been referred to by several different pseudonyms, such as glycated haemoglobin, glycosylated haemoglobin and HbA1c. However there has been an effort since 2001 to standardise the name of the test and A1C is becoming the most widely used and accepted term for the test. As well as the standardisation of the name of the test, there has been considerable worldwide effort to harmonise the calibration of the test. In 1993 when the DCCT was first published there was wide variability among methods with the result that values of 4.0% and 8.1% could be obtained for the same sample. Many bodies have worked with manufacturers of the tests to standardise the results and this has resulted in a dramatic improvement in comparability of A1C values between laboratories and between methods.

Point of Care Testing for A1C

Point of Care Testing is not a new phenomenon and began, as is generally accepted, with urine testing at the bedside. However the development of large centralised laboratories brought about economies of scale, improved Quality Assurance and the introduction of the large automated assay platforms that are commonplace today. There is nevertheless a place and time where POCT is appropriate for a number of reasons. For example, there is an undoubted benefit to the Patient who can have their test carried out whilst they wait to see the Physician who can then discuss the results with them and agree an appropriate course of treatment. There are currently a number of POCTs available for the measurement of A1C worldwide and it is recognised that this market is set to expand greatly over next few years. In the UK four of these POCT systems were evaluated in 2003 by the Medical Devices Agency on behalf of the NHS. The results of these evaluations can be found in their reports, MHRA 03016 for the DCA 2000+ (Bayer) and in MDA 02098 for the A1C Now test (Metrika Inc), Glycosal (Provalis Diagnostics Ltd) and Nycocard (Axis-Shield Ltd). A new generation of POCTs are becoming available which have addressed some of the perceived disadvantages of earlier systems and which, in particular, recognise the need for improved Quality Assurance and the traceability of results. The Quo-Test PoC system will be introduced later this year with an A1C test as the first in a number of assays for use at the Point of Care.

The Quo-Test System

The Quo-Test system consists of a small, lightweight instrument and a disposable cartridge which contains all the reagents required for a single test. The instrument is a

sophisticated fluorimeter and dual wavelength photometer and requires no calibration or operator maintenance. A small printer is also supplied so that a hard copy of the result can be kept with the Patient's records and a bar-code reader can also be attached to the instrument for the input of lot-specific test cartridge information.

To run an A1C test, the operator simply collects a tiny drop of blood from a finger-prick using the small plastic capillary supplied with every test cartridge. The capillary is then dropped into a hole in the top of the test cartridge that is then placed into the test zone of the instrument. To start the test the operator simply closes the instrument door. No other actions are required and the result is displayed 3 minutes later.

The instrument is lightweight, portable and self-calibrating and the test is simple, convenient and fast to carry out. This makes Quo-Test A1C ideal for PoC use in clinics and Doctors offices or surgeries.

Quo-Test A1C Technology

Quotient Diagnostics have developed a new system for the measurement of A1C in capillary or venous blood. Whereas most current methods utilise a separation step to differentiate the A1C fraction from other forms of haemoglobin, the Quo-test assay is a homogenous system with no separation or fractionation of the blood. The technology used in the test is known as Fluorescence Quenching and was first invented by Dr Ray Edwards and his colleagues at St Bartholomew's hospital. Quotient Diagnostics subsequently acquired the worldwide rights to the patent and have modified the technology to fit the requirements of a Point of Care test.

The Fluorescence Quenching assay uses a fluorophore conjugated to m amino-phenyl boronic acid. The specific interaction of the 1, 2 cis-diol groups of glycated haemoglobin with the boronic anion is well understood and the first commercial affinity chromatography assay for A1C using this method was described as early as 1981. A number of large automated laboratory systems also use this method for the measurement of A1C. The Quo-Test A1C assay therefore uses a well-established method for the binding of A1C in the sample, however the novelty in the assay comes from the method used to detect the binding of boronic acid to A1C.

The fluorophore, which is conjugated to the phenyl boronic acid, performs two functions; firstly, when a test cartridge is placed into the instrument, the amount of fluorescence in the buffer is measured as a baseline. When the blood sample is automatically added to the fluorescent buffer, the haemoglobin in the sample immediately reduces the fluorescence due to an inner filter effect. Importantly, this reduction in fluorescence is directly proportional to the concentration of haemoglobin in the sample. Therefore, by measuring the baseline fluorescence of the buffer and the fluorescence after the blood sample has been added, it is possible to accurately determine the total haemoglobin concentration in the sample.

Secondly, as soon as the blood sample has been added to the buffer in the test cartridge, the specific binding of the fluorophore-boronic acid conjugate to A1C begins. As the conjugate becomes bound to A1C, the fluorescence is quenched by the proximity of the coloured haemoglobin molecule. Again, the rate and degree of quenching are directly proportional to the A1C concentration in the sample. Curve-fitting algorithms are used to calculate the result, which is displayed on the large screen.

Assay Performance

Quo-Test A1C results are DCCT aligned, traceable to the IFCC reference method and the system has been compared to a number of laboratory-based methods to ensure that results

are accurate and precise. Correlation with these analysers exceeds $r = 0.97$ and the precision of the test, when measured over a twenty day period, gave a Coefficient of Variation (CV) for individual samples of less than 3 % CV. The assay is not affected by the main haemoglobin variants or by the presence of foetal or carbamylated haemoglobin.

Quality Assurance

One of the barriers for acceptance of POC assays has always been the perception that the quality of results obtained with this type of tests were not as good as results obtained from a laboratory. However, the new generation of POC tests which are starting to appear world-wide, often have the same degree of accuracy and precision as tests done in the Clinical Laboratory. There still remains the fact that POC tests, by their very nature, tend to be carried out away from the strict Quality Assurance procedures that are part and parcel of a large Biochemistry Laboratory. In order to rectify this issue the Quo-Test instrument has a QC function which can be switched on by the Laboratory Administrator when the instrument is installed. When the function is active, the instrument will require regular QC tests to be carried out before Patient samples can be run. QC controls for use with the system can be obtained from the Manufacturer and when the instrument requires a QC sample, the operator simply uses the bar-code scanner to input Control information from a bar code printed on the vial and then run the control as normal. At the end of the test, the result is calculated and compared with the acceptable range obtained from the bar code. If the control result was within acceptable limits, then the Operator can run further Patient samples. However, if the Control was out of range, then the instrument displays QC Lockout until an Administrator has intervened.

In addition to this QC function, the bar code scanner can also be used to input Operator and Patient ID when running a test. This information is stored in the instruments' memory together with the result, time, date and test cartridge lot number, ensuring that there is full traceability for the result of all tests performed on the system.

It is therefore apparent that the requirements for demonstrable and enforceable QC testing, which are a prerequisite for large automated laboratory equipment, are now being introduced into POC systems such as the Quo-Test A1C assay. This will ensure that tests carried out in remote areas away from the central laboratory now conform to the same exacting Quality standards as those performed in the Biochemistry lab.

The Quo-Test Biochemistry Platform

One of the most disturbing factors of Diabetes has been its' association with other life-style factors which have resulted in escalating rates of Diabetes (including impaired glucose tolerance), central (abdominal) obesity, hypertension and elevated blood lipids. The so-called "Deadly Quartet" of Diabetes.

Therefore carrying out an A1C test on a Patient at the POC does not obviate the necessity for the Physician to take a blood sample from the Patient in order to test for other important analytes. The Quo-Test system has therefore been designed to offer a full menu of biochemistry tests which are presently only available in the laboratory. In addition to the fluorimeter which is used for the A1C assay, the instrument also contains two photometer channels and is therefore a sensitive dual wave spectrophotometer. The test cartridge has been designed with simplicity in mind and is capable of carrying liquid reagents for enzyme cascade (e.g. cholesterol) and immunoturbidometric (e.g. hsCRP) assays. In the next few years, a number of new tests will be launched for use on the Quo-Test system enabling

Physicians' to obtain immediate results for these important risk factors so that they can discuss treatment and life-style changes with their Patients.

A1C Testing in Emerging Health care Markets

The Diabetes Atlas version 2 was issued by the International Diabetes Federation in 2003. In this comprehensive and wide-ranging report, the considerable toll that Diabetes exerts on the Health Care resources of most developing countries is highlighted. In addition, the rate at which new cases of Diabetes are emerging in these regions places an additional burden on countries already struggling with life-threatening diseases such as Malaria and HIV/AIDS. Whilst it must be stressed that the chronic lack of access to insulin and oral hypoglycaemic agents represents the main challenge for the Medical community in these regions, the lack of suitable Diagnostic equipment is also of concern. Quotient Diagnostics have therefore adapted their technology and produced the Quo-Test Lab instrument. This instrument contains the same fluorimeter and photometer technology as the POCT but has no moving parts. Whereas the POC system uses a cartridge with all the reagents contained within it for a single test, the Lab system comprises of a kit containing bulk reagent and enough test tubes for 100 tests. The user is required to pipette an appropriate amount of reagent into a tube prior to running a test and also to manually add a tiny amount of the Patient's blood. Calibration of each lot of bulk reagent is also required, however the result is available in 3 minutes and the accuracy and precision of the system is as good as a laboratory test. The great benefit to the user is that A1C tests can be done with minimal operator manipulation and at a fraction of the cost of PoC assays.

The Quo-Test PoC system will be available form the end of this year and the Lab version will be introduced shortly afterwards. Both systems will provide operators with Laboratory performance combined with the confidence of Quality Assurance.

Figure 1; Quo-Test Instrument and Test Cartridge



Figure 2; Quo-Test Lab Instrument

