HbA1c analysing – challenges for the laboratory – internal and external QC

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The uncertainty of a laboratory result is usually higher than expected by the general consumer of laboratory results, including the patients. It is a challenge for the laboratory to explain why a result never is exactly correct, but still useful for an intended clinical purpose.

The uncertainty of a result is estimated through a complete error analysis, including pre-analytical error components. The laboratory continuously monitor the performance of the assay as such in order to guarantee that the uncertainty of the reported results are within claimed limits. Internal quality control (QC) samples (expected values are determined and known prior to test) and external QC samples (for the laboratory blinded values) are used to check and describe the accuracy of the major analytic steps of a method over time. The internal and external QC are the pillars on which the monitoring of laboratory performance relies.

Assays for HbA1c have developed over years from assays with large uncertainties, to the current tests with a high degree of precision and trueness. The HbA1c results are now also used for the diagnosis of diabetes.

The external quality control material must be as commutable as possible and should have a target value achieved with a reference method procedure. For HbA1c are pools of fresh frozen native whole blood the best available sample material and is, with few exceptions, fully commutable. The pools should contain samples from at least 5 different patients. HbA1c is stable for up to a week in a cooled blood sample. If the logistics for sampling and distribution of samples to participating laboratories can be arranged within this period, fresh material can be used for external quality assessment. If logistics for collection and distribution of samples does not allow the use of fresh material, lyophilized material might have to be used, in spite of lack of commutability.

For tests used for the monitoring of diseases, the precision of the tests are often more important than the trueness. Long time stability of the internal QC material is therefore of prime importance. Lyophilized materials are often used because they meet the requirements of stability. Due to lack of commutability of lyophilized materials, method dependent target values and acceptance limits have to be assigned to the materials. Sometimes locally defined target values and acceptance limits have to be applied. Stabilized sample materials used for internal QC often show higher values for reproducibility than native samples do. Despite such shortcomings, stabilized materials are proper to use in order to identify changes over time in the performance of the assay. Internal QC material is usually provided by the manufacturer of the device. An identified problem is the lack of available internal QC material with target values close to the diagnostic limit 48 mmol/mol.

For a quantitative assays used to diagnose a condition defined by an agreed cut off level, the trueness of the assay might be more important than the precision. Diabetes is now diagnosed according to WHO by the finding of a repeated HbA1c value > 48 mmol/mol. A small bias of the HbA1c assay, e.g. 2 mmol/mol, will affect the prevalence of diabetes with 5 %.

The acceptance limits for external QC results might be decided with respect to uncertainty of the target value, and the allowable bias and imprecision of the assays. For Equalis EQA scheme the acceptance limits is defined as deviations less than +/- 3,5 mmol/mol from the target value with 95 % probability at the diagnostic limit 48 mmol/mol. This level of accuracy can be achieved with both hospital methods and point of care methods under good control.

A requirement for an accredited EQA scheme is that target values are set by accredited methods. Target values for HbA1c are offered by the European Reference Laboratory for Glycohemoglobin (ERL) in Holland, who offer measurements with the IFCC reference methods.