CAT
Critically Appraised Topic

HbA1c: will the HbA1c auto-analyzer HA-8160 (Menarini Diagnostics) imply a substantial improvement compared to the HA-8140?

Author: Apr. Inge Thoelen
Supervisor: Dr. Marc Moens/Dr. Jan Moerman
Search/methodology verified by: Dr. Johan Frans
Date: 14/07/2004
Expiry date: 14/07/2006

CLINICAL BOTTOM LINE

The Menarini HA-8160 HbA1c auto-analyzer has proven to be highly reliable, highly reproducible, technically simple to use, and appears a substantial improvement compared to the HA-8140.

Physician-endocrinologists were informed about the new HbA1c analyzer HA-8160 and its analytical performance characteristics. In addition, issues that had to be considered in the clinical decision making process were discussed, emphasizing the impact of assay imprecision and intra-individual biological variability on successive HbA1c results of the same patient. To ensure proper interpretation, we sought to report 95% confidence intervals along with the HbA1c result.

To provide simultaneous HbA1c availability with the patient visits, we agreed with the physician-endocrinologists on elaborating a fixed HbA1c daily assay schedule.

CLINICAL/DIAGNOSTIC SCENARIO

As early as 1955, researchers detected hemoglobin A1 (HbA1) as a fast-migrating component of Hb on gel electrophoresis. Using cation-exchange chromatography this fast-migrating portion was further fractionized into HbA1a, HbA1b, and HbA1c, with the latter accounting for 80% of HbA1. By the mid-1970s it became clear that HbA1 comprised several glycated hemoglobins and HbA1c in particular was the product of a non-enzymatic, irreversible posttranslational reaction between glucose and the aminoterminal valine of one or both β-chains of HbA (5, 10). Since red cells are freely permeable to glucose, HbA1c is formed at a rate dependent upon the prevailing blood glucose and consequently the level of HbA1c at a certain point in time reflects the glycemic history of the previous 120 days (average red cell lifespan). In the Diabetes Control and Complications Trial (DCCT) even a linear relationship was shown between HbA1c and mean blood glucose (22). Briefly, 6% HbA1c was shown to correspond to a mean blood glucose of 135 mg/dl and each 1% increase in HbA1c to a 35 mg/dl increase in mean blood glucose (5). Routine HbA1c testing is recommended in all patients with diabetes mellitus, not only to document their degree of glycemic control but also as a measure of risk for the development of diabetes complications (1, 5). There are many different HbA1c assay methods in current use (>30) but in all of these, HbA1c is expressed as a percentage of total hemoglobin.

In our laboratory, we used the Menarini HA-8140 for HbA1c analysis, which is based on reverse phase cation exchange 'High Performance Liquid Chromatography' (HPLC). On

account of our HA-8140 analytical performance scores, obtained in the HbA1c external quality assessment program (EKE) of the previous years, it became clear that this HbA1c analyzer scored unacceptable in the presence of carbamylated Hb (due to uremia). The HA-8140 analyzer was not able to separate carbamylated Hb from the HbA1c peak. The manufacturer Menarini therefore promoted the HA-8160 HbA1c analyzer (an upgrade of the HA-8140) which follows the same assay principle but has a lot of advantages above the HA-8140 (fully automated, faster, no interference of carbamylated and acetylated Hb, better reproducibilities,…). Hence, we started to think about replacing the HA-8140 with the HA-8160 but first evaluated the HA-8160 method in comparison with the HA-8140 in our laboratory.

QUESTION(S)

1) In which aspects will the use of the HA-8160 imply a substantial improvement compared to the HA-8140?
2) After assessing and judging the analytical performance characteristics of the HbA1c auto-analyzer HA-8160, can we conclude that the instrument meets the required criteria of analytical quality?
3) What is the impact of the HA-8160 analytical performance criteria on the clinical decision making process?

SEARCH TERMS

1) MeSH Database (PubMed): MeSH term: "Hemoglobin A, Glycosylated"
2) PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific)
3) Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/), National Guideline Clearinghouse (http://www.ngc.org/), Institute for Clinical Systems Improvement (http://www.icsi.org), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (http://www.update-software.com/cochrane/), Evidence Based Medicine Resource Center UZ Leuven (http://www.uzleuven.be/ebm/), Health Technology Assessment Database (http://www.york.ac.uk/inst/crd/htahp.htm)
4) National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/), International Federation of Clinical Chemistry (IFCC; http://www.ifcc.org/ifcc.asp), American Diabetes Association (ADA; http://www.diabetes.org/home.jsp), National Diabetes Information Clearinghouse (NDIC; http://diabetes.niddk.nih.gov/), Westgard QC (http://www.westgard.com), Clinical Laboratory Improvement Amendments (CLIA; http://www.cms.hhs.gov/clia/)
5) UpToDate Online version 12.2 (2004)
6) Diagnostisch kompas (College voor zorgverzekeringen, the Netherlands, 2003), Clinical Chemistry 4th edition (William J. Marshall; Mosby)

RELEVANT EVIDENCE/REFERENCES

1) American Diabetes Association. "**Standards of medical care in diabetes"** Diabetes Care. 2004 Jan;27(Suppl 1):S15-S35. (Guidelines)

2) McIntosh A, Hutchinson A, Home PD, Brown F, Bruce A, Damerell A, Davis R, Field R, Frost G, Marshall S, Roddick J, Tesfaye S, Withers H, Suckling R, Smith S, Griffin S, Kaltenthaler E, Peters J, Feder G. **"Clinical guidelines and evidence review for type 2**

diabetes: management of blood glucose" 2002 Sep; Sheffield: ScHARR, University of Sheffield (www.shef.ac.uk/guidelines/). (Guidelines)

3) Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. **"Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus"** Clin Chem. 2002 Mar;48(3):436-72. (Guidelines)

4) Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, Miedema K, Mosca A, Mauri P, Paroni R, Thienpont L, Umemoto M, Weykamp C; International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). **"Approved IFCC reference method for the measurement of HbA1c in human blood"** Clin Chem Lab Med. 2002 Jan;40(1):78-89. (Guideline)

5) Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, Sacks DB. **"Tests of glycemia in diabetes"** Diabetes Care. 2004 Jul;27(7):1761-73. (Review/Guidelines)

6) Jeffcoate SL. **"Diabetes control and complications: the role of glycated haemoglobin, 25 years on"** Diabet Med. 2004 Jul;21(7):657-65. (Review)

7) Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J, Zimmet P; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. **"Follow-up report on the diagnosis of diabetes mellitus"** Diabetes Care. 2003 Nov;26(11):3160-7. (Review)

8) Manley S. **"Haemoglobin A1c - a marker for complications of type 2 diabetes: the experience from the UK Prospective Diabetes Study (UKPDS)"** Clin Chem Lab Med. 2003 Sep;41(9):1182-90. (Review)

9) Price CP. **"Point-of-care testing in diabetes mellitus"** Clin Chem Lab Med. 2003 Sep;41(9):1213-9. (Review)

10) John WG. **"Haemoglobin A1c: analysis and standardisation"** Clin Chem Lab Med. 2003 Sep;41(9):1199-212. (Review)

11) Miedema K. **"Laboratory tests in diagnosis and management of diabetes mellitus. Practical considerations"** Clin Chem Lab Med. 2003 Sep;41(9):1259-65. (Review)

12) Rose E, Ketchell D. **"Does daily monitoring of blood glucose predict hemoglobin A1c levels?"** J Fam Pract. 2003 Jun;52(6):485-90; discussion 490. (Review)

13) Greenberg RA, Sacks DB. **"Screening for diabetes: is it warranted?"** Clin Chim Acta. 2002 Jan;315(1-2):61-9. (Review)

14) Krishnamurti U, Steffes MW. **"Glycohemoglobin: a primary predictor of the development or reversal of complications of diabetes mellitus"** Clin Chem. 2001;47(7):1157-65. (Review)

15) Kilpatrick ES. **"HbA1c measurement"** J Clin Pathol. 2004 Apr;57(4):344-5. (Editorial)

16) Manley S, John WG, Marshall S. **"Introduction of IFCC reference method for calibration of HbA: implications for clinical care"** Diabet Med. 2004 Jul;21(7):673-6. (Original article)

17) Miller CD, Barnes CS, Phillips LS, Ziemer DC, Gallina DL, Cook CB, Maryman SD, El-Kebbi IM. **"Rapid A1c availability improves clinical decision-making in an urban primary care clinic"** Diabetes Care. 2003 Apr;26(4):1158-63. (Original article)

18) Gerlo E. **"De bepaling van hemoglobine A1c"** Folia Diagnostica. 2003;12e jaargang:41-48. (Original article)

19) CDC Diabetes Cost-effectiveness Group. **"Cost-effectiveness of intensive glycemic control, intensified hypertension control, and serum cholesterol level reduction for type 2 diabetes"** JAMA. 2002 May 15;287(19):2542-51. (Original article)

20) Cagliero E, Levina EV, Nathan DM. **"Immediate feedback of HbA1c levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients"** Diabetes Care. 1999 Nov;22(11):1785-9. (Original article)

21) UK Prospective Diabetes Study (UKPDS) Group. **"Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33)"** Lancet. 1998 Sep 12;352(9131):837-53. (Original article)

22) The Diabetes Control and Complications Trial Research Group (DCCT). **"The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus"** N Engl J Med. 1993 Sep 30;329(14):977-86. (Original article)

23) McCulloch DK. **"Estimation of blood glucose control in diabetes mellitus"** UpToDate online 12.2;2004.

24) Rose BD, McCulloch DK. **"Glycemic control and vascular complications in type 1 diabetes mellitus"** UpToDate online 12.2;2004.

25) McCulloch DK. **"Glycemic control and vascular complications in type 2 diabetes mellitus"** UpToDate online 12.2;2004.

26) John WG, Weykamp C, Galanello R, Jou JM, Maggio A. **"Multi-national evaluation of the fully automated Arkray HA-8160 haemoglobin A1c analyser"** Clin Chem Lab Med. 2002 Oct;40(10):S106. (Poster Abstract)

APPRAISAL

1) <u>Analytical performance characteristics (analytical validation report)</u>

   1.1 Preanalytical considerations (patient variables, sample stability)
   <u>Patient variables</u>
   There are no clinically significant effects of age, sex, ethnicity, or season on HbA1c measurement results. Results are also not significantly affected by acute illness. There is no substantial effect of food intake or recent physical exertion on test results (3).
   Any condition that shortens erythrocyte survival (hemolytic anemia, hemoglobino-pathies (e.g. HbSS, HbSC,…)) or decreases mean erythrocyte age (recovery from acute blood loss) falsely lowers HbA1c results. Iron-deficiency anemia and splenectomy falsely increase HbA1c test results probably through prolonged erythrocyte survival (3, 10, 18, 23).
   Several hemoglobin variants (HbS, C, D, …), fetal hemoglobin (HbF), and chemically modified derivatives of hemoglobin (carbamylated Hb in the case of uremia; acetylated

Hb in the case of chronic ingestion of salicylates; pre-A1c or labile HbA1c which is the reversible intermediate Schiff base in the formation of HbA1c and which is highly responsive to short-term fluctuations in plasma glucose) interfere with some assay methods. In the presence of Hb variants and elevated levels of HbF (children younger than 6 months of age, "hereditary persistence of fetal hemoglobin",…) HbA1c should be calculated as percentage of HbA and not of total Hb (3, 10, 18, 23).

Icteric (jaundice) and lactescent (hyperlipidemia) specimens can also cause false elevation of HbA1c with some methods (3, 10).

The within-subject and between-subject biological variation for HbA1c are estimated to amount to 1.9% and 4.0% respectively (www.westgard.com/biodatabase1.htm). About two-thirds of the between-subject variation is suggested not to be glycemia related but due to genetic factors (enzymatic deglycation, genetically controlled erythrocyte fragility) (6).

<u>Sample stability</u>

There are no special collection conditions for HbA1c assays; the patient does not have to be fasting. Blood is preferably collected into EDTA tubes but may also be collected into heparinised or fluoride oxalate tubes. The latter anticoagulant however may affect methods based on charge separation. Sample stability is assay method specific. In general, whole blood samples are stable for up to 1 week at 4°C. Hemolysates stored at -70°C are stable for much longer periods (at least 1 year) (3, 10, 11).

1.2 Analytical considerations (reproducibility, accuracy, correlation, linearity, reference range)

<u>Reproducibility</u>

Recommendations ADA (American Diabetes Association): "Laboratories should use HbA1c assay methods with an interassay coefficient of variation (CV) <4% (ideally <3%)." (Level of evidence: C; cfr. Attachment 1, Table 1.1) (3, 5)

Both the within-run and between-run reproducibility of the HbA1c auto-analyzer HA-8160 were evaluated using whole blood samples. The within-run imprecision was tested by analyzing 22 whole blood samples twice in a single assay and subsequently a CV per sample was calculated. The mean CV was 0,50% and could be used as a very good estimate for the within-run reproducibility of the HA-8160 (Table 2.2, Figure 2.2). The between-run imprecision was assessed on two levels of HbA1c (level 1: mean value = 5.9%; level 2: mean value = 11%) in 21 runs and the resulting CV's were 1,58% and 0,88% for level 1 and 2 respectively (Table 2.2, Figure 2.1). CV's were scored according to the judgment criteria of the HbA1c external quality assessment program 2003 (EKE 2003) and compared to the total CV of the HA-8140 (CV HA-8140 = 2.60%) (Table 2.1, Table 2.2). The HA-8160 was found to have an excellent reproducibility both within- and between-run, to have substantial better scores for reproducibility than the HA-8140, and to comply with the recommendations of the ADA as stated above.

<u>Accuracy (bias)</u>

Recommendations ADA: "Laboratories should be aware of potential interferences, including hemoglobinopathies, that may affect HbA1c test results. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population." (Level of evidence: A) (3)

Accuracy of the HA-8160 was tested with external quality assessment material regained from EKE 2003 (kindly provided by the Scientific Institute of Public Health Belgium). Deviation from target was assessed on three levels and additionally interferences of carbamylated Hb and Hb variants (HbS, HbC) were examined. Deviations on the HA-8160 were found to be good to excellent and highly accurate

results were produced in the presence of carbamylated Hb or Hb variants (Table 2.2). For comparison, the HA-8140 scored unacceptable in the presence of carbamylated Hb.

Correlation with current method

As Table 2.2 and Figure 2.3 show, there was a good correlation between the HA-8160 and HA-8140 test results (R=0.9912). However, 30 samples were initially analyzed in the correlation study but 8 outliers were removed before interpretation because of falsely elevated an falsely low results on the HA-8140 due to interference of carbamylated Hb and increased HbF respectively. These interferences are eliminated on the HA-8160 since carbamylated Hb is eluted separately from the stable A1c peak off the HA-8160 column and the HbF peak is not integrated anymore in the total area of Hb.

Linearity

Linearity was evaluated by gradually diluting a 5.6% HbA1c sample in a 11.5% sample. Linearity was excellent throughout the clinically important range (Table 2.2, Figure 2.4).

Reference range

Recommendations NCCLS: "A laboratory should determine its own reference interval according to NCCLS guidelines (NCCLS Document C28A) even if the manufacturer has provided one."

We analyzed samples of 20 non-diabetic test participants. Only one of the 'normal' samples had a result (6.1%) outside the company reference range (4.0%-6.0%) and so we accepted these HbA1c values as 'normal' values. The calculated mean +/- 2 standard deviations (SD) of our 20 'normal' samples resulted however in a reference interval of 5.0%-6.0%. In a multi-national evaluation of the HA-8160 a corresponding smaller reference interval for HbA1c was found (4.6%-5.8%; 26). For NGSP-certified assay methods (NGSP: National Glycohemoglobin Standardization Program) like the HA-8160 assay, reference intervals should not deviate significantly from the 4.0%-6.0% range. Note however that the upper limit, which is clinically the most important reference limit for HbA1c, remains the same in our 'normal' population.

1.3 Analytical range

The resolution of the HA-8160 HbA1c auto-analyzer is 0.1% and peaks as low as 0.1% can be detected. The analytical range is consequently 0.1%-100%.

1.4 Turn around time (TAT)

In diabetes monitoring mode, throughput is 2.9 minutes per sample (4 minutes per sample for the HA-8140). A priming cycle of 6 minutes 36 seconds precedes the first sample measurement.

1.5 KAL (clinical tolerance limits)

Biological variation is a good basis for deriving analytical quality specifications that satisfy general medical needs. For monitoring a patient's condition (such as in the case of HbA1c), analytical variation has to be maintained below half the within-subject component of biological variation. To determine the patient status with regard to population-based reference intervals, analytical bias must be maintained below a quarter of the within- plus between- subject components. The optimum, desirable, and minimum quality specifications for analytical imprecision (I), bias (B) and total error (TE), expressed in percentages, are calculated from the within- and between-subject components of variation, expressed in coefficients of variation (CV_w and CV_g, respectively), using the following formulas (www.westgard.com/biodatabase1.htm):

- $I < k\ CV_w$ (desirable: k= 0.50; optimum: k=0.25; minimum: k=0.75)
- $B < m\ (CV_w^2 + CV_g^2)^{1/2}$ (desirable: m=0.250; optimum: m=0.125; minimum: m=0.375)

- $TE < 1.65 \, k \, CV_w + m \, (CV_w^2 + CV_g^2)^{1/2}$

The theoretical KAL are defined by the total error, so for HbA1c with a CV_w of 1.9% and a CV_g of 4.0% the desirable, optimum, and minimum KAL are 2.7%, 1.3%, and 4% respectively. In practice, these theoretical KAL are too strict for current HbA1c assay methods (for which an ideal CV <3% is recommended; see above). This is partly due to the fact that CV_w and CV_g are determined based on a 'normal' non-diabetic population while biological variations in diabetic patient populations were found to be much higher (10). In our laboratory, these KAL are therefore currently not used in the HbA1c internal quality assessment. Each day one of the two control materials (with high and low mean HbA1c value) is included at the beginning of the run. Imprecision and accuracy are next evaluated according to Westgard rules (http://www.westgard. com/mltirule.htm) via the internal quality management software program QC-Today (version 2.03.35, Instrumentation Laboratory, Belgium). Stop limits for HbA1c are set as the mean ± 3,5 standard deviations. Additionally, our laboratory participates in an external HbA1c proficiency-testing program (EKE; http://www.iph.fgov.be/ClinBiol/ NL/EEQ.htm), as recommended by the ADA.

2) Diagnostic performance

   2.1 *Sensitivity, specificity*
   At present, the ADA does not recommend the use of HbA1c as a diagnostic test for diabetes. The primary reason for this decision is a lack of standardized methodology resulting in varying non-diabetic reference ranges among laboratories (3,7). However, the use of HbA1c for the diagnosis of diabetes has been extensively considered since its introduction as an index for glycemia, and there is still considerable controversy surrounding this issue. A meta-analysis showed that, when using a statistical cut point of 2 SD's above the non-diabetic mean HbA1c value to diagnose diabetes, as defined by the 2-hour plasma glucose, a variety of HbA1c assays had a mean sensitivity of 66% and a specificity of 98%, which compares favorably to the fasting plasma glucose (7, 13). Yet, the ADA believes that it is still premature to add HbA1c to the group of tests used for the definitive diagnosis of diabetes and that it is best continuing to use the HbA1c test as a monitor for the effectiveness of glycemic therapy and as an indicator for when therapy needs to be modified.

   2.2 *Likelihood ratio's (LR)*
   Not really applicable for HbA1c since it is not used as a diagnostic test, though using the mean sensitivity and specificity from a meta-analysis (7), likelihood ratio's can be calculated as follows:
   LR_{+ve} = sensitivity/(1-specificity) = 0.66/(1-0.98) = 33
   LR_{-ve} = (1-sensitivity)/specificity = (1-0.66)/0.98 = 0.35
   The likelihood ratios express the odds that a given finding (here: positive or negative test result) would occur in a person with, as opposed to without, a particular condition.

   2.3 *NND (number needed to diagnose)*
   NND = 1/[sensitivity - (1-specificity)] = 1/[0.66 – (1-0.98)] = 1.56
   The NND combines sensitivity and specificity in a single term and can be a useful tool to compare various diagnostic tests with regard to the same condition. The ideal NND is 1. A NND of 1.56 for HbA1c is acceptable but further efforts need to be done to standardize the HbA1c assay worldwide before this measurement could be routinely employed for diagnosing diabetes (7).

3) Clinical impact

Recommendations ADA: "HbA1c should be measured routinely in all patients with diabetes mellitus to document their degree of glycemic control. Treatment goals should be based on the results of prospective randomized clinical trials such as the DCCT (22) and UKPDS (21). These trials have documented the relationship between glycemic control, as quantified by serial determinations of HbA1c, and risks for the development and progression of chronic complications of diabetes." (Level of evidence: A) (3)

3.1 *Diagnostic aspect*

Blood and urine glucose and urine ketone testing, which are important tests of glycemia in diabetes, provide useful information for day-to-day management of diabetes. However, these tests cannot provide the patient and health care team with an objective measure of glycemia over an extended period of time. Measurement of glycated proteins, primarily HbA1c, has added a new dimension to the assessment of glycemia. With a single measurement, a HbA1c test can quantify average glycemia over the past 3-4 months, thereby complementing day-to-day testing (5). HbA1c is used both as an index of mean blood glucose and as a measure of risk for the development of diabetes complications (3, 5, 12; cfr. 3.3). Today, HbA1c is the only test recommended for the assessment of long-term glycemic control. The use of other glycated proteins (e.g. serum fructosamine assay as an index for glycated serum proteins, mainly albumin) as an alternative for HbA1c testing has been well studied, but apart from several disadvantages (higher CV_w, short-term measure of glycemia (2 weeks), adjustment needed for abnormal serum albumin concentration) their clinical utility has not been clearly established yet, and there is no conclusive evidence that relates their concentration to the chronic complications of diabetes. Further studies are needed to determine their clinical usefulness (3, 5, 23).

3.2 *Treatment*

HbA1c measurements are now a routine component of the clinical management of patients with diabetes mellitus (diabetes type 1/2). Evaluation of treatment regimens is essentially based on HbA1c concentrations. Recommendations from the ADA concerning clinical management are as follows (1, 2, 3):
- Perform the HbA1c test at least two times a year in patients who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients whose therapy has changed or who are not meeting glycemic goals. (Level of evidence: E)
- Develop or adjust the management plan to achieve normal or near-normal glycemia with an HbA1c goal of <7%. (B)
- More stringent goals (i.e., a normal HbA1c, <6%) can be considered in individual patients. (B)
- Less stringent treatment goals may be appropriate for patients with a history of severe hypoglycemia, patients with limited life expectancies, very young children or older adults, and individuals with comorbid conditions. (E)
In the case of gestational diabetes mellitus, maternal metabolic surveillance should be directed at detecting hyperglycemia severe enough to increase risks to the fetus. Daily self-monitoring of blood glucose appears to be superior to intermittent office monitoring of plasma glucose or HbA1c (1).

3.3 *Health outcome*

Glycemic control is fundamental to the management of diabetes. Prospective randomized clinical trials such as the DCCT and the UKPDS have shown that improved glycemic control is associated with sustained decreased rates of

microvascular complications such as nephropathy, retinopathy, and neuropathy (Level of evidence: A; 1, 8, 14, 21, 22, 24, 25). In these trials intensive treatment regimens that reduced average HbA1c to ~7% were associated with fewer long-term microvascular complications. In addition, epidemiological studies support the potential of intensive glycemic control in the reduction of cardiovascular disease and cardiovascular mortality (macrovascular complications e.g. myocardial infarction; level of evidence: B; 1, 8, 14, 25). Finally, aggressive glycemic management with insulin may reduce morbidity in patients with severe acute illness, perioperatively and following myocardial infarction (Level of evidence: B; 1).

4) Organizational impact

   4.1 Impact in the hospital
   HbA1c is used to monitor long-term glycemic control. Like this, it has little impact on hospitalized patients (e.g. length of stay) since day-to-day monitoring of the blood glucose is then preferred, especially for patients with diabetic ketoacidosis or in a hyperosmolar hyperglycemic state (1). In these latter conditions, HbA1c testing may be useful to determine whether these acute episodes are the culmination of an evolutionary process in previously undiagnosed or poorly controlled diabetes or truly acute episodes in an otherwise well-controlled patient. Beyond that, HbA1c testing has little impact on hospital logistics or time management of hospital personnel.

   4.2 Is HbA1c incorporated in Clinical Practice Recommendations/Guidelines?
   As mentioned above, HbA1c plays a crucial role in the clinical management of diabetics (especially in out-patients) and is therefore widely incorporated in international clinical practice recommendations and guidelines for diabetes care (1, 2, 3, 5).

5) Cost impact: in and outside the laboratory

   5.1 Actual cost
   To determine the 'true' cost for a cost object like a HbA1c measurement one has to include not only the cost for the analysis as it is (instrument, reagents, maintenance, control material, sampling tubes), but also the cost of labor (laboratory personnel) and the overhead cost (indirect costs like e.g. the administration costs; principle of Activity Based Costing). In our laboratory for HbA1c, there is a special arrangement with Menarini company. We do not buy the HA-8160 analyzer and accessory reagents but we just pay a fixed price per sample measured (counter on the instrument): 2.735 € (VAT included)/test. Additional costs, which are not included in the contract, are control material for internal quality assessment (0.067 €/test) and printing paper (0.054 €/test). Next, there is the cost of material for collection of the blood sample: EDTA tube (0.096 €), half of the cost of a needle (fluoride oxalate tube for blood glucose is mostly drawn with the same needle; 0.041 €). Subsequently, we have the cost of personnel: 3 x 0.5 €/minute (two minutes for collection of the blood sample and one minute for the analysis; 1.5 €/test). Finally, we have to consider the overhead cost which has been calculated to amount to approximately 10% of the subtotal cost. Altogether, the total cost for one HbA1c measurement is 4.94 € Concerning the honoraria for HbA1c measurement provided by the RIZIV, HbA1c is categorized as a 'B 250' test (1.75 €) and the fixed honorarium per prescription for outpatients is 20.14 € This means the laboratory gets 21.89 € from the RIZIV per HbA1c prescription/measurement when it is prescribed solely. The total profit for one HbA1c measurement consequently amounts to 16.95 €

5.2 Reimbursement

HbA1c measurement is fully reimbursed by the patient sickness fund (patient personal fee = 0 €) in the case of diabetes mellitus, cystic fibrosis related diabetes, and diabetes resulting from chronic pancreatitis. Since March 1995, it is no longer reimbursed if HbA1c testing is applied for screening for diabetes because the ADA does not recommend the use of HbA1c as a diagnostic/screening test for diabetes (cfr. 2.1).

5.3 Profit elsewhere in the hospital

The HA-8160 produces more accurate and more precise HbA1c results compared to the HA-8140 which could lead to improved therapy adjustments to achieve normoglycemia in patients with diabetes mellitus and subsequently to improved glycemic control. Cost-effectiveness of intensive glycemic control relating to type 2 diabetes has been assessed by the CDC Diabetes Cost-effectiveness Group (CDC: Centers for Disease Control and Prevention; 19). They evaluated whether the benefits (measured in quality-adjusted life-years (QALY)) for type 2 diabetes of intensive glycemic control, intensified hypertension control, and reduction in serum cholesterol level, justified the costs. They concluded that intensified hypertension control reduces costs and improves health outcomes relative to moderate hypertension control. Intensive glycemic control and reduction in serum cholesterol however increase costs but improve health outcomes. For intensive glycemic control, the cost per QALY increased with age at diagnosis (19).

6) Decision making

6.1 Impact of HbA1c on the clinical decision making process and patient management

The laboratory should work closely with physicians who order HbA1c testing. Proper interpretation of test results requires an understanding of the assay method, including its known interferences. For example, if the assay method is affected by uremia, the physician should be made aware of this (3, 5). In addition, throughout the clinical decision making process physicians should translate HbA1c test results to both mean glycemia and outcome risks considering HbA1c kinetics (i.e., the rate of change in HbA1c with a change in glycemia), specific patient factors (high and low 'glycators', renal glucose threshold), and specific assay limitations (e.g. assay imprecision) (3, 5, 15). Concerning assay imprecision, physicians should take into account the 95% confidence interval (CI) limits of a HbA1c test result before interpreting the result as significantly changed with respect to the preceding HbA1c value. For example, with an assay imprecision for the HA-8160 of ~1.5% at 7% HbA1c (cfr. Table 2.2), a succeeding HbA1c value outside the 7% ± 0.2% (95% CI limits) range can be considered as a significant change. However, considering in addition a within-subject biological variability of at least 1.9% (probably higher in diabetic patients) it is recommended not to judge absolute changes up to 0,5% HbA1c as clinically relevant (3). In practice, clinically significant changes that should draw attention are ± 1% HbA1c (3, 10).

Recent studies suggest that immediate feedback to patients at the time of the clinic visit with HbA1c test results improves their long-term glycemic control since rapid HbA1c availability increased the frequency of intensification of therapy (17, 20). It is possible to achieve the goal of having HbA1c results at the time of the patient encounter with the physician, by either having the patient send in a blood sample shortly before the scheduled clinic visit or by having a rapid HbA1c assay system (cfr. 1.4). Regarding the latter, the use of point-of-care testing for HbA1c has been considered (9). Several instruments for HbA1c point-of-care testing have been

developed, based on immunological reactions or affinity chromatography, but presenting with much higher imprecisions than laboratory HPLC (10). The added benefit of rapid HbA1c measurements may however be modest in settings where intensification of therapy is already aggressive (17). To provide simultaneous HbA1c availability with the patient visits in our hospital, agreements between the laboratory and the requesting physicians should be made regarding time and frequency of daily HbA1c runs (e.g. two runs at 9.00h and 10.00h in the morning only on weekdays). One disadvantage of laboratory HbA1c HPLC-analyzers (HA-8140/8160) over laboratory HbA1c immunoassays (e.g. Cobas Integra) relates to the fact that for HPLC it is most economical when samples are collected in one single run than performing runs for each sample separately. However, STAT measurements are possible on the HA-8160 which basically means that normal runs in progress can be interrupted to measure a sample in the 'STAT port' when a quick result is needed.

6.2 Overexploitation/underutilization of the HbA1c assay

In the US, surveys have shown that the recommended frequency of HbA1c testing (cfr. 3.2) is achieved in no more than half of the diabetic patients (11). So, there is rather an underutilization of the HbA1c assay.

In our hospital ± 80% of the diabetic patients are assumed to meet the recommended criteria of HbA1c testing frequency. Figure 3.1 shows the HbA1c result distribution in our hospital of 194 HbA1c measurements performed from 16 to 30 June 2004. The majority (15+54+61/194 = 67%) of our diabetic patient population seems to achieve good to excellent glycemic control.

COMMENTS

Standardization of HbA1c

Recommendations ADA: "Laboratories should use only HbA1c assay methods that are certified by the National Glycohemoglobin Standardization Program (NGSP) as traceable to the DCCT reference." (Level of evidence: B) (3)
There are many different HbA1c assay methods in current use (>30). Assay principles are either based on charge differences between glycated and non-glycated components (HPLC, electrophoresis) or on structural differences (immunoassays, affinity chromatography) (10). Generally, results of methods using different assay principles show excellent correlation and none should be considered the 'best' method. However, reported HbA1c results from the same blood sample could differ considerably among methods unless they are standardized to a common reference. Since the DCCT (and later the UKPDS, cfr. 3.3) had determined the relationship between specific HbA1c values and long-term outcome risks in diabetic patients, the NGSP was initiated in 1996 (developed under the auspices of the American Association for Clinical Chemistry) to standardize HbA1c test results among laboratories to DCCT-equivalent values. This way, results could be directly related to these studies, and therefore to long-term micro- and macrovascular complications of diabetes mellitus. The DCCT reference is a HPLC cation-exchange method and is an NCCLS-designated comparison method (5). Today, the DCCT standard is widely applied in the USA, Europe, and Australia but some countries initiated their own national HbA1c standardization program (Sweden, Japan). For global harmonization and at an international level, the IFCC established in 1995 a HbA1c working group to develop both a new reference method and pure standards (4, 16). This new reference method involves measurement of HbA1c by mass spectrometry or capillary electrophoresis, calibrated with mixtures of highly purified HbA1c and HbA0. Due to the higher specificity of the new reference method, results are approximately 2% HbA1c lower

than results generated with current DCCT-standardized methods. However, the relationship between IFCC-numbers and DCCT-numbers appears to be linear ("DCCT = 0.915 x IFCC + 2.15"; 4, 5, 16). In the future, the IFCC reference method is going to replace the current NGSP anchor and will be adopted as a global anchor for calibration of HbA1c but with continued reporting of DCCT-numbers until further studies are completed.

TO DO/ACTIONS

1) HA-8160 method validation in comparison with the HA-8140
2) Providing physician-endocrinologists with some basic assay information about the HA-8160 (type of assay method, non-diabetic reference range, potential assay interferences, and assay performance characteristics (e.g. imprecision,…))
3) Checking the possibility of reporting 95% confidence intervals along with the HbA1c test result to ensure proper interpretation (feasible via the future LIS-system (GLIMS) which is going to be implemented before the end of 2004?)
4) Reaching agreement with the physician-endocrinologists regarding the HbA1c daily assay schedule (time and frequency) to provide simultaneous HbA1c availability with the patient visits

ATTACHMENTS

Attachment 1

Table 1.1: ADA evidence grading system for clinical practice recommendations.

Level of evidence	Description
A	Clear evidence from well-conducted, generalizable, randomized controlled trials that are adequately powered, including:
	▪Evidence from a well-conducted multicenter trial
	▪Evidence from a metaanalysis that incorporates quality ratings in the analysis
	▪Compelling nonexperimental evidence, i.e., "all or none" rule developed by Center for Evidence Based Medicine at Oxford[1]
	Supportive evidence from well-conducted randomized controlled trials that are adequately powered, including:
	▪Evidence from a well-conducted trial at one or more institutions
	▪Evidence from a metaanalysis that incorporates quality ratings in the analysis
B	Supportive evidence from well-conducted cohort studies
	▪Evidence from a well-conducted prospective cohort study or registry
	▪Evidence from a well-conducted prospective cohort study
	▪Evidence from a well-conducted metaanalysis of cohort studies
	Supportive evidence from a well-conducted case-control study
C	Supportive evidence from poorly controlled or uncontrolled studies
	▪Evidence from randomized clinical trials with one or more major or three or more minor methodologic flaws that could invalidate the results
	▪Evidence from observational studies with high potential for bias (such as case series with comparison to historical controls)
	▪Evidence from case series or case reports
	Conflicting evidence, with the weight of evidence supporting the recommendation
E	Expert consensus or clinical experience

[1] Either all patients died prior to therapy and at least some survived with therapy, or some patients died without therapy and none died with therapy. Example: use of insulin in the treatment of DKA (diabetic ketoacidosis).

Attachment 2

Table 2.1: Judgment criteria of the HbA1c external quality assessment program 2003 (EKE 2003) (http://www.iph.fgov.be/ClinBiol/NL/EEQ.htm).

Characteristic	Excellent	Good	Acceptable	Poor	Unacceptable
Deviation from DCCT-target	<0,3%	0,3%-0,5%	0,51%-0,80%	0,81%-1,20%	>1,20%
Reproducibility (CV)	<2,0%	2,0%-3,5%	3,51%-4,50%	4,51%-6,00%	>6,00%
Linearity (R)	>0,9950	0,9901-0,9950	0,9801-0,9900	0,9700-0,9800	<0,9700

Table 2.2: Analytical validation report of the HbA1c auto-analyzer HA-8160 in comparison with the HA-8140: summary.

Characteristic	HA-8160	Interpretation[1]	HA-8140	Interpretation[1]
Correlation with HA-8140 (N=22)	R=0,9912	Good	/	/
Within-run reproducibility	CV = 0,50%	Excellent	/	/
Between-run reproducibility level 1 (5,9%)	CV = 1,58%	Excellent	Total CV = 2,60%	Good
Between-run reproducibility level 2 (11%)	CV = 0,88%	Excellent		
Linearity	R=0,9986	Excellent	R=0,9937	Good
Deviation from target (5,2%)	+0,1%	Excellent	-0,2%	Excellent
Deviation from target (8,1%)	+0,2%	Excellent	-0,3%	Good
Deviation from target (10,3%)	+0,3%	Good	-0,3%	Good
Interference of 1,5% carbamylated Hb	-0,3%	Good	+1,3%	Unacceptable
Interference of 3% carbamylated Hb	-0,2%	Excellent	+0,4%	Good
Interference of HbC-variant (HbAC)	-0,1%	Excellent	+0,2%	Excellent
Interference of HbS-variant (HbAS)	-0,4%	Good	0,0%	Excellent
Reference range (n=20)	1 outlier (6,1%)	5%-6%[2]	/	/

[1] Interpretation according to the judgment criteria of the HbA1c external quality assessment program 2003 (EKE 2003) (cfr. Table 2.1).
[2] Reference interval determined on HbA1c results from 20 non-diabetic test participants.

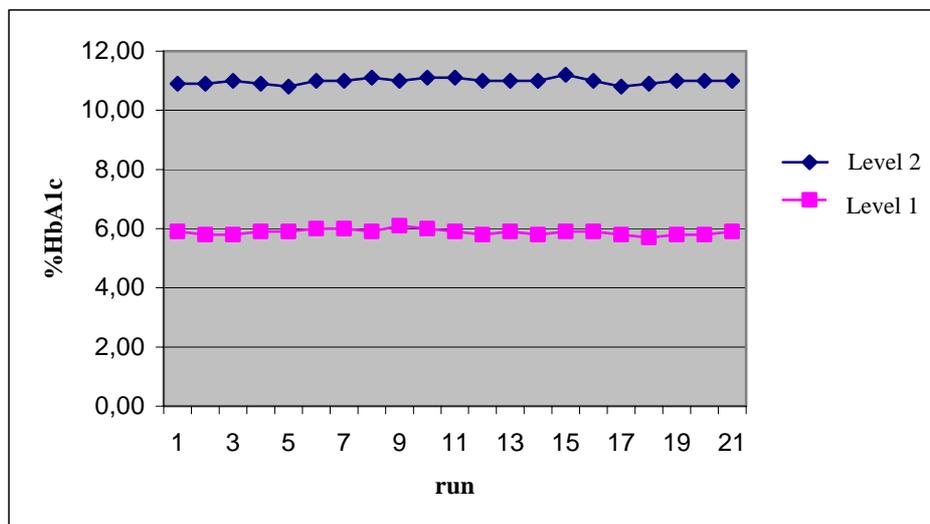**Figure 2.1:** Between-run reproducibility of the HA-8160.

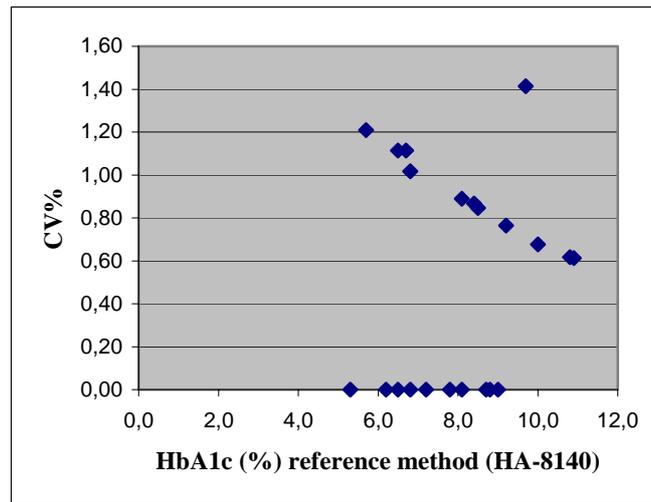**Figure 2.2:** Within-run reproducibility of the HA-8160.

Figure 2.3: Correlation between the HA-8160 and the HA-8140 test results.
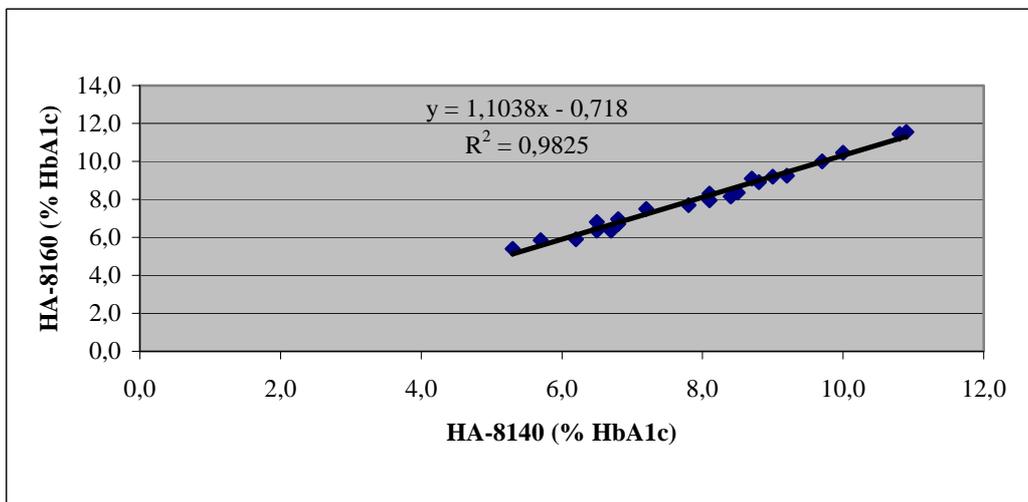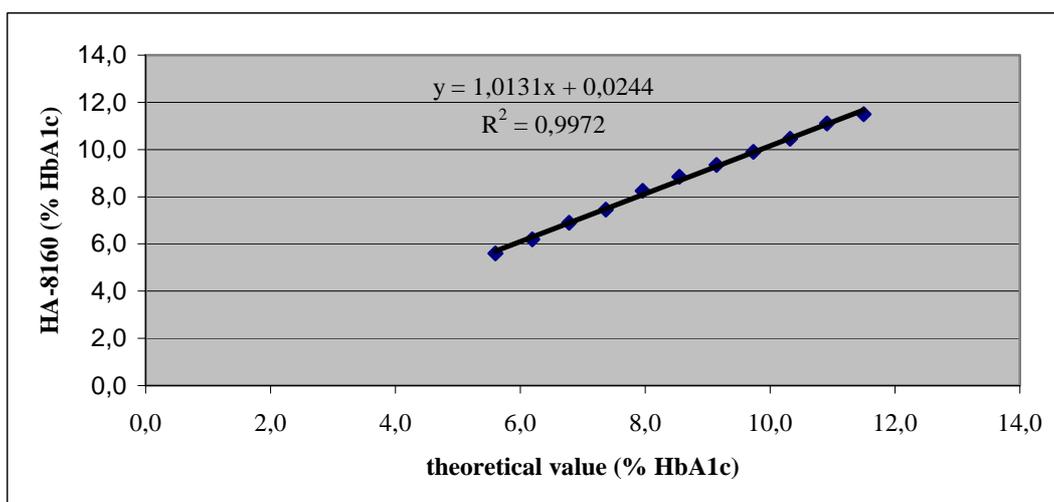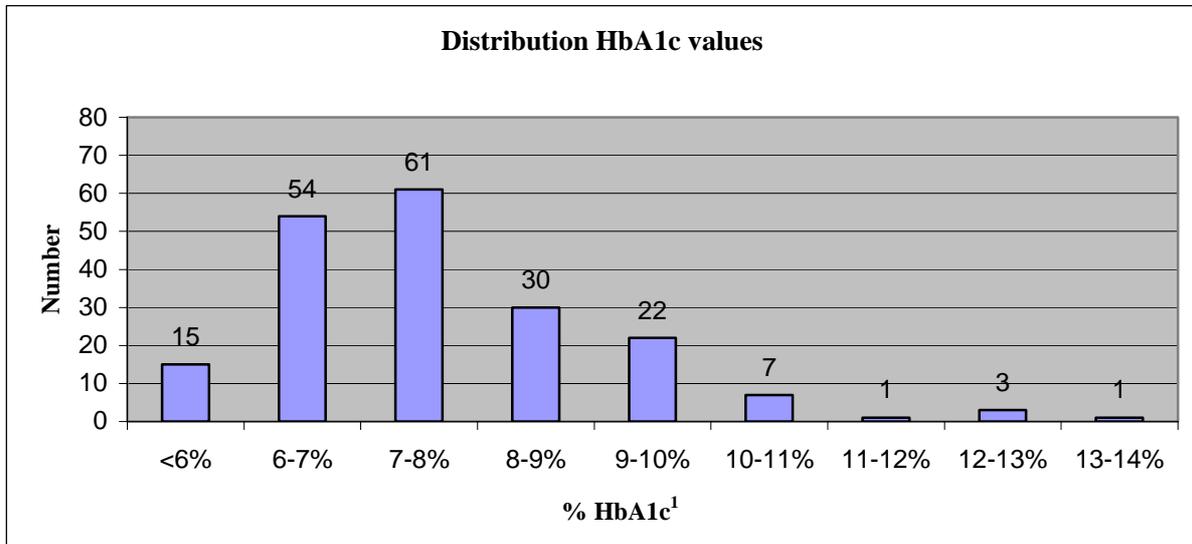

Figure 2.4: Linearity of the HA-8160.

Attachment 3

Figure 3.1: HbA1c result distribution in our hospital (Imelda Hospital Bonheiden) (194 HbA1c measurements performed from 16 to 30 June 2004; interpretations of HbA1c values are consistent with international recommendations).

[1]Interpretation: - <7%: excellent
                     - 7%-8%: good
                     - 8%-9%: moderate
                     - >9%: poor