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6.1.1.1 Discrepancy in HbA_{1c} Measurements Performed at Different Local Laboratories and at a Selected Central Reference Laboratory

O. Nørgaard Jensen, N. de Fine Olivarius,* P. Hyltoft Petersen,
N. A. Klitgaard, O. Blaabjerg and M. Hørder

Department of Clinical Chemistry, Odense University Hospital, Odense, Denmark
**Central Research Unit for General Practice, Panum Institute, Copenhagen, Denmark*

ABSTRACT

As participants in a general practice intervention study, 66 patients had their HbA_{1c} measured both at a local and at a selected central reference laboratory. A discrepancy in the results was observed, as 97% of the results measured locally were lower than the centrally determined results. Bias (as calculated from mean value of measured HbA_{1c}) between local laboratories and the central laboratory was measured to – 1.47 % HbA_{1c}. A bias of this magnitude gave "problems" both to the general practitioners, patients and laboratories.

To reduce the "problems" a bias of 0.5% HbA_{1c} is estimated to be acceptable. But, to avoid these "problems" totally, a bias of 0.25% HbA_{1c} is estimated to be the highest allowed bias. For HbA_{1c}, a control system for both control of method standardisation and for specificity is described.

CLINICAL SITUATION

In the Danish study "Diabetes Care in General Practice", 250 general practitioners participate in the intervention group (1). The purpose of the study is to implement and to document the effect of a detailed concept for treatment of diabetic patients ≥40 years of age at the time of diagnosis. The metabolic regulation over time is examined by measuring the fraction of HbA_{1c} at least once a year at a single central reference laboratory in all patients. The patients are classified according to the measured HbA_{1c} into three categories:

$$HbA_{1c} \leq 0.07 \sim \text{good control}$$
$$0.07 < HbA_{1c} \leq 0.085 \sim \text{acceptable control}$$
$$HbA_{1c} > 0.085 \sim \text{poor control}$$

However, some general practitioners prefer to have a measurement of HbA_{1c} done at their local laboratory as well as at the reference laboratory (Fig. 1). But they are freqently confronted with discrepancies between the results from the two measurements, where the result from the reference laboratory is highest, and often the reason for classifying the patient in a less favorable category, as according to the study protocol the central results were conclusive for classification and treatment of all patients. In 66 patients HbA_{1c} was measured both centrally and locally, and no more than 120 days passed between the two measurements and at least 300 days had passed since the day of diagnosis. It turned out that 35 of 66 patients were classified in a poorer category by the reference laboratory, and no one in a better. In 20 patients the classification changed from good to acceptable, in 11 patients from acceptable to poor and in 4 patients from good to poor regulation.

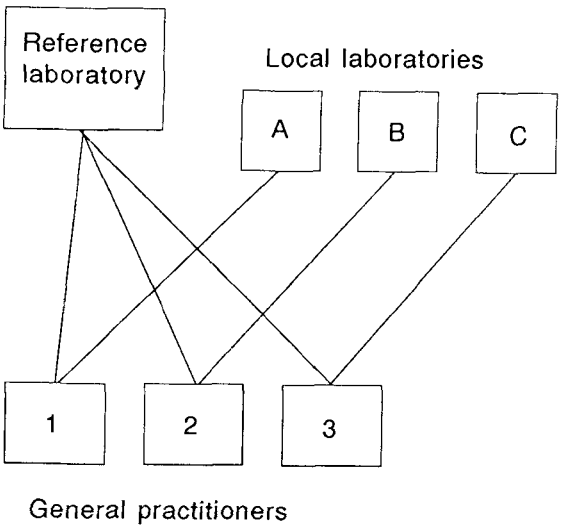
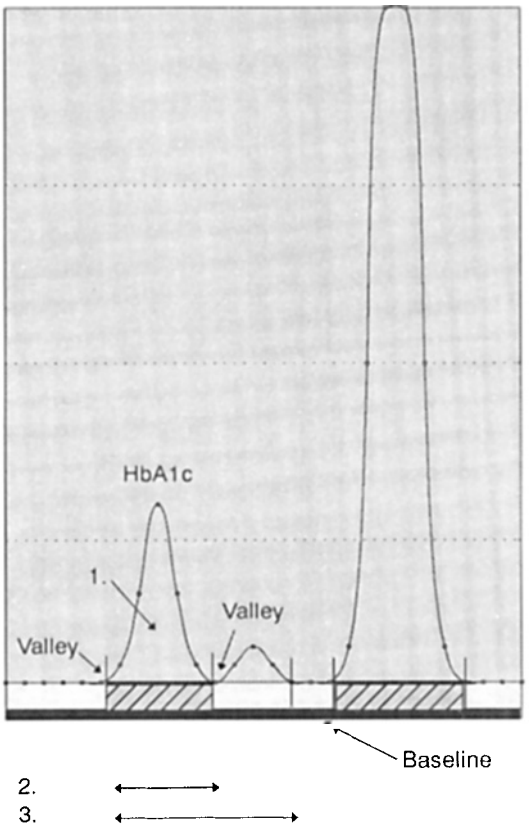


Figure 1. According to study protocol, HbA_{1c} is measured at least once a year at the selected reference laboratory in all patients. In addition, however, some general practitioners prefer to have measurements of HbA_{1c} done at their local laboratory.

CHARACTERISTICS OF THE METHODS

Two procedures of analyses were used. a) High Performance Liquid Chromatography (HPLC) and b) Low Pressure Liquid Chromatography. For explanations of the difference in results it is important to mention that when (HPLC) is used as method of analysis, three different principles for calculating the results are used. 1. Integration from "valley-valley" would yield the lowest result. 2. Integration to "the baseline" would yield a higher result. 3. Integration including the small peak close to the HbA_{1c} peak, would yield even higher results. (Fig. 2).



Figur 2. Illustration of a chromatogram. The fraction of HbA_{1c} is calculated as the area of the HbA_{1c} peak in proportion to the total area of all peaks. Three ways of calculating are used:
1) integration from Valley- Valley,
2) integration to the basisline,
3) integration to the basisline including the peak next to HbA_{1c} .

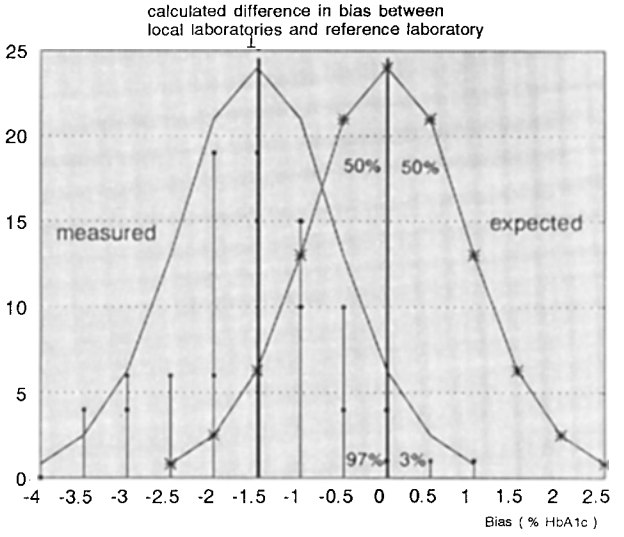
MODEL FOR EVALUATION OF QUALITY SPECIFICATIONS

None of the models available can be used directly. Therefore, a new method had to be developed. Here the agreement and disagreement between local laboratories and the reference laboratory was estimated - **and the maximum acceptable percentage of disagreement is the measure for maximum acceptable analytical bias.**

The presumptions are that differences between the local and central measurements are distributed according to the expected (only biological within-subject variation, $s_{w\text{-}s}$, and a negligible analytical precision) mean difference (~ bias). $s_{w\text{-}s}$ is about 0.6% HbA_{1c} (2) and as average estimated s_A = 0.3% HbA_{1c} , which according to the formula: presumed standard deviation, for differences, s_{pre} gives:

$$s_{pre} = \sqrt{2\,(s_{w\text{-}s}^2 + s_A^2)} = 0.95\ \%\ HbA_{1c}$$

The actual measured standard deviation, s_{meas}, is 0.83, which is fully in accordance with the presumptions. This means that the estimated and actually measured standard deviations are well matched and it indicates that variation in bias among the local laboratories is negligible. Bias as calculated from mean value of measured HbA_{1c} between the local laboratories and the central laboratory is – 1.47% HbA_{1c} (Fig.3).



Figur 3. Measured and expected difference in bias of HbA_{1c}. (–) Measured standard deviation, s_{meas} = 0.83. Measured bias, B_{meas} (local – reference laboratory) = –1.47% HbA_{1c}. (97% of local results were lower than the centrally measured results).(*) Expected standard deviation, s_{exp} = 0.95. Expected bias, B_{exp} = 0% HbA_{1c}.

EVALUATION OF QUALITY SPECIFICATIONS

From the present situation, where a bias $|B_A|$ = – 1,47% HbA_{1c} caused 97% of the locally determined results to be lower and 3% to be higher than the results from the reference laboratory, the reaction from the general practitioners was frustration. The results from the reference laboratory were conclusive for the classification and by that for the choice of treatment. Figur 4 demonstrates the magnitude of disagreement (in form of percentage of positive or negative differences) as function of analytical bias in % HbA_{1c}. With a bias of – 0.5% HbA_{1c} , 75% of the local results would be lower than the results of the reference laboratory. To reduce frustrations about differences in results, this must be considered to be the maximum allowable bias. To avoid frustrations totally, however, the bias should be less than – 0.25% HbA_{1c}, where the results would be 65% and 35%, respectively. The general practitioners would probably not recognize a bias of this small magnitude, and furthermore it would secure reliable control and treatment for the patients.

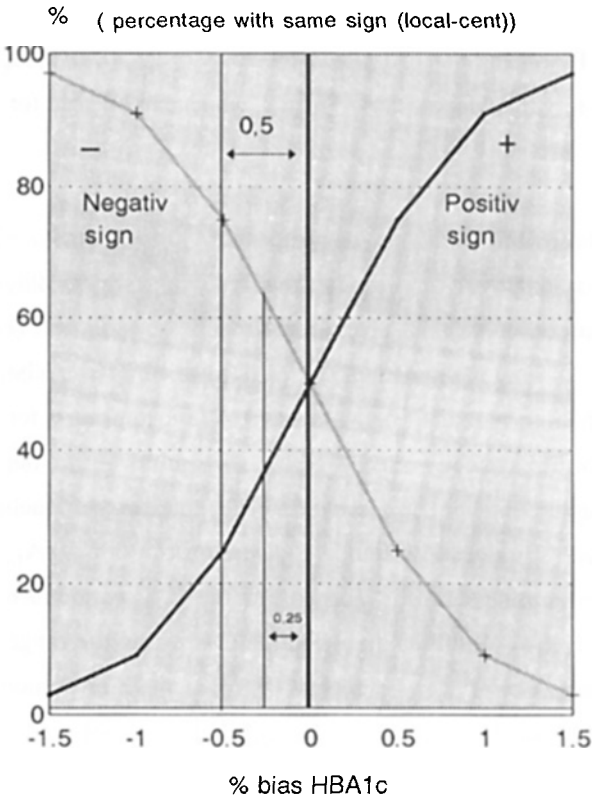


Figure 4. The distribution of differences (local – central) in proportion to % bias HbA_{1c}. With a bias of – 0.5% HbA_{1c} (acceptable), 75% of the local determined results would be lower and 25% higher than central determined. With a bias of – 0.25% HbA_{1c} (ideal), it would be 65% and 35%, respectively.

DESIGN OF CONTROL SYSTEM

For glycated HbA_{1c} , there is no international accepted calibrator and/or standardized method. In order to improve quality, both a standardization and an external control system, usable for all methods of glycated HbA_{1c} , is necessary. It is well described that by a combination of "affinity and cation-exchange chromatography", it is possible to separate and isolate each glycated fraction of Hb and non-glycated Hb. By saturating Hb with CO (carbon monooxide) the fractions keep stable over time. (5, 6). This method can be used for developing both a calibrator and a control material. By affinity chromatography non-glycated Hb (HbA_0) is separated from the glycated fractions of Hb. By cation-exchange chromatography, the HbA_{1c} fraction is isolated. To be used for testing specificity, cord-blood (75-90% HbF) (4) is treated in the same way to isolate fetal haemoglobin, HbF.

To prepare **a calibrator** with exactly known content of HbA_{1c} , the pure fractions (HbA_{1c}, HbA_0) are upconcentrated. Before mixing, they are standardized by measuring an

determining the exact content of each fraction by spectrophotometri. For CO-saturated Hb, we used ϵ_{419} = 174000 (8, 9). By mixing the two concentrated pure fractions, it is possible to prepare a calibrator with target value, e.g. 10% HbA_{1c}, which can be used for **standardization.**

As an external control system, a set of control samples was prepared. 1) for control of method standardization and proportionality 4 samples are prepared with 0%, 4%, 8%, 12% HbA_{1c}, respectively. 2) for control of specificity, one sample, identical to, the 4% sample is applied with, 0%, 2%, 4% of fetal haemoglobin (HbF). HbF is reported to be in the magnitude of 0.5% of total Hb in normal adult individuals. (3, 4). Furthermore, for the different HPLC-systems, this control system could be a tool for optimizing of the integration procedure (Valley-Valley or baseline). If we want to calculate bias for each individual laboratory with an average reproducibility standard deviation of 0.3% HbA_{1c}, approximately 6 independent measurements are needed to get the 0.90 confidence interval − 0.25 to + 0.25% HbA_{1c}. If this is fullfilled an estimated bias within the range − 0.25 to + 0.25% HbA_{1c} would guarantee at a probability of 0.95 that the bias do not exceed + or − 0.5% HbA_{1c}.

In order to guarantee the ideal maximum bias of + or − 0.25% HbA_{1c}, the estimated bias should be within the range of − 0.12 to + 0.12% HbA_{1c} performed with about 18 independent measurements. If measurements are proportional and the method is specific, then the control results from the three levels can be combined and fewer runs are needed.

DISCUSSION

HbA_{1c} measurements are important in the treatment of diabetes mellitus. A bias of the observed magnitude (− 1.47% HbA_{1c}) has consequences for both the general practitioners, patients and the laboratories. The number of methods for HbA_{1c} increases. Two new immunological methods which use HbA_{1c} specific monoklonal antibodies are commercially available. (3, 7). It is therefore important to standardizise the methods for HbA_{1c} analyses. The model developed for the investigation of present data seems to be valid, even if only bias is examined.

It looks like it is possible to establish an external control system with control samples for evaluation of both standardization and specificity − and with a relatively small number

of measurements, determine bias with a confidence, which guarantee the quality in agreement with the specifications.

CONCLUSIONS

To secure that the clinicans interpret the conditions of the patients in the same way, the demand (the clinical specifications) is agreement in the results (discrepancy less than 65/35 (fig.4)). To secure the analytical specifications a bias of − 0.25 to + 0.25 %HbA_{1c} (maximum +/− 0.5 %HbA_{1c}) is demanded.

The control system consists of "samples" for control of standardization and for control of specificity and it is possible to guarantee the quality in agreement with the specifications.

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Correspondence:
O. Nørgaard Jensen
Department of Clinical Chemistry
Odense University Hospital
DK - 5000 Odense C
Danmark