

# MULTICENTER EVALUATION OF A FULLY AUTOMATED WHOLE BLOOD GLYCOHEMOGLOBIN (HbA1c%) ASSAY ON THERMO SCIENTIFIC KONELAB CLINICAL CHEMISTRY ANALYZERS

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## Introduction

The determination of HbA1c is a good indicator to estimate the average blood glucose concentration in diabetes. The concentration of glycosylated hemoglobin is directly proportional to the mean concentration of glucose and it represents the integrated value for glucose over the preceding 6 to 8 weeks.

To measure HbA1c% immunoturbidometrically, a hemolyzed whole blood sample is needed. Usually the hemolyzing is done manually. The manual pipetting, mixing and incubation process is time consuming and it adds several risks to the overall process. The total variation is easily increased due to the operator to operator variation, and the handling of non-labelled secondary tubes of hemolyzed samples creates the risk of misidentification.

With the new method on the Thermo Scientific Konelab clinical chemistry analyzers it is possible to perform an automated sample pretreatment directly from the primary tubes on the analyzer. That is called on-board hemolyzation in this poster.

In this study we have compared HbA1c results analyzed with the new automated pretreatment feature, on-board hemolyzing, to the results analyzed after a carefully controlled manual hemolyzing. The similar evaluation procedure was carried out in three different laboratories, running three different Konelab models, Konelab 20i, Konelab 30i and Konelab 60i. In the final comparison study between the on-board and manual hemolyzation the results from the three different laboratories were pooled for final calculations.

In the precision study four pooled whole blood samples were analyzed twice a day during 20 days in Konelab 60i clinical chemistry analyzer. Comparison to another on-board hemolyzation method was made on Konelab 60i and on Integra 800 Roche Tina-quant HemoglobinA1c Gen.2 with European Reference Laboratory for Glycohemoglobin (Method: ESRL #9).

## Materials and Methods

### Instrument

Thermo Scientific Konelab clinical chemistry analyzers (Konelab 20i, 30i and 60i), Sw versions 7.1 or 7.1.1, Thermo Fisher Scientific, Vantaa, Finland

Roche Cobas Integra 800 (European Reference Laboratory for Glycohemoglobin, The Netherlands), Mannheim, Germany

### Reagents

HbA1c assay kit, Ref 981658, Thermo Fisher Scientific, Vantaa, Finland. HbA1c Pretreatment Liquid – a kit for on-board hemolyzing, Ref 981924 Thermo Fisher Scientific, Vantaa, Finland

Hemolyzing reagent – a reagent for manual hemolyzing, Ref 981659, Thermo Fisher Scientific, Vantaa, Finland.

Roche Tina-quant HemoglobinA1c Gen.2, Roche Diagnostic, Mannheim, Germany

All the reagents for the on-board hemolyzation and the HbA1c assay are ready for use liquids in the barcoded system vessels. The calibrators (four vials) are included in the HbA1c reagent kit in Thermo Scientific methods. In the comparison study on Konelab analyzers the only difference between the on-board and manual methods was the hemolyzation step.

### Controls

HbA1c Control Normal, Ref nro 981695, Thermo Fisher Scientific, Vantaa, Finland

HbA1c Control Abnormal, Ref nro 981696, Thermo Fisher Scientific, Vantaa, Finland

### Methods

Konelab analyzers were equipped with Sw versions 7.1.1 or 7.1. Both of these versions were able to process automatically the pretreatment of whole blood samples for HbA1c test. The pretreatment process includes mixing of the whole blood sample and on-board hemolyzing. From the hemolyzed aliquot total hemoglobin (Hb) and glycohemoglobin (HbA1c) are measured and HbA1c% calculated automatically. Total hemoglobin is measured photometrically and HbA1c determination is based on the turbidimetric inhibition immunoassay. The measuring range for HbA1c% is 4–17% (DCCT/NGSP values) at a normal Hb concentration of 150 g/l. The assay applications for HbA1c% on Konelab analyzers are equal for both on-board and manually hemolyzed samples. To perform on-board hemolyzing, a new pretreatment application, a HbA1c Pretreatment Liquid kit for automated use and a dedicated segment for sample loading are needed. This special sample segment has a barcode for automatic identification and for the operator an easily identified violet color instead of black used in normal sample segments. With the pretreatment segment the operator can request the analyzer to perform on-board hemolyzing from primary tubes. The normal segment is used for samples not to be pretreated on-board (manually hemolyzed samples, calibrators and controls).

The manual hemolyzing is needed in special cases.

1. If the result of Hb- or HbA1c-determination is out of measuring range, the analyzer is not able to make a secondary dilution from the primary tube automatically.
2. If the viscosity of the sample is abnormally high, for example in samples with extremely high hemoglobin concentration (over 200 g/l) or when clots exist in the sample, the sample aspiration integrity is not secured in the automatic analyzer dispensing.
3. If the sedimentation rate of the sample is very high, the instrument is not able to aspirate enough erythrocytes to hemolyzing, causing the results of Hb- and HbA1c-tests to fall below the measuring range.

For the precision study the analyzer performed on-board pretreatment step for all four precision samples two times separately, of each hemolyzate the Hb- and HbA1c-tests were performed as duplicates.

## Samples

The used samples were whole blood patient samples EDTA as the anticoagulant.

For comparison study each whole blood sample was hemolyzed both manually and on the Konelab analyzers automatically on-board. Prior to manual hemolyzing the samples were mixed well and gently with a test tube rocker (30 min). The same mixing step was performed also before the primary tubes were placed in the pretreatment segment for on-board hemolyzing. Both hemolysates of each whole blood sample were measured within 60 min of each other.

For the precision study whole blood EDTA-samples were mixed to four pools depending on their HbA1c(%) result. Pools were divided into 3 ml aliquots and they were stored in a freezer during this study. For every precision run new samples (two vials of each pool) were thawed at room temperature (one hour) and mixed well before analyzing.

The samples used for comparison study with European Reference Laboratory for Glycohemoglobin (ERL), were frozen EDTA whole blood. Sample set batch was 042008A from ERL, The Netherlands.

## Results

In the method comparison study the samples (n=187, total from three different Konelab models) were hemolyzed both automatically on-board and using the manual method. Hb- and HbA1c-determinations were run as duplicates from these two hemolysates and HbA1c%-results were calculated by the analyzer. Averages of duplicate HbA1c% were calculated and these results were compared in Figure 1. Bias and bias% between the manual and automated methods on Konelab clinical chemistry analyzers are in Figure 2.

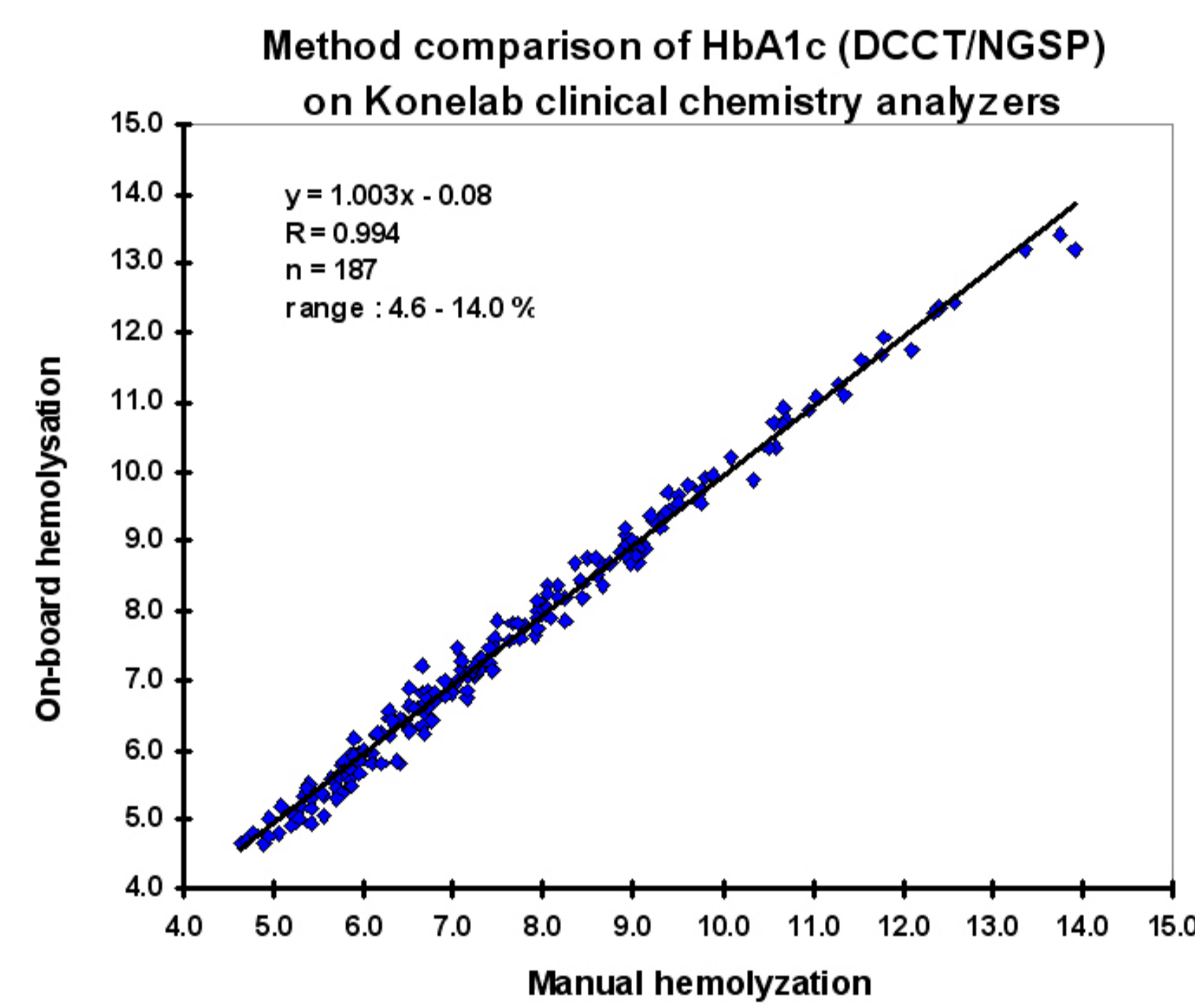


Figure 1 : Method comparison between the manual and the on-board hemolyzing in HbA1c test on Konelab 20i, 30i and 60i clinical chemistry analyzers

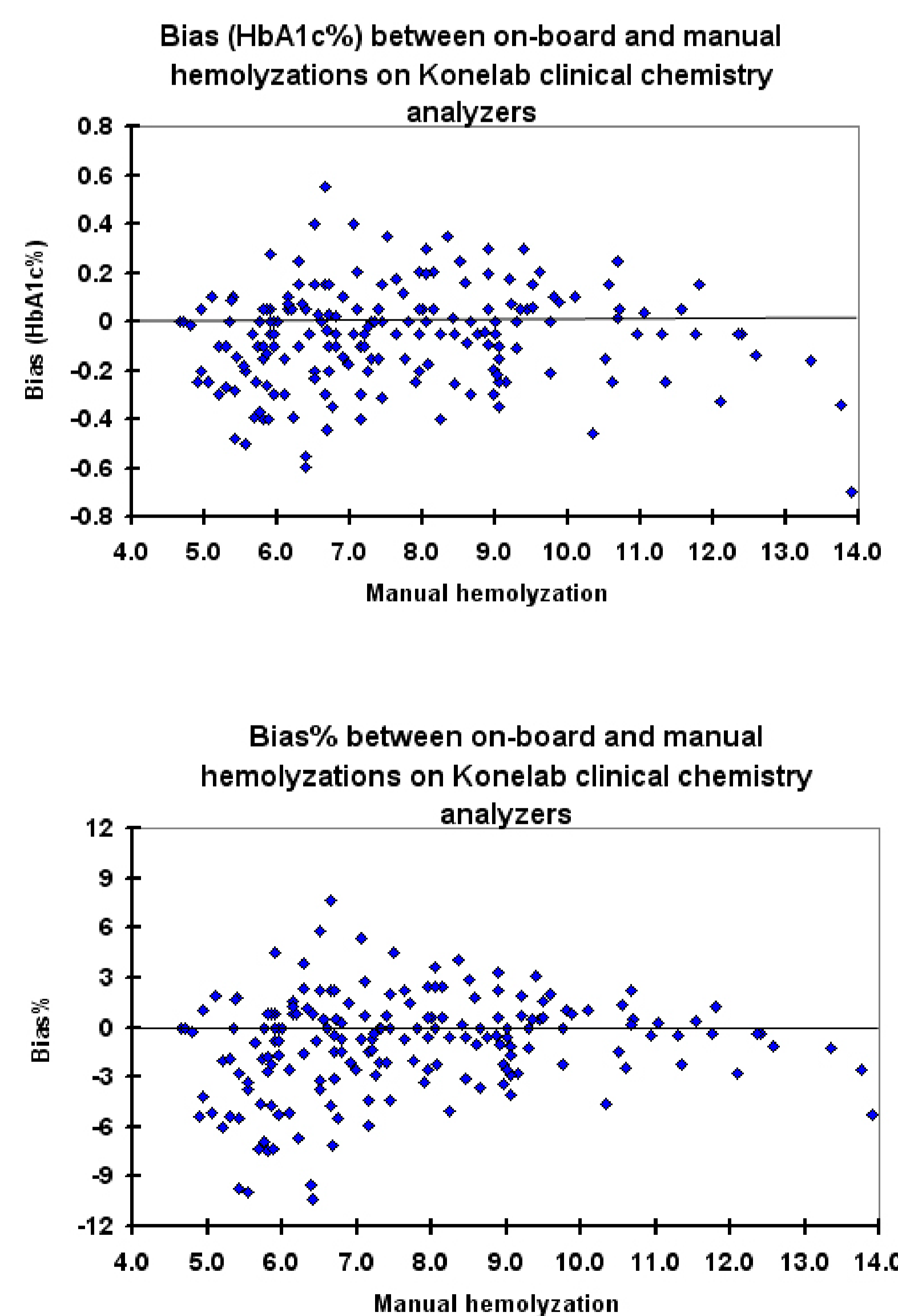


Figure 2 : Bias and the bias% between manual and on-board hemolyzation HbA1c methods (n=187) on Konelab 20i, 30i and 60i clinical chemistry analyzers

In the method comparison study on Konelab 60i with another whole blood method (ESRL#9, Roche, Tina-quant HemoglobinA1c Gen.2 on Integra 800) samples (n=40) were hemolyzed by automatically on-board in both methods. Hb- and HbA1c-determinations were run as duplicates from these two methods and HbA1c%-results were calculated by the analyzer. All measured results were compared in Figure 3. Bias and Bias % between two on-board hemolyzation HbA1c methods are in Figure 4.

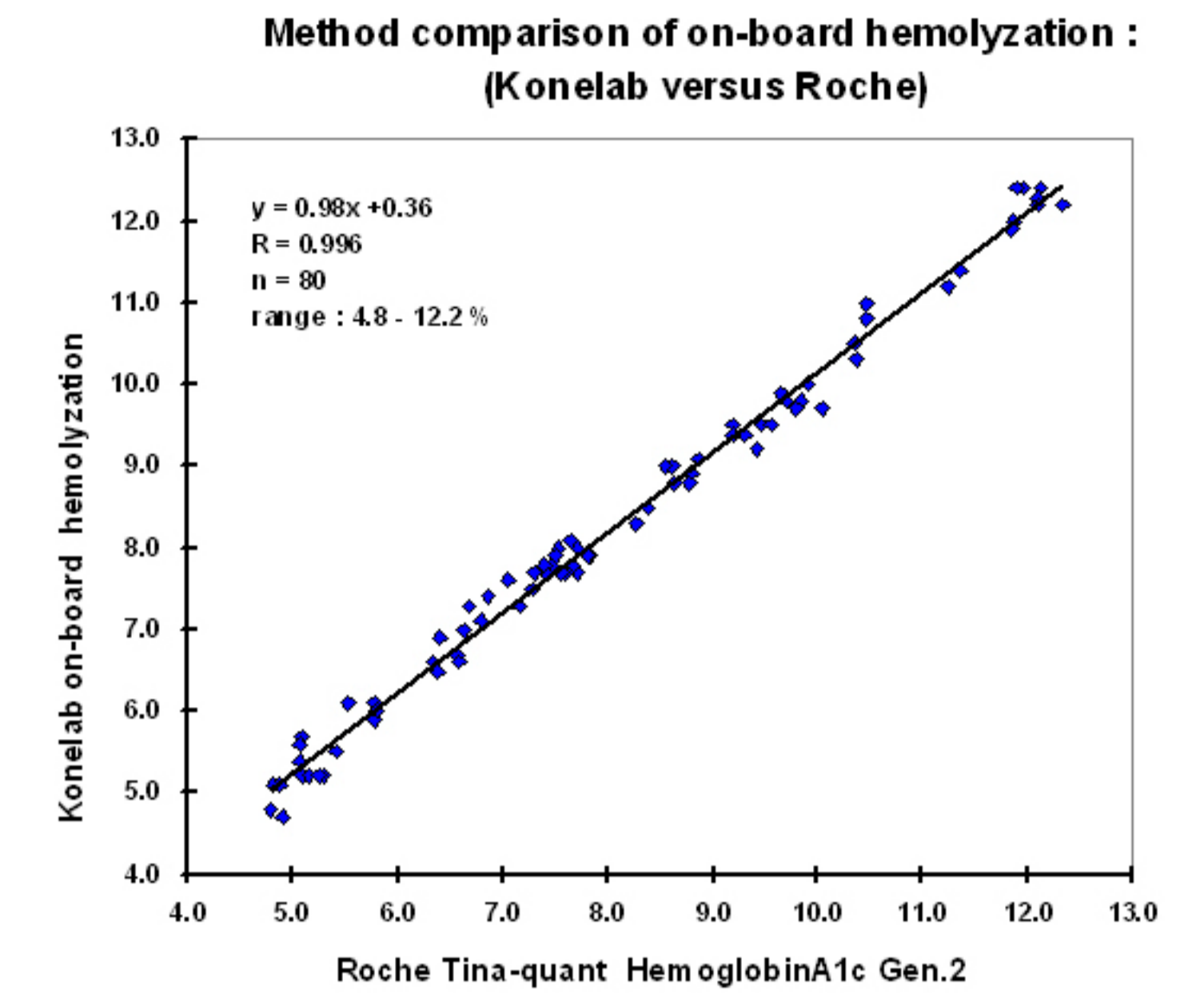


Figure 3 : Method comparison between the two on-board hemolyzation methods, Konelab 60i versus Roche Integra 800 (n=40, as duplicates)

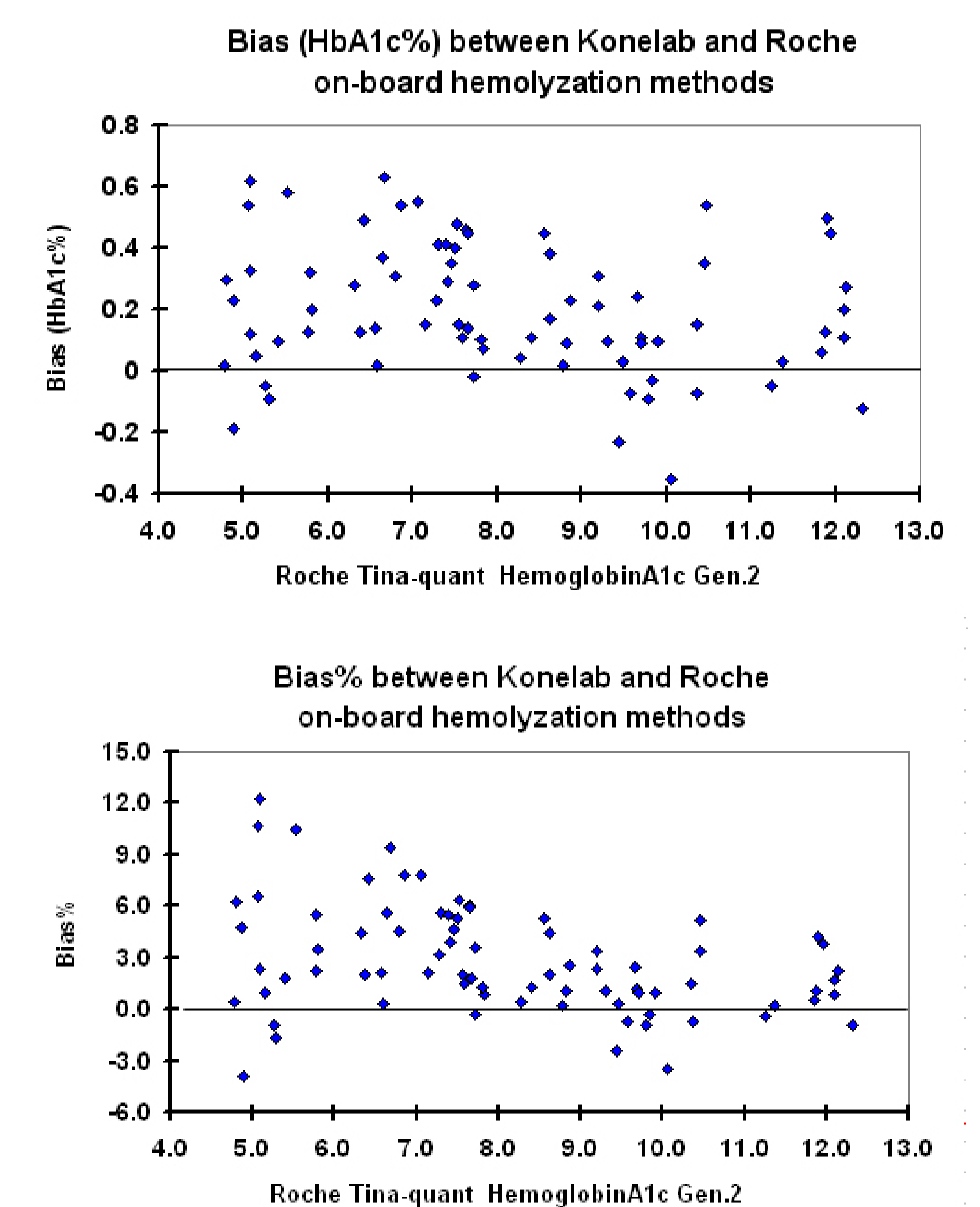


Figure 4 : Bias and bias% between two on-board hemolyzation HbA1c methods (n=40, as duplicates)

In the precision study two on-board hemolyzations per day were performed with the analyzer Konelab 60i during 20 days. The precision samples (n=4) were pooled whole blood and HbA1c% was measured as duplicate from each hemolyzate. CV% were calculated from average of duplicated HbA1c%-results. The results are shown in Figure 5.

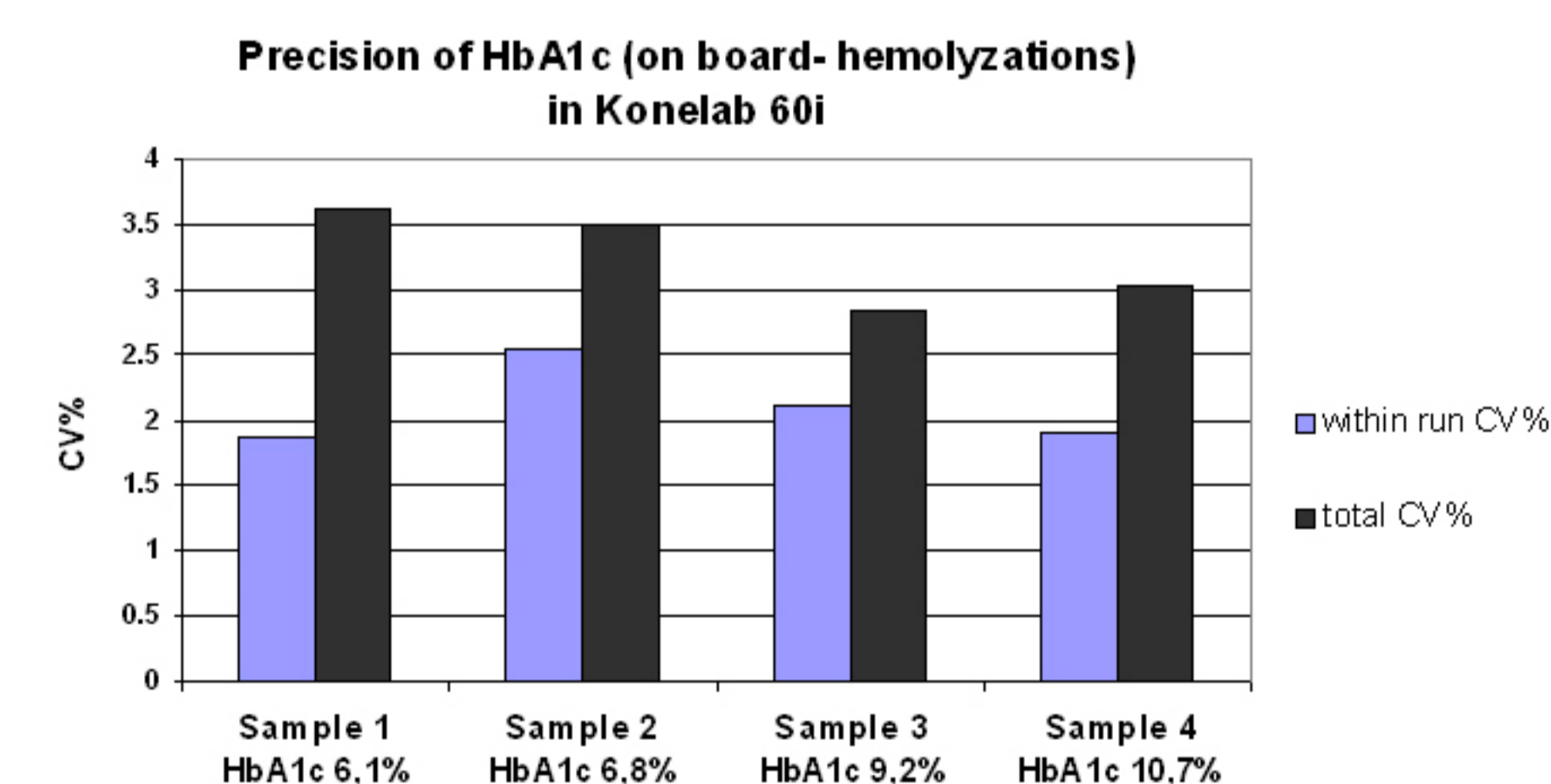


Figure 5 : The precision study of the automated on-board method

## Conclusion

The correlations between the manual and the automated on-board hemolyzing and between two different on-board hemolyzation method were excellent. The precision of the evaluated on-board hemolyzing was good.

The new application is user-friendly, reliable and cost effective, saving both in labor costs and consumable costs needed for manual pretreatment. We conclude that the fully automated whole blood HbA1c test is an excellent method on the family of Thermo Scientific Konelab clinical chemistry analyzers.

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