Hemo_Control

A system for measurement of B—Haemoglobin, manufactured by EKF-diagnostic GmbH in Germany

Report from a premarketing evaluation, organised by SKUP "Scandinavian Evaluation of Laboratory Equipment for Primary Health Care". The evaluation was ordered by MEDimport AS in Norway

SKUP/2004/29

SKUP in Norway, NOKLUS, Bergen, phone. +47 55 58 67 02, fax +47 55 58 67 10
SKUP in Sweden, EQUALIS AB, Uppsala, phone. +46 18 69 31 64, fax +46 18 69 31 46

PREMARKETING EVALUATION OF THE Hemo_Control HAEMOGLOBIN MEASURING SYSTEM

TABLE OF CONTENTS

Summary

The Hemo_Control haemoglobin measuring system (Hemo_Control), manufactured by EKF-diagnostic GmbH in Germany, is intended for determination of the haemoglobin concentration in human blood. Hemo_Control consists of an absorption photometer called Hemo_Control Photometer and of Hemo_Control Microcuvettes that contain dried reagents. In the cuvette, haemoglobin is converted to azide methaemoglobin, a coloured product that is measured bichromatically in the photometer. The sample volume is 10 µL. The sample can be drawn directly into the Hemo_Control Microcuvette from a capillary puncture. The cuvette is read directly in the Hemo_Control Photometer. The measuring range is 0 — 256 g/L (0.00 — 15.89 mmol/L).
The first part of this evaluation was performed under standardised and optimal conditions, i.e. by experienced laboratory technologists in a hospital laboratory. The second part was performed under ”real life” conditions by staff at two primary care centres. The analytical quality goal derived from biological variation was set to allow a total error of up to ±5 %.

Results
The within-series precision with venous EDTA samples in the hospital laboratory was good. The CV was around 1 %. When the imprecision was measured between days the CV figures did not increase. There was a negative bias, but negligible small, relative to the Comparison Method on Coulter LH 750. The total error was less than ±5 %. The results achieved in the hospital laboratory fulfil the analytical quality goal.
The precision with venous samples at the primary care centres was also good. The CV was 0.7 and 1.5 % respectively. There was a positive bias, but negligible small, relative to the Comparison Method. These results also fulfil the analytical quality goal with a total error of less than ±5 %.
Using capillary samples taken in the finger the imprecision was, as expected, higher.
At Centre B the imprecision was acceptable, with CV 2.8 %. At Centre A the imprecision was too high, with CV 5.5 %. The quality goal was therefore not attained with capillary samples.
It is a complicating fact that the haemoglobin concentration in capillary blood is not representative for the haemoglobin concentrations in venous blood. These pre-analytical sources of error are not only valid for Hemo_Control, but for all instruments using capillary samples for measuring B—Haemoglobin.

Practical points of view
All personnel involved in the evaluation summarised their opinion about the Hemo_Control system as being quick and easy to use. They also thought the instrument was small and neat.

Conclusion
Hemo_Control showed, when using venous samples, good precision and only small deviations from the results of the Comparison Method. The bias was small and negligible. The total error was less than ±5 %. The quality goal is attained with venous samples.
The quality goal was not attained with capillary samples, mainly due to non-representative haemoglobin concentrations in capillary puncture blood and to poor precision. Acceptable precision can be obtained with skilful sample collection, but the non-representative concentrations appear impossible to avoid. These pre-analytical sources of error with capillary samples are valid not only for Hemo_Control, but for all instruments measuring haemoglobin.
Hemo_Control is quick and easy to use and well suited for the Primary health care.

Planning of the evaluation

Scandinavian Evaluation of Laboratory Equipment for Primary Health Care, SKUP, received a request for a premarketing evaluation of the Hemo_Control haemoglobin measuring system, manufactured by EKF-diagnostic GmbH in Germany, by Kjell Myrseth, representative of MEDimport AS in October 2003. At the time of the request, the system had not been marketed in Scandinavia.

The protocol for the evaluation was set up in December 2003, following the guidelines "*Evaluation of analytic instruments. Guidelines particularly designed for evaluation of instruments in primary health care*" [1]. The evaluation of Hemo_Control is a complete evaluation according to the guidelines. The evaluation was carried out during February to May 2004.

The evaluation comprised the following studies:

In a hospital laboratory:
- Within-day imprecision
- Between-day imprecision
- Correlation between Hemo_Control and the designated Comparison Method results from venous samples
- Practical viewpoints from the users

In two primary care centres:
- Within-day imprecision
- Correlation between Hemo_Control and the designated Comparison Method results from venous and capillary samples
- Practical viewpoints from the users

After an inquiry by SKUP, the Department of Clinical Chemistry at the University Hospital MAS in Malmö, Sweden, made the hospital part of the evaluation. At this hospital laboratory haemoglobin is analysed on a cell counter from Beckman Coulter, Coulter LH 750.

The evaluation in the primary care was carried out at Horten Helsesenter in southern Norway and at Havna Legekontor in Bodø in northern Norway. At Horten Helsesenter there are four doctors and six co-workers. Five of the co-workers share the laboratory work. They are nurses and medical secretaries. Four of the co-workers participated in the evaluation of Hemo_Control. Horten Helsesenter analyse the routine haematology samples on a cell counter. At Havna Legesenter in Bodø there are three doctors and five co-workers. The co-workers are nurses and medical secretaries. Four of the co-workers participated in the evaluation. At Havna they analyse haemoglobin on HemoCue in their routine work.

Contracts were made between SKUP and the Department of Clinical Chemistry, and between SKUP and MEDimport AS.

A survey of the persons responsible for the various parts of the evaluation is given in Table 1.

Table 1. Persons responsible for various parts of this evaluation

Birgitta Alemo	Laboratory technologist and instructor	Leader of the evaluation in the Department of Clinical Chemistry at the University Hospital MAS in Malmö, Sweden
Eva Mauritzson	Laboratory technologist and instructor	Operator of the evaluation in the Department of Clinical Chemistry at the University Hospital MAS in Malmö, Sweden
Anne Lise Saga	Laboratory technologist and instructor	Instructor in Vestfold district in Norway and for the evaluation at Horten Helsesenter.
Mette Nyberg	Nurse	Contact person for the evaluation at Horten Helsesenter in Norway. Together with three co-workers, Mette carried out the measurements with the Hemo_Control system.
Rigmor Lind	Laboratory technologist and instructor	Instructor in Nordland district in Norway and for the evaluation at Havna Legekontor.
Vigdis Hansen	Medical secretary	Contact person for the evaluation at Havna Legekontor in Norway. Together with three co-workers, Vigdis carried out the measurements with the Hemo_Control system.
Kjell Myrseth	Market Manager	Representative for MEDimport AS in Norway
Grete Monsen	Laboratory technologist, Project manager for SKUP, NOKLUS, Norway	Responsible for the evaluation. Author of this report, in cooperation with Arne Mårtensson.
Arne Mårtensson	Clinical biochemist, Co-ordinator for SKUP in Sweden, EQUALIS AB	Responsible for the evaluation. Author of this report, in cooperation with Grete Monsen.

Grete Monsen and Arne Mårtensson worked out the preliminary protocol of the evaluation in co-operation with Kjell Myrseth and the participating laboratory. The protocol was also thoroughly discussed and finally agreed upon at a start-up meeting at the Department of Clinical Chemistry in Malmö 2004-01-23 with the following participants:
Kjell Myrseth, Arne Mårtensson, Birgitta Alemo and Eva Mauritzson.

A preliminary report has been sent to Kjell Myrseth, MEDimport AS, and forwarded to EKF-diagnostic GmbH in Germany. They have discussed and commented the preliminary report and this final report is then written.

Preparations

Validation of trueness of the Comparison Method

Before the evaluation, the Comparison Method on the Coulter LH 750 in the hospital laboratory was validated with the ICSH reference method for haemoglobin [2].
The measurements with the ICSH reference method were made at the Department of Clinical Chemistry, Helsingborg Hospital, Sweden. Twenty samples were measured with both methods. The comparison showed that low values with the Coulter method were slightly too high and high values were slightly too low. The following correction formula was then used to adjust all original Coulter values (x) to corrected values (y).
$y = 1.028x - 1.8$
All Coulter values used as comparison values in this evaluation has been corrected according to the formula. The deviation diagram in Figure 1 demonstrates the agreement between ICSH and Coulter after the correction.

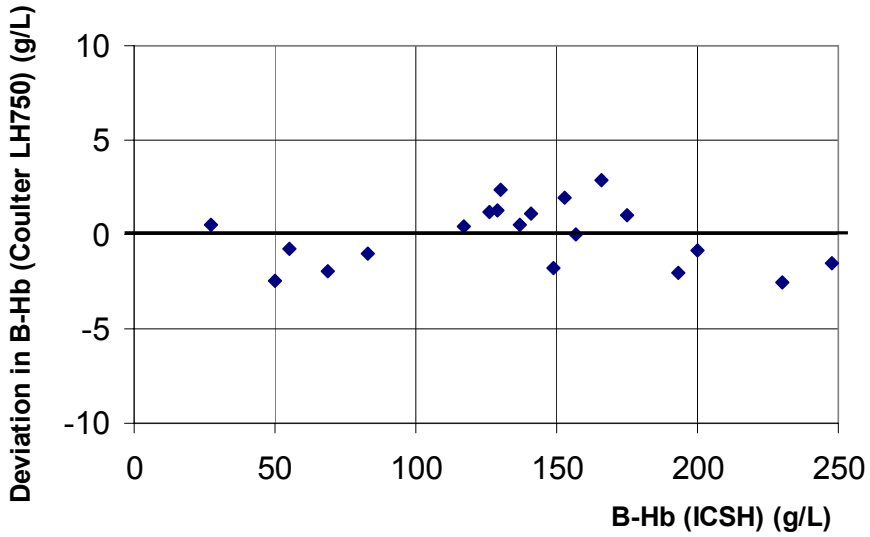

Figure 1. The corrected Comparison Method compared with the ICSH reference method. n = 20

Test of the concordance of the Hemo_Control instruments

The parallel evaluation in the hospital laboratory and at the primary care centres required three Hemo_Control Photometers in use and one instrument as backup. According to the SKUP standard protocol, instrument concordance must be documented before the evaluation. The four instruments were placed next to each other in the laboratory at Malmö University Hospital. Three patient samples with low, medium and high B—Haemoglobin concentration were selected among the routine samples. The three samples were analyzed ten times on each Hemo_Control Photometer.
According to the manufacturer of Hemo_Control, the imprecision of the Hemo_Control is ≤2 %. It was suggested from SKUP that the CV was allowed to increase by 30 % as a maximum, when the average imprecision of the four individual instruments was compared with the total imprecision of the results from all four instruments. If the CV increased more, the instruments did not fulfil the concordance requirement. In that case calculations and comparisons of the mean values for the individual instruments should determine which

instrument that showed mean values significantly deviating from the others. MEDimport AS should be contacted for exchange of the deviating instrument. MEDimport AS accepted the suggestion from SKUP.
The results of the concordance test are shown in Table 2 and 3.
Raw data is shown in Attachment 1.

Table 2. Results of the concordance test

	Instrument number			
	3000-04-0002	3000-03-0400	3000-03-0403	3000-04-0001
Sample no. 1 (n = 10)				
B—Haemoglobin mean (g/L)	72.1	72.7	74.3	73.6
CV (%)	1.9	2.0	1.7	2.1
Sample no. 2 (n = 10)				
B—Haemoglobin mean (g/L)	130.7	131.6	131.8	131.7
CV (%)	1.4	1.2	1.4	1.1
Sample no. 3 (n = 10)				
B—Haemoglobin mean (g/L)	185.3	185.5	187.4	184.6
CV (%)	1.4	1.0	1.0	1.2

Table 3. Results of the concordance test. ANOVA calculation.
The effect on total CV when using several instruments instead of a single instrument.

Sample no.	**B—Haemoglobin mean (g/L)**	**Within-instrument CV component (%)**	**Between-instrument CV component (%)**	**Total CV (%)**	**Increase of CV (%)**
1	73.2	1.9	1.2	2.3	17
2	131.5	1.3	0.0	1.3	0
3	185.7	1.1	0.5	1.3	11

"Within-instrument CV component" refers to the mean contribution to the "Total CV" originating from the within instrument imprecision.
"Between-instrument CV component" refers to the mean contribution to the "Total CV" originating from the between instruments imprecision. The within-instrument imprecision is not included in this figure.
"Increase of CV" refers to the increase in percent of the CV from "Within-instrument CV component " to "Total CV". The acceptance limit for this increase was set by SKUP to maximum 30 %.

The defined requirements for concordance among the Hemo_Control instruments were amply fulfilled. All instruments showed about the same imprecision, but as there was just a need for three instruments in the evaluation, instrument no. 3000-04-0002 was randomly excluded from the evaluation. This instrument was kept for backup and would only have been used if one of the other instruments had given problems, which was not the case.

Materials and methods

Hemo_Control

Hemo_Control consists of an absorption photometer called Hemo_Control Photometer and of disposable Hemo_Control Microcuvettes, which contain dried reagents.

Blood samples are drawn from a venous blood sample or directly from a finger prick into the cuvettes by capillary force. The Hemo_Control Microcuvette filled with 10 µL sample can be measured immediately or within 10 minutes at the latest. The cuvette is placed in the drawer of the Hemo_Control Photometer. Blood components react with the dried reagents in the microcuvette. Sodium deoxycholate haemolyses the erythrocytes. Sodium nitrite converts the released haemoglobin to methaemoglobin, which reacts with sodium azide to form azide methaemoglobin. The chemical reaction in the cuvette generally takes less than 45 — 60 seconds. During this time the absorption value changes constantly. At the end of the reaction the absorption value remains constant for several minutes. The end point of the reaction is measured bichromatically at the wavelengths 570 and 880 nm. The second wavelength is used to compensate for interference that might be caused by blood components, like chylomicrons or leukocytes, or scratches on the surface of the cuvette. The haemoglobin concentration is calculated automatically and the result is shown on a liquid crystal display. The measuring range is 0 — 256 g/L (0.00 — 15.89 mmol/L).

Vanzetti first described this method principle in 1966 [3]. The Vanzetti method is also known as the azide methaemoglobin method.

A Control Cuvette is available for an electronic check of the Hemo_Control Photometer. To check the whole measuring system, including the Hemo_Control Microcuvettes, suitable Control Solutions at three different levels are available from the manufacturer.

The factor for unit recalculation from gram B—Haemoglobin per litre to mmol B—Haemoglobin (Fe) per litre is 0.620 559. This factor is derived from the molecular weight 16 114.5 of the haemoglobin subunit containing one iron atom [2]. This factor is used both in the Hemo_Control software and in SKUP's recalculations in this report

Technical data of the Hemo_Control Photometer is shown in Table 4.
Technical data of the Hemo_Control Microcuvette is shown in Table 5.

Table 4. Technical data of the Hemo_Control Photometer as provided by the manufacturer

Measuring procedure	Optical absorption photometry
Light source	Dual-Color-LED 570 / 880 nm
Dominant wavelength of light source	1st wavelength 570 ±5 nm 2nd wavelength 880 ±10 nm
Spectral half value width of the light source	1st wavelength 15 ±3 nm 2nd wavelength 50 nm
Receiver	Photodiode 350 — 820 nm
Measuring range	0 — 256 g/L
Sample material	Venous, arterial or capillary human blood
Sample carrier	EKF Microcuvette
Sample size	10 µL
Linearity*	0 — 200 g/L ±3 g/L, >200 g/L ±7 g/L
Average measuring time	Depending on concentration, 30 — 60 s
Power supply	Main adaptor: Input: 100 — 250 V AC / 50 — 60 Hz Output: 6 V DC Integrated battery: Voltage 2.4 V Capacity: 1500 mAh (ca. 100 h operating time)
Power take up	Maximal: 3 W Typically: 1.2 W Minimal: 30 mW
Interface	Printer (RS 232 C)
Measured data storage	Up to 100 results, including date and time
Environmental temperature	Room temperature (15 — 40ºC)
Dimensions (LxWxH)	160 mm x 160 mm x 68 mm
Weight	ca. 700 g

* According to the manufacturer "linearity" specify the maximum bias from the true concentration in the high and low concentration range.

Table 5. Technical data of the Hemo_Control Microcuvette as provided by the manufacturer

Type	Microcuvette, coated with reagents for determining the haemoglobin in venous, arterial or capillary blood.
Volume in the cuvette cavity	10 µL
Reagents	Sodium deoxycholate, sodium nitrite, sodium azide, non-reactive additives
Material	Polystyrene
Storage	room temperature(15 — 30 ºC), dry storage in the original containers
Dimensions (LxWxH)	ca. 35 mm x 24 mm x 4 mm

Product information

Hemo_Control is manufactured by:
EKF-diagnostic GmbH
Ebendorfer Chaussee 3
39179 Barleben
Germany
Internet: http://www.ekf-diagnostic.de

Suppliers of Hemo_Control in the Scandinavian countries:

Denmark:	Norway:	Sweden:
—	MEDimport AS Postboks 2513 3702 Skien Phone: +47 35 91 37 37 Fax: +47 35 91 37 38 e-mail: kjell.myrseth@medimport.no Internet: www.medicus.no/import/	Handelshuset Medic AB Källbäcksrydsgatan 30B 507 31 Brämhult Phone: +46 33 - 23 00 99 Fax: +46 33 - 23 00 28 Mobile: +46 709 - 43 90 90 e-mail: mikael.andreasson@hhmedic.se

In this pre-marketing evaluation the following Hemo_Control equipment was used:

Photometers: serial no. 3000-03-0400 in the hospital laboratory
serial no. 3000-03-0403 at Primary Care Centre A
serial no. 3000-04-0001 at Primary Care Centre B
serial no. 3000-04-0002 back-up instrument

Microcuvettes: lot no. 034107

Control Solutions: low: 18-1-B248, normal: 117-2-B314 and high: 117-3-B314

Comparison Method

The routine method for B—Haemoglobin in the Department of Clinical Chemistry at the University Hospital MAS in Malmö, was after correction used as the designated Comparison Method. Note that the original Comparison Method values have been corrected before they are used for comparison as described under the section "Validation of trueness of the Comparison Method". It is a photometric method that measures cyanmethaemoglobin.
The method is implemented on the Beckman Coulter LH 750 cell counter, with reagents and calibrator from Beckman Coulter.

EDTA blood is diluted in an alkaline buffer containing detergent and potassium cyanide (KCN). Erythrocytes are haemolysed by the reagent and haemoglobin is released. Haemoglobin (Fe^{2+}) is first oxidised to methaemoglobin (Fe^{3+}) and then a cyanide complex, cyanmethaemoglobin, is formed. The light absorption of the cyanmethaemoglobin is measured at 525 nm (compared to 540 nm that is usual in other instruments).

Coulter LH 750 Comparison Method product information

Instrument — from Beckman Coulter
Coulter LH 750 cell counter,
serial no. AF 33299 (LH 1)

Reagents — from Beckman Coulter

Calibrator — from Beckman Coulter
PN 7504535-C, Lot no. 4758
PN 7504535-D, Lot no. 4766
PN 7504535-D, Lot no. 4769
PN 7504535-D, Lot no. 4775

Internal quality controls — from Beckman Coulter
5C Coulter IQAP, article no. 754719

Start date	Level	Lot no.
2004-01-23	Abnormal I	870400
	Normal	885400
	Abnormal II	861500
2004-02-23	Abnormal I	870900
	Normal	886000
	Abnormal II	862000
2004-03-23	Abnormal I	871500
	Normal	886700
	Abnormal II	862500
2004-05-03	Abnormal I	872100
	Normal	887300
	Abnormal II	863200

Quality assurance of the Coulter Comparison Method

Internal quality control

Three levels of internal quality control material from Beckman Coulter were measured once a day during the evaluation period. The quality control material is stabilized whole blood, which means limited expiration time and frequent lot changes. Two different lots of the Abnormal 1 and Normal control, and three lots of the Abnormal 2 control are included in the calculation of Between-day CV in Table 6. The number of lots is less than the total number of lots because some lots were not used in sufficient number to be included in the calculations. The tolerance limits set by Beckman Coulter were ±2 g/L, ±4 g/L and ±5 g/L for the three levels. The different lots at each level have similar, but not exactly the same assigned haemoglobin concentration. For each level, a $CV_{within\ lot}$ is calculated for the different lots, and subsequently an average and pooled CV is calculated from the different $CV_{within\ lot}$. All results has been corrected according to description in the previous section "Validation of trueness of the Comparison Method" and than compared with the tolerance limits. All the corrected results, except one on the normal level, were inside the tolerance limits.
A summary of the precision obtained on the Coulter instrument is shown in Table 6.

Table 6. Internal quality control results with the Coulter Comparison Method. Between-day imprecision.

Level	Number of control material lots	Number of results	B—Haemoglobin mean* (g/L)	Between-day CV_{pooled} (%)	Bias for B—Hb* (g/L)
Abnormal 1	2	38	51.6	1.2	–0.3
Normal	2	40	133.0	0.8	+1.4
Abnormal II	3	37	167.0	0.8	+2.7

* The values in these columns are shown after correction of the Comparison method.
"Between-day CV_{pooled}" includes the within-series imprecision.
"Bias" is the mean deviation of the results from the assigned value.

External quality control

The Department of Clinical Chemistry in Malmö participates in a Swedish proficiency-testing scheme provided by EQUALIS. Results from most cell counters (n=240) in Sweden are reported to this scheme. The inter-laboratory variation is usually about 1.7 — 2.0 % CV. The sample materials in the scheme have no assigned values determined by a reference measurement procedure. The single participant is usually compared with the mean of all participating laboratories.
The results from the Comparison Method in this scheme from the period before, during and after the evaluation are shown in Table 7.

Table 7. External quality control results with the Coulter Comparison Method

Survey year-week	B—Haemoglobin, mean of all laboratories (g/L)	B—Haemoglobin, Comparison Method* (g/L)
2003-39	154	156
2003-43	143	146
2003-47	135	138
2003-51	111	112
2004-04	154	158
2004-08	112	—
2004-12	133	135
2004-16	147	(136)
2004-20	140	140
2004-24	130	132
Mean	137.5	139.6

* The Comparison Method values are shown after correction as described in the previous section "Validation of trueness of the Comparison Method".
The values from 2004-08 and 2004-16 are not included in the column means.

For the survey 2004-08 there is no result from the Clinical Chemistry Department in Malmö. In the survey 2004-16 the Malmö laboratory reported an outlier compared to the other participants in the survey. The values for these two surveys are excluded when the mean of each column is calculated.

Within-series imprecision for the Coulter Comparison Method
The within-series imprecision for the Coulter Comparison Method was calculated from duplicate results from 105 venous patient samples. The classification of the results into the three haemoglobin concentration levels was done according to the values on Hemo_Control. The result is shown in Table 8. The raw data is shown in Attachment 2.

Table 8. Within-series imprecision for the Coulter Comparison Method
Venous patient samples in the hospital laboratory

Level	B—Haemoglobin Interval (g/L)	Excluded results*	B—Haemoglobin Mean value (g/L)	n	CV (%) (95 % confidence interval)
Low	20 — 110	3	73.3	38	1.0 (0.8 — 1.2)
Medium	111 — 150	0	131.2	41	0.7 (0.6 — 0.9)
High	151 — 252	2	184.9	26	0.5 (0.4 — 0.7)
All	20 — 255	5	123.5	105	0.7 (0.6 — 0.8)

* Please refer to Table 11 for explanation of excluded results.

Other products used in the evaluation

DiffSafe was used to transfer venous blood from the EDTA tubes to the Hemo_Control cuvettes. DiffSafe was supplied from Alpha Scientific Corp, US. Product designation: B-D 366005.

The following vacuum tubes were used for sample collection of the venous samples:

In the hospital laboratory:
Vacuette® EDTA tubes. Draw volume: 3 mL blood. Content: 114 µL of 8 % liquid K_3EDTA. Item no.: 454086. In these tubes the collected whole blood is diluted 3,8 % by the EDTA solution. Supplier: Greiner Bio-One, Austria.

At Primary Care Centre A:
Vacutainer™ EDTA tubes. Draw volume: 4 mL blood. Content: 7.2 mg anhydrous K_2EDTA. Catalogue no.: 368861. Supplier: Diagnostics, USA

At Primary Care Centre B:
Vacuette® EDTA tubes. Draw volume: 4 mL blood. Content: 7.2 mg anhydrous K_3EDTA. Item no.: 454021. Supplier: Greiner Bio-One, Austria.

The use of vacuum tubes with liquid EDTA in an evaluation has some consequences. When comparing venous results on two different methods, the dilution effect is the same for both methods and negligible, but when comparing venous results with capillary results the dilution effect is important to take into account. However, in this evaluation were capillary samples only taken at the primary care centres and none of the centres use tubes with liquid EDTA.

Evaluation procedures

Quality control of the Hemo_Control instruments
The optical function of the Hemo_Control Photometers was checked daily during the evaluation period with the special Control Cuvettes with assigned target values by the manufacturer. As an internal quality control for Hemo_Control, the Control Solutions from the manufacturer were measured daily at three levels in the hospital laboratory and at two levels at the primary care centres.

Evaluation in a hospital laboratory
The first part of this evaluation was performed under standardised and optimal conditions, i.e. by experienced laboratory technologists in a department of clinical chemistry at a hospital. To investigate imprecision and correlation according to the protocol, 100 patient blood samples were needed. The samples were collected in 3 mL EDTA tubes. The samples were measured in the routine with the Coulter Comparison Method, and then a selection was done to get results covering the whole measuring range of Hemo_Control. Only properly collected and handled tubes were used. To get samples with really low and high haemoglobin concentrations, some samples were manipulated by removing red blood cells or plasma.
In this way, the following 20 samples were made:

- Ten samples with B—Haemoglobin values between 30 — 80 g/L
- Five samples with B—Haemoglobin values between 170 — 200 g/L
- Ten samples with B—Haemoglobin values between 200 — 250 g/L

After analysing these 100 samples, two deviating results were found among those with the lowest haemoglobin concentrations. Therefore it was decided to prepare another ten samples with concentrations between 20 — 40 g/L. As can be seen later in the result section these extra results showed that the two deviating results were random errors and there is no systematic error at this low level. A total of 110 samples were measured during the period 2004-01-27 until 2004-03-02.

The EDTA tubes were mixed for at least ten minutes before the Hemo_Control measurements. Putting DiffSafe into the stopper of the EDTA tube facilitated the transfer of the sample to the Hemo_Control cuvette. DiffSafe makes it possible to safely drip blood from the tube onto a hydrofobic film. From the drops on the film the sample was sucked by capillary force into the Hemo_Control cuvette. The cuvette was read on the Hemo_Control Photometer within 10 minutes. Analyses were done in duplicates. After the Hemo_Control measurements, the samples were measured a second time on the Coulter instrument. All measurements on the same sample were performed within the same day.

In cases where the difference in result between the two methods were more than 6 g/L, a printout detailing measuring parameters and a scatter diagram of the different cell populations was obtained from the Coulter to assist in further investigations.

To estimate the between-day variation, the EDTA tubes were measured once again on Hemo_Control on one of the following days. Example: The first six samples were measured for the first time at Day 1. Two of these samples were also measured at Day 2, two were measured at Day 3 and the last two were measured at Day 4. This procedure was repeated with the succeeding samples on other days. As Hemo_Control is neither recalibrated between-series nor between-days, the between-day variation was expected to be the same as the within-series variation. Therefore the between-day variation was measured on a limited number of samples. The first 20 patient samples were used.

Evaluation in primary care

The second part of this evaluation was performed under real life conditions by staff at two primary care centres. At each centre 40 patients were randomly chosen for sample collection during the evaluation period. All patients had rested in sitting position before sample collection, but the period of sitting varied. Both venous EDTA samples and capillary samples were taken. The venous EDTA samples were measured on Hemo_Control and consecutively sent to the hospital laboratory in Malmö. In Malmö, the samples were stored in a refrigerator until analysis with the Coulter Comparison Method. No sample was more than four days old when measured on the Coulter.

The imprecision with capillary samples is expected to be approximately 2 — 3 times higher than the variation with venous samples. This is due to the pre-analytical conditions related to the capillary sampling technique. According to the protocol, the imprecision with capillary samples therefore was evaluated after the first 20 samples. The total number of capillary samples that was to be taken was depending on the precision achieved. If the CV-result was as expected, the Primary Care Centre finished the capillary sample taking after the first 20 results. If the imprecision was higher than expected, they should continue until 40 samples were taken.

The finger pricks were performed with the device that the primary care centres usually use in their routine work. At least two drops of blood were wiped off before sampling. Duplicate samples were taken from the same prick. The finger prick area was wiped off between the two sample withdrawals. The haemoglobin result was read on the Hemo_Control Photometer within 10 minutes, according to the instruction manual.

A summary of the total number of measurements in the evaluation is shown in Table 9.

Table 9. Summary of the measurements done in the evaluation

	Hospital laboratory		Primary care	
	Malmö		Centre A	Centre B
	Comparison Method	Hemo_ Control	Hemo_ Control	Hemo_ Control
Validation of the Comparison Method with the ICSH reference method *20 venous patient samples in duplicates*	40	—	—	—
Concordance of the Hemo_Control instruments, *10 replicates on 3 patient samples*	—	30	—	—
Within-series imprecision, method comparison and total error, *duplicates on 110 venous patient samples*	220	220	—	—
Within-series imprecision, method comparison and total error, *duplicates on 40 venous patient samples*	160	—	80	80
Within-series imprecision, method comparison and total error, *duplicates on capillary patient samples, 40 at Centre A and 20 at Centre B*	—	—	80	40
Between-day imprecision, internal quality control, *daily replicates on control samples on 3 levels in the hospital laboratory and on 2 levels in the primary care*	115	70	20	30
Between-day imprecision, *A third replicate on 20 venous samples*	—	20	—	—
Total number of measurements, approximately	535	340	180	150

Analytical quality specifications

There are no generally agreed analytical quality specifications for the precision of the B—Haemoglobin analysis. Models to derive goals for analytical quality based on biological variation are gaining increasing acceptance [4]. From data on biological variation such as within-subject-CV and between-subject-CV, models have been developed to calculate specifications for desirable quality, expressed as desirable imprecision, bias and total error. The term ”total-error” is used for the combined effects of imprecision and bias, and the ”desirable-total-error” is the interval around a true value covering 95 % of the results. The word ”allowable” is better than ”desirable” indicating that the figures for imprecision, bias and error should be as low as possible. However the expressions from the literature are kept.

For B—Haemoglobin in venous blood, Sebastián-Gámbaro et al. [5] estimate the within-subject-CV to 3.4 %, and the between-subject-CV to 6.2 %. Ricos et al. [6] estimate the within-subject-CV to 2.8 %, the between-subject-CV to 6.6 % and calculate from these figures desirable-imprecision-CV to less than 1.4 %, desirable-bias to less than 1.8 % and desirable-total-error to less than ±4.1 % ($p < 0.05$). The formula used is:

Desirable-total-error ($p < 0.05$) < 1.65 * desirable-imprecision + desirable-bias.

The difference between the results from the evaluated method and the Comparison Method is completely explained by error in the investigated method only if a comparison method has no error. In this evaluation, the effects of imprecision and bias in the Comparison Method also have to be considered. As described later, the within-series-imprecision of the Comparison Method varies between CV 0.8 and 1.2 % for different control materials. Calculated from duplicate values with patient samples the within-series-imprecision is about 0.7 %.

To compensate for errors in the Comparison Method, the desirable-total-error is expanded in this study. If the within-series-imprecision of the used comparison method is set to 1.0 %, the desirable-total-error should theoretically be expanded from ±4.1 % (according to Ricos) to ±5 % ($p < 0.05$). The analytical quality goal was therefore set to ±5 %. These figures are used as tolerance limits in the deviation diagrams in this report. The limits have been drawn as stippled lines.

Results and discussion

Evaluation in a hospital laboratory

Internal quality control results
The results from internal quality control on Hemo_Control in the hospital laboratory are shown in Table 10.
The raw data is shown in Attachment 3.

Table 10. Internal quality control results, Hemo_Control, in the hospital laboratory

Control name	B—Haemoglobin (g/L)		n	CV (%) (95 % confidence interval)
	Assigned value	Average result		
EKF, Low	80 ±5	80.3	16	1.2 (0.9 — 1.8)
EKF, Normal	120 ±7	117.4	26	0.9 (0.7 — 1.3)
EKF, High	160 ±9	158.3	26	1.2 (0.9 — 1.6)
Control Cuvette	118 ±3	118.5	22	0.6 (0.5 — 0.9)

All the internal quality control results were within the acceptance limits set by the manufacturer. The CV values are also satisfying.

Excluded results in the calculations and in the diagram
The within-series imprecision on Hemo_Control was calculated from the differences between duplicate determinations of venous EDTA samples. The bias to the Coulter Comparison Method was calculated from the differences between the mean of the duplicates from the two methods.
The samples were selected among patient samples sent to the hospital laboratory. Some samples were manipulated to give low results and some were manipulated to give high results, as described in the section “Evaluation procedures”. The values were divided into three groups according to haemoglobin concentrations measured on Hemo_Control before the calculations. The differences were tested for outliers at each level according to Burnett [7] with repeated truncations.

Totally there were 110 results. Five of these results were excluded as statistical outliers or of other reasons. These results and the different reasons for the exclusions are shown in Table 11.

Table 11. Excluded results

Sample no.	B—Haemoglobin (g/L)							Reasons for exclusion
	Hemo_Control			Coulter			Difference H_C – Coulter	
	1	2	Mean	1	2	Mean		
42	29	34	31.5	34.2	35.2	34.7	–3.2	H_C 1 ≠ H_C 2
43	26	27	26.5	33.2	33.2	33.2	–6.7	H_C ≠ Coul.
44	108	107	107.5	112.3	110.3	111.3	–3.8	H_C ≠ Coul.
93	high	high	high	255.2	255.2	255.2	—	H_C too high
97	221	220	220.5	218.2	224.4	221.3	–0.8	Coul.1 ≠ Coul.2

Explanation of reasons for exclusion:
H_C 1 ≠ H_C 2 — Statistically too big difference between the two Hemo_Control values
H_C ≠ Coul. — Statistically too big difference between the Hemo_Control Mean and the Coulter Mean
H_C too high — Results above the measuring range of Hemo_Control
Coul.1 ≠ Coul.2 — Statistically too big difference between the two Coulter values

Two samples, number 43 and 44, gave big differences between the mean of duplicate values from Hemo_Control and the mean of duplicate values from Coulter. In an attempt to explain the differences between the two methods, the cell counter results were checked more carefully. No explanation to the differences was found.

The scope of the calculations of precision and bias is to give measures on typical results. All the outliers above are therefore excluded, leaving 105 results in these calculations. The total error diagram should show both systematic and random errors. Therefore are in this case only the results from sample number 93 and 97 excluded, leaving 108 results to be shown in the diagram.

Within-series imprecision

The within-series imprecision for Hemo_Control with venous EDTA samples was calculated from 105 duplicate results. The result is shown in Table 12.
The raw data is shown in Attachment 2.

Table 12. Within-series imprecision, Hemo_Control
Venous patient samples in the hospital laboratory

Level group	B—Haemoglobin Interval (g/L)	Excluded results*	B—Haemoglobin Mean value (g/L)	n	CV (%) (95 % confidence interval)
Low	20 — 110	3	72.8	38	1.1 (0.9 — 1.4)
Medium	111 — 150	0	130.7	41	0.9 (0.7 — 1.1)
High	151 — 252	2	185.2	26	0.9 (0.7 — 1.3)
All	20 — 252	5	123.2	105	1.0 (0.9 — 1.1)

* Please refer to Table 11 for explanation of excluded results.

These results could be compared with within-series imprecision for the Coulter Comparison Method with the venous patient samples, which was 1.0 %, 0.7 % and 0.5 % at the low, medium and high level.

Between-day imprecision
The between-day imprecision for Hemo_Control was calculated from the results of duplicate determinations of venous EDTA samples from 20 patients at Malmö University Hospital.
The between-day imprecision in Table 13 includes the within-series imprecision.
Raw data is shown in Attachment 4.

Table 13. Between-day imprecision*, Hemo_Control.
Venous patient samples in the hospital laboratory

B—Haemoglobin Interval (g/L)	B—Haemoglobin Mean value (g/L)	n	CV* (%) (95 % confidence interval)
83 — 175	129.6	20	0.7 (0.5 — 1.1)

* The between-day imprecision includes the within-series imprecision.

Bias
The bias for Hemo_Control compared to the Coulter Comparison Method was calculated from duplicate results from 105 venous EDTA samples.
The result is shown in Table 14.
Raw data is shown in Attachment 2.

Table 14. Bias, Hemo_Control. Venous patient samples in the hospital laboratory

Level group	B—Haemoglobin Interval (g/L)	Excluded results*	B—Haemoglobin Mean value (g/L)	n	Mean difference Hemo_Control – Comparison Method (95 % confidence interval) (g/L)
Low	20 — 110	3	72.8	38	–0.5 (–0.8 — –0.2)
Medium	111 — 150	0	130.7	41	–0.5 (–1.0 — ±0.0)
High	151 — 252	2	185.2	26	+0.3 (–0.6 — +1.1)
All	20 — 252	5	123.2	105	–0.3 (–0.6 — ±0.0)

* Please refer to Table 11 for explanation of excluded results.

Total error
The total error is a combination of systematic and random error. The total error with Hemo_Control obtained under standardised and optimal conditions in the Clinical Chemistry Department is illustrated in Figure 2. Venous samples were measured on Hemo_Control and on the Coulter instrument. The x-axis shows the average values of duplicates on the Coulter Comparison Method. The y-axis shows the deviations in gram per litre between the first single value with Hemo_Control and the average value of duplicates with the Comparison Method. The tolerance limits at ±5 % are shown as stippled lines in the diagram.
Raw data is shown in Attachment 2.

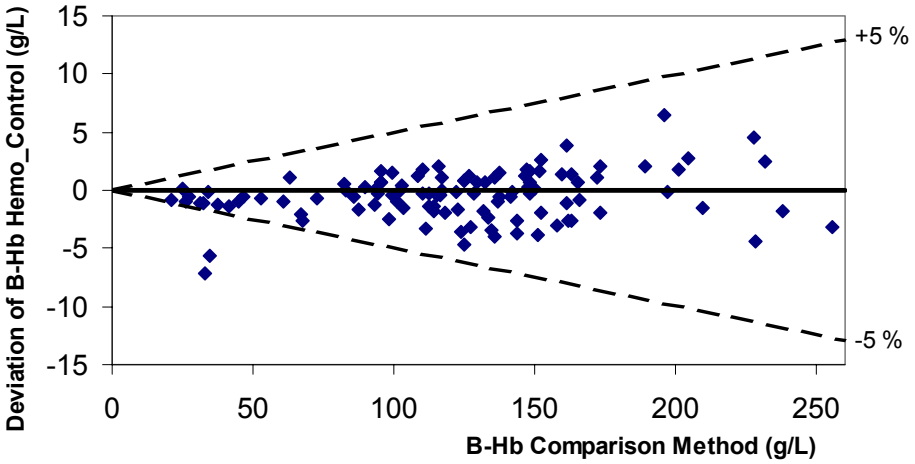

Figure 2. Total error. Venous samples in the hospital laboratory. n = 108
Deviation of the Hemo_Control results to the Coulter Comparison Method results.
The y-axis shows the deviation of the first Hemo_Control result from the corresponding duplicate mean with the Comparison Method.
The x-axis shows the duplicate mean with the Comparison Method.
The tolerance limits for the total error are shown as stippled lines.
Why the two deviating results should not be excluded in the diagram is explained under "Excluded results in the calculations and in the diagram" in the text.

Discussion of the results in the hospital laboratory

Imprecision with venous samples
Table 12 shows that the within-series imprecision for Hemo_Control with venous EDTA samples in the Clinical Chemistry Department was good. The CV was around 1.0 % at all three concentration levels. Table 13 shows that the between-day imprecision for Hemo_Control with venous samples in the Clinical Chemistry Department was good. The CV was 0.7 % in our limited series of results. The between-day imprecision includes the within-series imprecision. There is no proved difference between within-series imprecision and between-day imprecision.

Bias with venous samples
Table 14 shows that the Hemo_Control results with venous samples show a small negative bias compared to the Coulter Comparison Method at low and medium levels and a small positive bias at high concentrations. At the low haemoglobin concentration level the small bias is statistically significant, but it has no clinical importance.

Total error with venous samples
Figure 2 confirms that the Hemo_Control results with venous samples show good agreement with the Coulter Comparison Method. The tolerance limits for the total error of ±5 % are shown as stippled lines in the deviation diagrams. Only two out of 108 results were outside these tolerance limits. 98 % of the results were within the tolerance limits. The Hemo_Control results are slightly lower than the Comparison Method at the low haemoglobin level. The small deviation has no clinical importance. The results obtained with venous samples on Hemo_Control under standardised and optimal conditions in the Clinical Chemistry Department fulfil the quality goals.

Evaluation at two primary care centres

Internal quality control results
The results from internal quality control on Hemo_Control at the two primary care centres are shown in Table 15.
The raw data is shown in Attachment 5.

Table 15. Internal quality control results, Hemo_Control, at the primary care centres

Control name	B—Haemoglobin (g/L)		n	CV (%) (95 % confidence interval)
	Assigned value	Average result		
Primary Care Centre A:				
EKF, Normal	120 ±7	119.4	9	0.7 (0.5 — 1.4)
EKF, High	160 ±9	158.6	9	1.0 (0.7 — 2.0)
Primary Care Centre B:				
EKF, Normal	120 ±7	118.6	17	0.6 (0.4 — 0.9)
EKF, High	160 ±9	157.8	17	0.6 (0.4 — 0.9)

All the internal quality control results were within the acceptance limits set by the manufacturer. The CV values are also satisfying.

Within-series imprecision

Within-series imprecision was calculated from the results of duplicate determinations with venous samples from 40 patients at each of the two primary care centres. In addition, 40 capillary samples at Centre A and 20 capillary samples at Centre B were analysed.
Each capillary sample duplicate was collected from the same skin puncture. In the calculations the values were divided into two groups according to B—Haemoglobin concentrations.
The differences were tested for outliers in each group according to Burnett [7]. No outliers were found.
The within-series imprecision with venous samples is summarised in Table 16.
The within-series imprecision with capillary samples is summarised in Table 17.
Raw data is shown in Attachment 6 and 7.

Table 16. Within-series imprecision. Venous samples at Primary Care Centre A and B.

Level group	**B—Haemoglobin Range (g/L)**	**B—Haemoglobin Mean value (g/L)**	**n**	**CV (%)** **(95 % confidence interval)**
Primary Care Centre A:				
Low <140	94 — 139	128	18	1.4 (1.0 — 2.1)
High ≥140	140 — 170	152	22	1.5 (1.2 — 2.2)
All	94 — 170	141	40	1.5 (1.2 — 1.9)
Primary Care Centre B:				
Low <140	78 — 139	127	20	0.7 (0.5 — 1.0)
High ≥140	140 — 154	147	20	0.8 (0.6 — 1.1)
All	78 — 154	137	40	0.7 (0.6 — 0.9)

Table 17. Within-series imprecision. Capillary samples at Primary Care Centre A and B.

Level group	**B—Haemoglobin Range (g/L)**	**B—Haemoglobin Mean value (g/L)**	**n**	**CV (%)** **(95 % confidence interval)**
Primary Care Centre A:				
Low <140	96 — 139	126	17	6.3 (4.7 — 9.7)
High ≥140	140 — 186	154	22	5.1 (4.0 — 7.2)
All	96 — 186	142	39	5.5 (4.6 — 7.2)
Primary Care Centre B:				
All	82 — 154	135	20	2.8 (2.2 — 4.1)

Bias

The bias for venous and capillary sample results obtained with Hemo_Control compared to those obtained with Coulter was calculated from mean values of duplicate determinations. The calculations were carried out in the same way as described for the hospital laboratory, except that the values were divided into less number of level groups instead, because the limited number of results.

The results obtained for bias are shown in Table 18 and Table 19.

Raw data is shown in Attachments 6 and 7.

Table 18. Bias. Venous samples at Primary Care Centre A and B

Level group	**B—Haemoglobin Range (g/L)**	**B—Haemoglobin Mean value (g/L)**	**n**	**Bias Hemo_Control – Comparison Method (95 % confidence interval) (g/L)**
Primary Care Centre A:				
Low <140	92 — 138	128	18	+0.3 (–0.5 — +1.1)
High ≥140	141 — 167	151	22	+1.2 (–0.2 — +2.5)
All	92 — 167	141	40	+0.8 (±0.0 — +1.6)
Primary Care Centre B:				
Low <140	78 — 139	127	22	+0.3 (–0.2 — +0.9)
High ≥140	140 — 156	146	18	+1.2 (+0.3 — +2.2)
All	78 — 156	136	40	+0.7 (+0.2 — +1.2)
Primary Care Centre A and B:				
All	78 — 167	139	80	+0.8 (+0.3 — +1.2)

Table 19. Bias. Capillary samples at Primary Care Centre A and B

Level group	**B—Haemoglobin Range (g/L)**	**B—Haemoglobin Mean value (g/L)**	**n**	**Bias Hemo_Control – Comparison Method (95 % confidence interval) (g/L)**
Primary Care Centre A:				
Low <140	93.0 — 139.5	125	16	–2.6 (–5.1 — –0.1)
High ≥140	140.0 — 181.0	153	23	+3.2 (–0.7 — +7.1)
All	93.0 — 181.0	142	39	+0.8 (–1.8 — +3.2)
Primary Care Centre B:				
All	80.0 — 153.0	135	20	+0.8 (–0.8 — +2.4)
Primary Care Centre A and B:				
All	80.0 — 153.0	139	59	+0.8 (–1.0 — +2.6)

Total error

The deviation diagrams, Figure 3 and 4, show the total error (bias and imprecision together) for the results obtained with Hemo_Control versus those obtained with the Coulter Comparison Method. Figure 3 shows the results with 80 venous samples from the two primary care centres, and Figure 4 shows the results with 59 capillary samples. The two diagrams are drawn according to the same principles, except that the scale of the y-axis in Figure 4 is larger, because the capillary results are more scattered. It must also be emphasised, that most of the capillary results in Figure 4 come from Centre A, which had poorer CV than Centre B. According to the protocol, the CV-results achieved after 20 capillary results were evaluated before taking a decision whether they were to continue taking capillary samples or not. Centre B got an acceptable CV with the capillary samples and was therefore told that they could stop taking capillary samples after the first 20 results.

Total error diagram with venous sample results is shown in Figure 3.

Total error diagram with capillary sample results is shown in Figure 4.

Raw data is shown in Attachments 6 and 7.

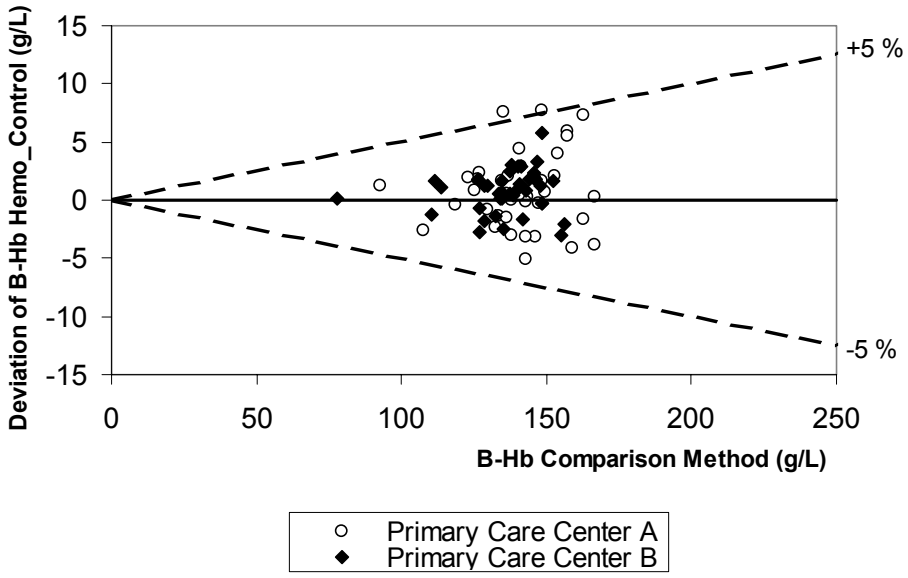

Figure 3. Total error. Venous sample results at the primary care centres. n=80
Hemo_Control venous sample results at Centre A and B (40 results from each) compared with venous Coulter Comparison Method results. The y-axis shows the deviation of the first Hemo_Control result from the corresponding mean of the duplicate results with the Comparison Method.
The x-axis shows the mean concentration of the duplicate results with the Comparison Method.
The tolerance limits for the total error are shown as stippled lines.

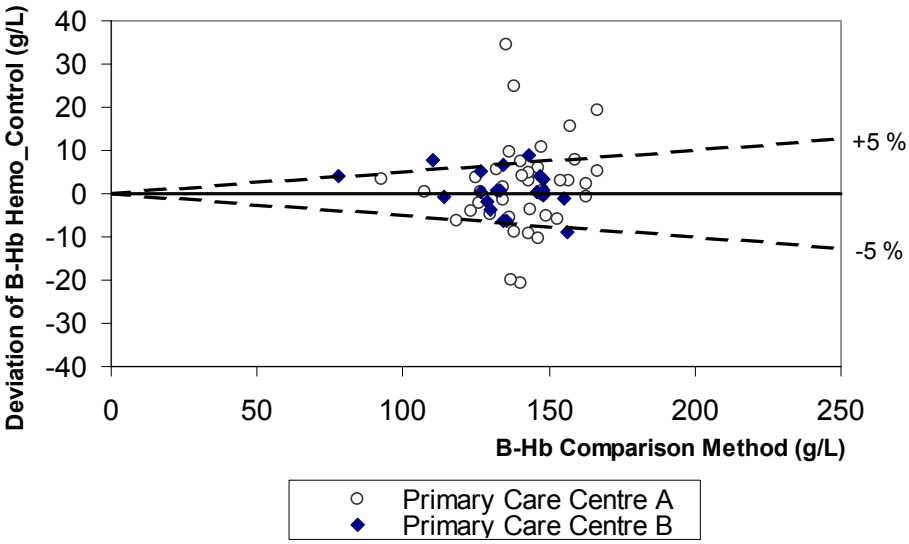

Figure 4. Total error. Capillary sample results at the primary care centres. n=59
Hemo_Control capillary sample results at Primary Care Centre A and B compared with venous Coulter Comparison Method results. The y-axis shows the deviation of the first Hemo_Control result from the corresponding mean of the duplicate results with the Comparison Method.
The x-axis shows the mean concentration of the duplicate results with the Comparison Method.
The tolerance limits for the total error are shown as stippled lines.

Discussion of the results at the two primary care centres

Imprecision with venous samples
The within-series precision obtained on Hemo_Control with venous samples at Primary Care Centre A and B is good (Table 16). The CV is about 1 %, slightly higher than 1 % at Centre A and slightly lower than 1 % at Centre B. The CV-values were the same at different levels of haemoglobin concentrations. The results at Centre B are as good as the results achieved under optimal conditions in the hospital laboratory.

Imprecision with capillary samples
As predicted, the imprecision achieved with capillary samples (Table 17) is higher than the results with venous samples. The CV that was calculated after the first 20 capillary results at Centre B was 2.8 %. This variation is approximately 2 — 3 times higher than the variation with venous samples, which is as expected. According to the protocol, Centre B therefore ended the investigation after the first 20 capillary samples. At Centre A, however, the CV with capillary samples was higher than expected, more than three times the CV of venous samples. At Centre A, they therefore continued the capillary sample taking until they reached 40 results, as described in the protocol. This resulted in a CV between 5 and 6 %.
Proper capillary sampling for haemoglobin measurements is difficult but essential to minimize the deviations from venous results. The imprecision with capillary samples will usually increase, compared to imprecision achieved with venous samples. The poor capillary CV at centre A is probably associated with the sampling technique and the skill of the sample collector. This centre was probably not as familiar with capillary sampling as was centre B. The result can also vary depending on the type of lancet used, and on the physiological conditions in the puncture area. This pre-analytical source of error are valid not only for Hemo_Control, but for all instruments measuring haemoglobin in capillary samples. See further discussion about the problems with capillary samples under the coming section “Total error”.

Bias with venous samples
The haemoglobin results with venous samples on Hemo_Control at the two primary care centres show a slightly positive bias relative to the Comparison Method. At Centre A the bias between the two methods is approximately +0.8 g/L, but statistically not significant.
The bias at Centre B is of the same size, but statistically significant due to a lower imprecision at Centre B. The overall bias for all Hemo_Control results at the two primary care centres is +0.8 g/L. The bias is significantly different from zero, but hardly of clinical importance.

Bias with capillary samples
The Hemo_Control results with capillary samples show about the same bias relative to the Comparison Method as the results from venous samples do. At Centre A, the bias is negative at the low level and positive at the high level and at two levels together. The bias for all results from Centre B is also positive. The overall bias for all capillary results at the two primary care centres is however not significantly different from zero.

Total error with venous samples
The results from the venous samples are spread fairly even about the zero-line in the diagram, Figure 3. No systematic difference is revealed by visual inspection of the deviation diagram. The tolerance limits for a total error of ±5 % are shown as stippled lines. Of all the results are 97.5 % within these limits, signifying that the haemoglobin results with Hemo_Control at Primary Care Centres A and B, when using venous samples, fulfil the quality goal that was agreed upon for this evaluation.

Total error with capillary samples
The capillary sample results deviate more, and only 75 % of these results are within the tolerance limits. The quality goal was therefore not attained with capillary samples The main reasons seems to be that the haemoglobin concentrations in capillary puncture blood is not representative for the concentration in venous blood and the poor precision at capillary sample collection. Acceptable precision can be obtained with skilful sample collection, but the non-representative concentrations in capillary samples appear impossible to avoid.
The problem is that some sampling sites produce puncture blood with higher haemoglobin concentration than in venous blood and that other sampling sites produce blood with lower haemoglobin concentration than venous blood.

These pre-analytical sources of error are valid not only for Hemo_Control, but for all instruments measuring haemoglobin in capillary samples. See also Attachment 8, which is an extract from a previous SKUP report evaluating Biotest Hemoglobin Measuring System [8]. The extract contains some general observations from measuring haemoglobin in capillary samples.

Discussion of all results

Imprecision with venous samples
The within-series precision obtained on Hemo_Control with venous samples is good enough at all sites in the evaluation. However, the difference in precision between the two primary care centres is obvious and shows that skilfulness when filling the measuring cuvette is important to get the best result.

Imprecision with capillary samples
The within-series precision obtained on Hemo_Control with capillary samples is poorer than with venous samples and good enough only at one of the two primary care centres.
The difference in precision between the two primary care centres shows that skilfulness in capillary sampling is a requirement to get acceptable results.

Bias with venous samples
The Hemo_Control results with venous samples show a slightly negative bias in the hospital laboratory and a slightly positive bias at the two primary care centres. The discrepancy may be explained by the sample selection that is different in the hospital and in the primary care and also by the fact that there was a small difference between the instruments in the concordance test. The overall judgment of the venous results is that there is no significant bias.

Bias with capillary samples
The Hemo_Control results with capillary samples show no bias that is significantly different from zero.

Total error with venous samples
The Hemo_Control results with venous samples fulfil the quality goals with a total error of less than ±5 %.

Total error with capillary samples
The Hemo_Control results with capillary samples do not fulfil the quality goals with a total error of less than ±5 %. This was probably caused by the non-representative haemoglobin concentrations in capillary puncture blood and also by the poor precision at capillary sample collection and thus not due to Hemo_Control per se.

Practical points of view

There were no major problems with the instruments during the evaluation period. Sometimes, one of the two primary care centres had problems with turning on the instrument. To get started, they had to restart the instrument with the restart button at the underside of the device. Points of view expressed during the practical use of Hemo_Control under standardised and optimal conditions and in primary health care are shown below.

Positive comments
- The system is easy to use
- The instrument is small and elegant
- The cuvettes are easily filled
- The results are shown quickly
- The touch screen is easy to work with, and it is easy to program the different functions
- The display is easy to read and understand
- There were no problems concerning the hygienic aspect when working with the device
- A minimum of maintenance is needed
- The training by MEDimport was good
- The manual is easy to read and understand

Negative comments
- The control cuvette is kept outside the instrument (can get lost)

References

1. N.G. Christensen, G. Monsen, S. Sandberg. Utprøving av analyseinstrumenter. En veiledning spesielt beregnet for utprøving av instrumenter for primærhelsetjenesten. Alma Mater Forlag 1997, ISBN 82-419-0230-1.

2. "Recommendations for reference method for haemoglobinometry in human blood (ICSH standard 1995) and specifications for international haemoglobincyanide standard (4th edition)"; Expert panel. J. Clin. Pathology 49:4, 271—274 (1996).

3. G. Vanzetti, An azide-methemoglobin method for hemoglobin determination in blood, Am. J. Lab. & Clin. Med. 67 (1966) 116.

4. C.G. Fraser & P. Hyltoft Petersen, Quality goals in external quality assessment are best based on biology, Scand J Clin Lab Invest 1993; 53 suppl 212. Chapter I. Quality planning.

5. M.Á. Sebastián-Gambaro et al., Intra- and Inter-Individual Biological Variability Data Bank, Eur J Clin Chem Clin Biochem 1997; 35(11): 845—852. This data bank for biological variation is republished on the internet address: http://www.westgard.com

6. C. Ricós et al., Current databases on biological variation, Scand J Clin Lab Invest 1999, 59: 491—500

7. R. W. Burnett, Accurate Estimation of Standard Deviations for Quantitative Methods used in Clinical Chemistry, Clin Chem, vol. 21, No. 13, 1975, page 1935—1938.

8. SKUP report SKUP/2001/17 evaluating Biotest Hemoglobin Measuring System. The complete report is available on the internet address: http://www.skup.nu

Attachments

1. Raw data, validation of trueness of the Coulter Comparison Method
2. Raw data, concordance test
3. Raw data, internal quality control, Hemo_Control, hospital laboratory
4. Raw data, venous patient samples, hospital laboratory
5. Raw data, between-day imprecision, Hemo_Control, hospital laboratory
6. Raw data, internal quality control, Hemo_Control, Primary Care Centre A and B
7. Raw data, venous and capillary patient samples, Primary Care Centre A
8. Raw data, venous and capillary patient samples, Primary Care Centre B
9. Extract from the SKUP report SKUP/2001/17. Some general observations from measuring haemoglobin in capillary samples

The raw data attachments are only included in the copy to MEDimport AS.

Attachment 9.
Extract from the SKUP report SKUP/2001/17.

This text has been slightly edited since first published
in the SKUP report, SKUP/2001/17, about Biotest Hemoglobin Measuring System.
The full report is available on Internet: www.skup.nu

SOME GENERAL EXPERIENCES FROM MEASURING B—HAEMOGLOBIN IN CAPILLARY SAMPLES

During this evaluation some general problems with capillary samples became obvious. The following discussion is valid not just for Biotest but for all instruments using capillary samples for determination of B—Haemoglobin. The literature about these problems is not elucidating and some comments might facilitate the reading of this report.

In this study the word ”capillary” is used frequently together with blood, puncture, sample and result. It should be pointed out that the main part of the blood in a capillary sample does not originate from the capillaries but from the arterioles. The capillaries have very small diameters and the bleeding from them is negligible.

The words ”analytical quality” are also used many times in this report. In that concept we include the errors from the preanalytical phase, that is the sample collection errors.

Experience 1:
The haemoglobin concentration in capillary puncture blood was homogenous
In our study it was possible for one of the primary care centres to obtain very low imprecision when the two samples were collected from the same capillary puncture.

This shows that blood from the capillary punctures in Primary Care Centre B was homogenous in haemoglobin concentration.

Experience 2:
The haemoglobin concentration in capillary puncture blood often deviated from that in venous blood
However, after proving that the capillary results were reproducible, we observed that they showed insufficient analytical quality according to the criteria set up in this evaluation. See deviation diagram in Figure 8. The capillary results scattered much more than the venous results. The capillary results are in some cases higher and in others lower than the corresponding venous results measured with the comparison method. This finding is not caused by high imprecision.

Deviating capillary results could also be seen when comparing capillary and venous results, both obtained with Biotest. The deviations are then seen as a big “range of differences”. As stated in table 17 the “range of differences” between capillary and venous results was in our study found to be –9.4 — +10.4 %.

Deviating results obtained with capillary samples are found not only in this study. Daae et al [5][6] found the “range of differences” between capillary and venous results to be –9.2 – +10.3 %. The type of anticoagulant in the collection tubes for the venous samples is not stated, but according to personal communication with Dr Daae were liquid EDTA tubes

used and the venous results were not corrected for dilution. That type of tube dilutes a venous sample by 1.2 %. The originally published results have here been recalculated and are here presented after correction for sample dilution in the venous collection tubes i.e. the reported mean difference between capillary and venous results +2.4 % is therefore corrected to +1.2 %. D.W. Pi et al [8] compared capillary results with venous results and found large individual differences. The standard deviation of the differences was as high as 7.8 g/L.

There are possible explanations for why haemoglobin in some capillary puncture blood is concentrated and in some other is diluted compared to venous blood. Daae et al [5] refer to flow dynamic rules leading to a concentration of big particles like the erythrocytes in the centre of narrow arterioles. On the other hand, other authors claim that the capillary puncture blood may be diluted with interstitial fluid. Either of these two factors may be dominant in the single capillary puncture blood depending on the conditions at the puncture site.

Our second experience is thus that the B—Haemoglobin concentration in capillary puncture blood often deviates from the concentration in venous blood from the same individual. The main part of the inaccuracy in capillary results does not arise when the sample is sucked up into the cuvette. It is a preanalytical error occurring already in the capillary puncture.

Experience 3:
The mean concentrations of haemoglobin in capillary and venous samples were the same.
In our study there are both capillary and venous Biotest results from totally 80 patients in the two Primary Care Centres. Raw data are given in Attachments 5 and 6. The vacuum tubes used for collection of venous samples contained liquid EDTA, which caused a dilution of 3.8 %. The raw values from venous blood have therefore been corrected for the dilution before the comparison with the concentrations in the capillary samples. The results from the comparison are presented in table 17.

Table 17. Comparison of the haemoglobin concentration in capillary and venous samples.

Analyte	**Range (g/L)**	**Mean value (g/L)**	**n**	**Mean difference capillary - venous**		**Range of differences capillary - venous (%)**
				(g/L)	**(%)**	
Capillary haemoglobin	99 — 175	139.9	80	+0.2 (–0.8 — +1.1)	+0.2 (–0.5 — +0.8)	–9.4 — +10.4
Venous haemoglobin	95 — 170	139.8	80			

All values in this table are corrected for sample dilution (3.8 %) in the venous collection tubes.
The 95 % confidence intervals for the mean differences are given in brackets.

Considering the confidence intervals there was no difference between the mean haemoglobin concentration in capillary and venous samples.

Our experience may not be valid for all capillary sampling techniques. In our study with our method of collecting the capillary samples after fingerpricks there was no difference between the mean haemoglobin concentration in capillary and venous samples. However, if other equipment and methods are used for the capillary punctures there may be a difference. E.g. we have collected the samples from the third to the fifth drop. There may be a difference if other drops are collected.

As mentioned before, there are contradictory statements in the literature on the difference between the mean haemoglobin concentration in capillary and venous samples. The mean concentration deviation in capillary samples has been reported to be +1.2 % [5] [6], –0.6 % [7], +3.2 g/L [8] and –2.7 g/L [9] respectively. Only in one case, +1.2 %, we now know that the difference have been calculated after correction for the dilution of the venous samples. The other references are therefore non-conclusive.

Anyhow, our third experience is, that despite that there were many individuals with big differences between the concentrations in the capillary and the venous sample, we could find no difference between the mean concentrations of haemoglobin in capillary and venous samples when results from many individuals were compared.

Practical consequences of our experiences

In the following paragraphs the practical consequences of our experiences are discussed. When B—Haemoglobin is measured in capillary samples there are thus two important problems to face:

1. Deviating concentration in the puncture blood
   The concentration in the capillary puncture blood may often be non-representative for the concentration in venous blood in the same individual. Even with optimal collection, the concentrations are in some puncture blood higher and in others lower than the corresponding venous concentrations. These preanalytical deviations may be up to ±7 g/L.

2. Sampling imprecision
   It is more difficult to collect capillary samples than venous samples. This usually leads to higher imprecision [7] [10]. The CV, for duplicate samples collected from the same puncture can often be around 3 %. That imprecision corresponds to a variation in the individual values up to ±8 g/L (95 % probability) at normal level of B—Haemoglobin.

It is common that these two sources of error are not separated. However, in this evaluation it was necessary to make this distinction for capillary samples to explain the paradox that high precision can be combined with an insufficient analytical quality.

<u>Considering these problems, highest analytical quality cannot be obtained with capillary samples. This is valid not only for Biotest, but for all instruments using capillary samples for measuring B—Haemoglobin.</u>

With venous samples only one of the sources of error is present. That is the sampling imprecision which gives a CV around 1 %. This variation corresponds (with 95 % probability) to a deviation of ±3 g/L in the normal range of B—Haemoglobin.

The requester of the B—Haemoglobin analysis has to consider whether the capillary analytical quality is good enough in the existing clinical situation. The uncertainty in a capillary result forces the requester to be more careful in interpreting the result. For example if there are two consecutive B—Haemoglobin results in the normal range for a patient there must be a difference (= critical difference) of at least 13 g/L to be sure (with 95 % probability) that the value is changed.

We have also noted an often-overlooked preanalytical source of error when measuring B—Haemoglobin in venous blood. Vacuum tubes for collection of venous samples contain dry or liquid EDTA. One common type of tube with liquid EDTA dilutes the blood 3.8 %. In case of a dilution effect, one should consider to recalculate the result before it is reported. Notice that this error is not revealed in proficiency testing schemes.

References of this attachment:

5. L.N.W. Daae, S. Halvorsen, P.M. Mathisen & K. Mironska, A comparison between haematological parameters in 'capillary' and venous blood from healthy adults, Scand J Clin Lab Invest 1988; 48: 723-726.

6. L.N.W. Daae, S. Halvorsen, P.M. Mathisen & K. Mironska, ERRATUM TECHNICAL NOTE: A comparison between haematological parameters in 'capillary' and venous blood from healthy adults, Scand J Clin Lab Invest 1989; 49: 305.

7. I.B. Hersleth, L.N.W. Daae, Hemoglobinmåling er mindre nøyaktig i kapillærblod enn i veneblod!, Bioingeniøren, 1995, nr 6/7, 7-8.

8. D.W. Pi, S.H. Krikler, T.G. Sparling, C.J. Carter and L.D. Wadsworth, Reappraisal of optimal hemoglobin standards for female blood donors in Canada, Transfusion, Vol. 34, No. 1–1994, page 7-10.

9. M. Sfez, P. Desruelle, G.F. Pennecot, P. Cohen-Bacrie, Translation of the french title: Interest in measuring haemoglobin during surgery. Evaluation of a colorimetric method (Hemocue®), Cah Anestesiol, vol 39, No 4, 1991, page 247-251.

10. A..M. Conway, R.F. Hinchliffe, J. Earland, L.M. Anderson, Measurement of haemoglobin using single drops of skin puncture blood: is precision acceptable? J Clin Pathol, 51 (3), 1998 Mar, page 248-250.