Biotest Hemoglobin Measuring System

A system to measure Blood–Haemoglobin, manufactured by Biotest Medizintechnik GmbH

Report from a premarketing evaluation, organised by "Scandinavian Evaluation of Laboratory Equipment for Primary Health Care", SKUP

SKUP/2001/17

SKUP Sweden, EQUALIS AB, Box 977, SE-751 09 Uppsala, Sweden, phone +46 18 69 31 64, fax +46 18 69 31 46

PREMARKETING EVALUATION OF THE BIOTEST HEMOGLOBIN MEASURING SYSTEM

TABLE OF CONTENTS

SUMMARY

The Biotest Hemoglobin Measuring System (Biotest) is used for determination of the haemoglobin concentration in human blood. Biotest consists of an absorption photometer called Biotest Hemoglobin Tester and of Biotest Hemoglobin disposable microcuvettes which contain dried reagents. In the cuvettes, haemoglobin is converted to azide methaemoglobin. The system measures the endpoint of the reaction bichromatically.

It is possible to draw the sample, 10 µL blood, directly into the Biotest cuvette from a capillary puncture. The cuvette can be read almost immediately in the Biotest Hemoglobin Tester. The measuring range is 0 – 256 g/L.

The first part of this premarketing evaluation has been performed by experts under ”standardised conditions”, i.e. by experienced laboratory technologists in a department of clinical chemistry. The second part was performed under ”real life conditions” by ordinary users of this kind of tests, i. e. by staff in two primary care centres.

The routine method for B–Haemoglobin in the Department of Clinical Chemistry, Malmö University Hospital, is based on photometric measurement of cyanmethaemoglobin in the cellcounter Coulter GenS. This method is accredited and was used as the designated comparison method in this evaluation.

Preliminary analytical quality goals for this evaluation were derived from biological variation and set to allow a total error of less than ±5 %.

Results

Testing with venous samples in the Department of Clinical Chemistry.
The within-series imprecision for Biotest with venous EDTA samples was good with CV around 0,7 %. The between-day imprecision was good. This CV was 1,2 %.
The linear correlation between Biotest and the comparison method was good with $r^2 = 1,00$.
The slope of the regression line was 0,989 and the intercept was -1,1 g/L.

Together, our findings indicate that the values from Biotest, with venous samples, are slightly lower, on average -1,9 % (or -2,6 g/L), than the values from the comparison method. However, this small deviation is of no clinical importance.
These individual results fulfil our analytical quality goals with a total error of less than ±5 %.

Testing with venous samples in Primary Care Centres.
These results are similar to those obtained in the Department of Clinical Chemistry.

The within-series imprecision in the Primary Care Centres was as good as in the Department of Clinical Chemistry. The CVs were 0,7 % and 0,4 % respectively.
The linear correlation between Biotest and the comparison method was good with $r^2 = 0,98$ and 0,99. The slopes of the regression lines were 1,057 and 0,984 respectively.
The intercepts were -10,7 and -1,2 g/L. The regression line obtained for one of the two Pri-mary Care Centres combines a comparatively bigger negative intercept with a steeper slope.

These Biotest values were lower, on the average -2,2 % (or -3,0 g/L), than the values obtained with the comparison method.
These individual results fulfil our analytical quality goals with a total error of less than ±5 %.

Testing with capillary samples in the Primary Care Centres.
The within-series imprecision calculated from duplicate values from the same capillary puncture was acceptable in both centres. However, the CV-values varied from 0,9 % in one centre to 3,0 % in the other. The difference illustrates that imprecision can vary depending on the type of lancet used, sampling technique and the skill of the sample collector.
The linear correlation between Biotest and the comparison method was less good with $r^2 = 0,93$ and 0,95. The slopes of the regression lines were 1,056 and 1,037 respectively. The intercepts were -5,0 and -3,2 g/L.

Biotest results obtained with capillary samples were lower, on average -1,9 % (or -3,0 g/L), than those of the comparison method with venous samples.
These individual results do not fulfil our analytical quality goals, which is a total error of less than ±5 %.

Some general experiences from measuring B–Haemoglobin in capillary samples
During this evaluation some general problems with capillary samples became obvious.
The main part of the inaccuracy in a capillary result does not arise when the sample is sucked up into the cuvette. It is a preanalytical error occurring already in the capillary puncture.
The haemoglobin concentration in capillary puncture blood is for some individuals higher and for others lower than the corresponding venous blood concentration. For this reason, B-Haemoglobin results from capillary samples are less reliable. This preanalytical error is valid not only for Biotest, but for all instruments using capillary samples for measuring B-Haemoglobin. This observation does not disqualify capillary samples for B-Haemoglobin, but the requester of the analysis has to consider whether the capillary analytical quality is good enough in the existing clinical situation.

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 points of view
All personnel involved in the evaluation summarised their opinion about the Biotest system as being quick and easy to use.

Conclusion
Biotest showed good precision when using venous samples. The within-series imprecision was around CV 0,7 %. The Biotest results had a good linear correlation with, and showed only small deviations from, the comparison method results, both in the Department of Clinical Chemistry and in the two Primary Care Centres. The bias was on average -2 % or -3 g/L.

Good duplicate precision can also be obtained with Biotest with capillary samples, but this requires proper sample collection. The within-series imprecision, calculated from duplicates collected from the same capillary puncture, was CV 0,9 % and 3,0 % respectively.
The bias was about the same as with venous samples. However, the haemoglobin concentrations in capillary puncture blood often deviate from that in the corresponding venous blood. For this reason, B-Haemoglobin results from capillary samples are less reliable. This preanalytical source of error is valid not only for Biotest, but for all instruments using capillary samples for measuring B-Haemoglobin.

Biotest is quick and easy to use.

PLANNING OF THE PRESENT STUDY

At the request of Biotest Medizintechnik GmbH, Scandinavian Evaluation of Laboratory Equipment for Primary Health Care, SKUP, has carried out a premarketing evaluation of the Biotest Hemoglobin Measuring System (henceforth called Biotest). At the time of the request, the system had not yet been marketed in Scandinavia.

This premarketing evaluation is a complete evaluation following the guidelines set out in the book *"Utprøving av analyseinstrumenter …"*, Alma Mater Forlag, Bergen Norway, 1997. English translation of the full title: ”Evaluation of analytic instruments. A guide particularly designed for evaluation of instruments in primary health care.”

The evaluation comprises the following studies:

In a department of clinical chemistry:
- Within-day imprecision
- Between-day imprecision
- Linear agreement of Biotest to a designated comparison method with venous samples
- Practical viewpoints from the users

In two primary care centres:
- Within-day imprecision
- Linear agreement between both capillary and venous samples with Biotest and venous samples with a designated comparison method
- Practical viewpoints from the users

After inquiry by SKUP Sweden, the Department of Clinical Chemistry at Malmö University Hospital accepted to carry out the analytical parts of the evaluation. Contracts were set up between SKUP Sweden, EQUALIS AB (External Quality Assurance for Laboratory Medicine In Sweden) and representatives from Biotest Medizintechnik GmbH and between SKUP Sweden, EQUALIS AB and the Department of Clinical Chemistry, Malmö University Hospital.

The protocol of the evaluation was worked out at a meeting with the following participants.
Dick Nelson, Representative of Biotest Medizintechnik GmbH
Tommy Forsell, Representative of Biotest Medizintechnik GmbH
Arne Mårtensson, Co-ordinator, SKUP Sweden, EQUALIS AB
Birgitta Alemo, Instructor, Department of Clinical Chemistry
Inger Karlberg, Laboratory Technologist, Department of Clinical Chemistry
Lilian Fink, Assistant Nurse, Lunden Primary Care Centre
Birgitta Nilsson, Laboratory Technologist, Granen Primary Care Centre

Birgitta Nilsson was later in the evaluation replaced by Ruth Herrlin, Assistant Nurse, Kirseberg Primary Care Centre. Birgitta Alemo and Inger Karlberg instructed in the Primary Care Centres. Inger Karlberg measured the samples in the Department of Clinical Chemistry, Malmö University Hospital. Lilian Fink and Ruth Herrlin collected the patient samples and performed the measurements with Biotest in the Primary Care Centres.

Birgitta Alemo, Inger Karlberg and Arne Mårtensson have written this report. Gunnar Nordin, Managing Director of EQUALIS and Ulf Rosén, Clinical Biochemist in the Department of Clinical Chemistry, Malmö University Hospital, made valuable contributions.

MATERIAL AND METHOD

Biotest

Biotest consists of an absorption photometer called Biotest Hemoglobin Tester and of disposable Biotest Hemoglobin Micro-cuvettes, which contain dried reagents. Capillary samples are usually drawn directly from a finger puncture or from an EDTA tube with venous blood into cuvettes by capillary force.

The filled Biotest cuvette is placed in the drawer of the Biotest Tester. In the micro cuvette, blood reacts with the reagents. Sodium deoxycholate haemolyses the erythrocytes and the haemoglobin is released. Sodium nitrite converts the haemoglobin into 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.

A test cuvette is available for electronic check of the Biotest Tester. To check the whole measuring system, including the Biotest cuvettes, a suitable quality control blood can be used. Biotest Medizintechnik GmbH approves Streck Para 4 for this purpose.

Technical data from the manufacturer is shown in table 1.

Table 1. Technical data for Biotest

Ambient temperature	15 - 35º C
Sample volume	10 µL
Measuring range	0 – 256 g/L
Linearity	0 – 200 g/L ±3 g/L > 200 g/L ±7 g/L
Measuring time	3 – 200 s
Power supply	Integrated NiMH accumulator 3,6 V
Main power adapter	Input 230 V AC, Output 9 V DC
Power consumption	Max. 30 mA, typically 15 mA
Operating time	Approximately 40 h with a fully charged accumulator and if in continual use.
Dimensions (open drawer)	W=12 cm (19 cm), H=6 cm, D=18,5 cm
Weight	475 g (without main power adapter)

Biotest product information

Biotest is manufactured by:
Biotest Medizintechnik GmbH
Industristrasse 19
D-63755 Alzenau, Germany
Phone: Int + 49 (0) 6023-9487-0
Fax: Int + 49 (0) 6023-9487-33
Internet: www.biotest-mt.de

Suppliers of Biotest in the Scandinavian countries:

Denmark:	Norway:	Sweden:
MEDINOR AS Langebjerg 35B P.O. Box 321 DK-4000 Roskilde	MEDINOR ASA Nils Hansens vei 4 Postboks 94, Bryn N-0611 Oslo	MEDINOR AB Box 1215 S-181 24 Lidingö
Phone: 70 15 10 41	Phone: 22 07 65 00	Phone: 08-544 812 00

During this premarketing evaluation the following Biotest Hemoglobin Testers were used:
serial no. 600-0100-0156 in the Department of Clinical Chemistry,
serial no. 600-0100-0206 in Primary Care Centre A and
serial no. 600-0100-0151 in Primary Care Centre B.

Biotest micro cuvettes, type “U”, from batch 31400900, were used for all samples.

Designated comparison method

The routine method for B–Haemoglobin in the Department of Clinical Chemistry, Malmö University Hospital, was used as the designated comparison method. This is a photometric method, which measures cyanmethaemoglobin. The method is implemented on the Beckman Coulter GenS System, with reagents and calibrator from Beckman Coulter.

The measuring principle in the GenS is as follows. EDTA blood is diluted in an alkaline buffer containing detergent and potassium cyanide (KCN). The erythrocytes are haemolysed by the reagent and the haemoglobin is denatured. Free ferroheme is oxidised to ferriheme and a cyanide complex is formed, ferriheme$(CN)_2$. The light absorption of the cyanide complex is measured at 525 nm.

Validation
Before the evaluation period, the GenS method was validated with the ICSH reference method [1]. The measurements with the ICSH reference method were made in the Department of Clinical Chemistry, Helsingborgs Hospital. 20 samples were measured in duplicates with both methods. See figures 1 and 2 and table 2.
Raw data are given in Attachment 1.

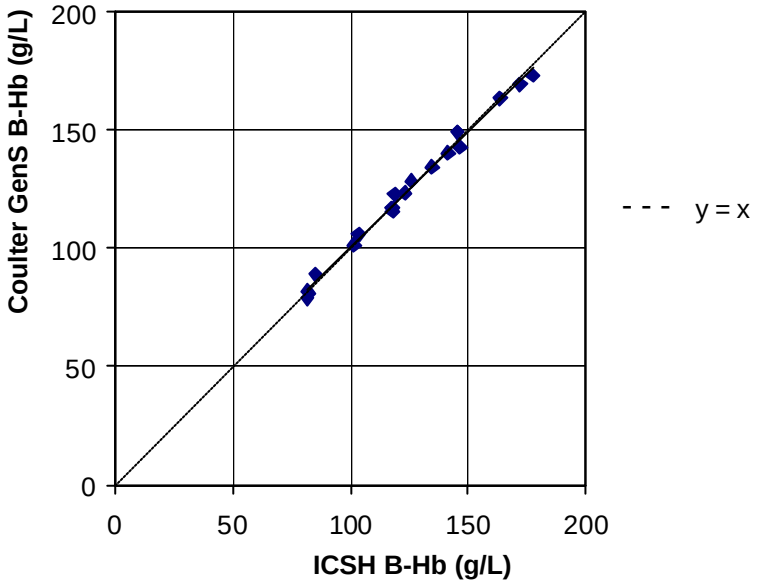

Figure 1. Coulter GenS method compared with the ICSH reference method.
Venous blood with both methods.
Mean values from duplicates. n = 20

Table 2. Calculation of linear agreement.
Coulter GenS method compared with the ICSH reference method.

Parameter	**Calculation**
Equation	y = 0,978 x + 2,7
Determination coefficient r^2 (95 % confidence interval)	0,99 (0,98 – 1,00)
Standard error, SE of residuals	2,5
Standard error in calculation of the slope of the regression line, Sea	0,02
Standard error in calculation of the intercept, SEb	0,6
Slope of the regression line (95 % confidence interval)	0,978 (0,936 – 1,020)
Intercept (95 % confidence interval)	+2,7 (-2,6 – +8,0)

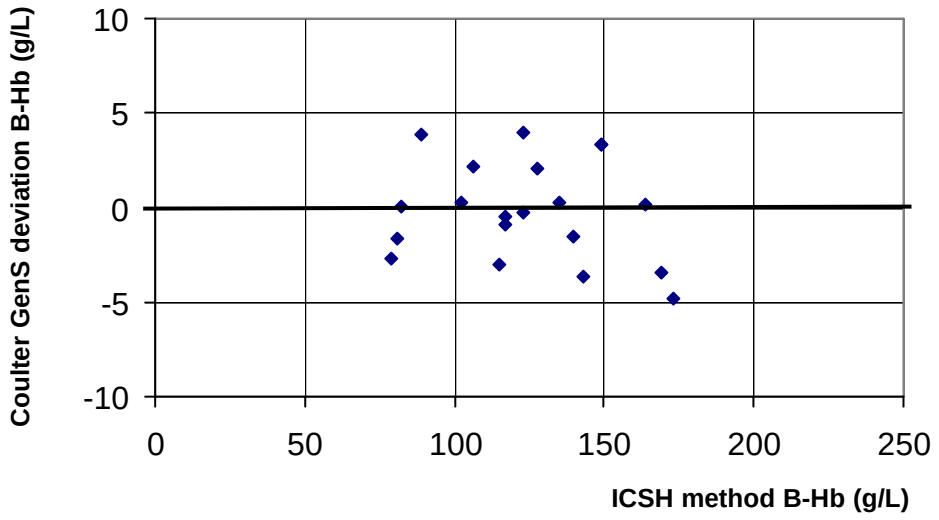

Figure 2. Coulter GenS method (single values) compared with the ICSH reference method (duplicate values. Venous blood with both methods. n = 20

The methods showed good agreement:
The slope is not significantly different from 1 and the intercept is not significantly different from 0 g/L.
The Coulter GenS method had a mean value of 124,3 g/L and a duplicate imprecision of CV = 0,2 %.
The ICSH method had a mean value of 124,3 g/L and a duplicate imprecision of CV = 0,3 %.

Quality assurance of the Coulter GenS comparison method during the evaluation period
Internal control samples from Beckman Coulter, at three levels, were measured every fourth hour according to a schedule. As an extra internal control, Streck Para 4, at two levels, were measured once a day. The results are summarised in table 3.

Table 3. Internal quality control results obtained with the Coulter GenS method at the Department of Clinical Chemistry, Malmö University Hospital.

Control name	Dates of analysis	B–Haemoglobin (g/L)		n	CV (%) (95 % confidence interval)
		Assigned*	Found*		
5C Coulter IQAP, Normal	2000.10.18 – 11.17 2000.11.17 – 12.13	155 – 160 – 165 156 – 161 – 166	157 – 159,8 – 162 159 – 160,8 – 163	42 40	0,7 (0,6 – 0,9) 0,7 (0,6 – 0,9)
5C Coulter IQAP, Abnormal I	2000.10.18 – 11.15 2000.11.17 – 12.13	126 – 130 – 134 127 – 131 – 135	128 – 129,9 – 132 131 – 132,5 – 134	44 48	0,6 (0,5 – 0,7) 0,7 (0,6 – 0,9)
5C Coulter IQAP, Abnormal II	2000.10.18 – 11.16 2000.11.17 – 12.13	48 – 50 – 52 49 – 51 – 53	50 – 50,4 – 52 49 – 51,0 – 52	38 36	1,1 (0,9 – 1,4) 1,2 (1,0 – 1,6)
Streck Para 4, Low	2000.10.18 – 12.13	54 – 60 – 66	59 – 60,5 – 62	40	1,2 (1,0 – 1,5)
Streck Para 4, Normal	2000.10.18 – 12.13	119 – 125 – 131	124 – 126,0 – 129	39	0,9 (0,8 – 1,2)

* The declared haemoglobin concentration is given with three values by the manufacturer; low limit for acceptance, expected mean, high limit for acceptance and the found haemoglobin concentration is also given with three values; lowest found result, mean of found results and highest found result.

The Department of Clinical Chemistry participates in a proficiency testing scheme provided by EQUALIS. All results in the scheme during the period of the premarketing evaluation are shown in table 4. The samples in this scheme have no assigned values determined with a reference method, but the overall mean of the 240 participating laboratories are shown in the table.

Table 4. Results from Coulter GenS in the Department of Clinical Chemistry, Malmö University Hospital, in EQUALIS proficiency testing scheme.

Date	Mean of all laboratories B–Haemoglobin (g/L)	Results from Coulter GenS in Malmö B–Haemoglobin (g/L)
2000 10 04	164	162
2000 11 01	149	148
2000 11 29	157	156
2000 12 20	120	120

Coulter GenS comparison method product information.

Instrument ..... Coulter GenS System, from Beckman Coulter, serial no. AB 39381.
Reagents ..... from Beckman Coulter Euro Diagnostics GmbH
Calibrator ..... S-CAL Kit
from Coulter Electronics LTD, PN 7547026, lot no 5755

Internal control..... 5C Coulter IQAP,
from Beckman Coulter, article no. 754719,
levels: Normal, lot no 88 44 00,
Abnormal I, lot no 87 17 00 and
Abnormal II, lot no 86 19 00.

Extra internal control..... Streck Para 4,
manufactured by Streck Laboratories Inc, US,
delivered by Electra-Box Diagnostica AB, Sweden, article no. 215406,
levels: Low, lot no 024 800 65 and
Normal, lot no 024 800 66

DiffSafe

DiffSafe from Alpha Scientific Corp, US; product designation: B-D 366005

Evaluation procedures

Three Biotest Hemoglobin Testers were used in this evaluation. Before the evaluation started the Testers were checked for giving similar results. Five patient samples with venous EDTA blood with different concentrations in the interval 50 – 200 g/L were selected, and each sample was drawn into three Biotest cuvettes. All 15 cuvettes were read in all three Biotest Testers. The mean values of all nine determinations for each sample were calculated. No single value should differ more than ±3 g/L from the mean value. No result exceeded this required limit and all three Biotest Testers were accepted.

The Biotest Testers were checked daily during the evaluation period with special control cuvettes assigned target values by the manufacturer.

As an internal control for Biotest, the same Streck Para 4 as used for the GenS comparison method was measured daily at two levels.

Evaluation under standardised conditions in the Department of Clinical Chemistry
To investigate imprecision and linear agreement, patient blood samples already measured in routine with the GenS comparison method were used. These samples were collected in 3 mL EDTA tubes (Vacuette, Greiner). Only properly handled tubes were used. Samples were selected to cover the whole measuring range of Biotest, including samples with really low and high haemoglobin concentrations;
five samples with B–Haemoglobin values between 30 – 80 g/L,
five samples between 170 – 200 g/L and
five samples between 200 – 250 g/L.
A total of 105 samples from 105 patients were measured in twenty days during the period 2000-10-18 – 2000-11-29.

The EDTA tubes were mixed for at least ten minutes before the Biotest measurements.
To transfer the sample from the tube to the Biotest cuvette the technique that is described in the manual for Biotest was used: The stopper in the EDTA tube was replaced with a device, DiffSafe. This makes it possible to safely drip blood from the tube. With DiffSafe a drop of blood was put on a hydrofobic film. From this drop the sample was sucked by capillary force into the Biotest cuvette. The cuvette was read in the Biotest Tester within 10 minutes. Analyses were done in duplicates.

If the difference was more than 6 g/L between Biotest and the GenS comparison method a printout was made from the Coulter GenS. This printout contains all parameter from the measurement and a scatter diagram showing different cell populations. This was done to make further investigation of deviating results possible.

After the Biotest measurements, the samples were measured a second time with the GenS comparison method. The measurements with the comparison method and Biotest were performed within the same day.

To estimate the between-day variation the EDTA tubes were measured once again with Biotest on the following days. As Biotest is not calibrated between-series or between-days, the between-day variation was expected to be the same as the within-series variation. Accordingly the between-day variation was measured on a limited series of samples, namely the first 20 patient samples measured

in the Department of Clinical Chemistry. Ten samples were measured at Day 1, five of these were measured again at Day 2 and another five were measured again at Day 3. This procedure was repeated other days with another ten samples.

Evaluation in primary care
In each of the two Primary Care Centres 40 outpatients were randomly chosen for sample collection during five days over the period 2000-11-02 – 2000-12-13. The patients were first given written information about the evaluation. Only patients consenting to the extra sample collection participated in the evaluation. The patients visited the doctor before samples were taken. All patients had rested in sitting position before sample collection, but the period of sitting varied. The capillary samples were collected before the venous ones.
Capillary samples were taken after a fingerprick.
To do the capillary puncture Haemolance® from Haemedic AB, Box 116, SE-226 21 Munka Ljungby, Sweden was used in both primary care centres. Two different models were used:
1. Normal flow, stick depth 1,8 mm, article number 50115
2. Low flow, stick depth 1,8 mm, article number 50125
Primary Care Centre A in most cases used the Low flow model and Primary Care Centre B in most cases used the Normal flow model.
The two or three first drops of blood were wiped off. Duplicate samples were taken from the same capillary puncture. After the first sample was taken, the blood was wiped off and a new drop of blood was used for sample number two.
The Biotest cuvette was read in the Biotest Tester within 10 minutes.

Venous samples were drawn in 3 mL EDTA tubes (Vacuette, Greiner). The measurements with Biotest were done in the same way as in the Department of Clinical Chemistry.
After the Biotest analysis the EDTA tube was transported to the Department of Clinical Chemistry. The samples were stored in a refrigerator until analysis with the comparison method the next day.

If the difference was more than 6 g/L between Biotest and the GenS comparison method a complete printout of the measurement was made from the Coulter GenS. This was done to make further investigation of deviating results possible.

ANALYTICAL QUALITY SPECIFICATIONS

Analytical quality specifications based on biological variation

The SKUP procedure includes a specification of the desirable analytical quality for the investigated method. There are no generally agreed analytical quality specifications for the precision of the B–Haemoglobin analysis or for the acceptable difference between results from capillary and venous blood.

Models to derive goals for analytical quality based on biological variation are gaining increasing acceptance [2]. From data on biological variation 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 M Á Sebastián-Gámbaro et al [3] estimate the within-subject-CV to 3,4 %, and the between-subject-CV to 6,2 %. C. Ricos et al [4] 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 investigated method and the comparison method is completely explained by error in the investigated method only if a comparison method have no error. In this evaluation, the effects of imprecision and bias in the comparison method also have to be considered. As will be shown later, the within-series-imprecision of the comparison method varies between CV 0,7 % and 1,2 % for different control materials. Calculated from duplicate values with patient samples the within-series-imprecision varies between CV 0,7 % and 0,5 %.

To compensate for the errors in the comparison method in this study the desirable-total-error is expanded. 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 % ($p<0,05$). Our analytical quality goals were therefore in this study 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.

B–Haemoglobin concentrations in capillary samples compared to venous samples

The literature presents few and contradictory statements on the difference in haemoglobin concentration in capillary and venous blood.

Some authors argue that the average concentration of haemoglobin is higher in capillary samples than in venous samples. For example, Daae et al [5] [6] compared the haematological parameters in capillary and venous samples from 40 healthy adult volunteers of both sexes and reported the following figures:

Table 5. Expected difference in B–Haemoglobin results from Daae et al

Analyte	Range (g/L)	Mean value (g/L)	n	Mean of differences capillary - venous (g/L)	(%)	Range of differences capillary - venous (%)
Capillary Haemoglobin	97 – 172	137,6	40	+3,2	+2,4	-9,2 – +10,3
Venous Haemoglobin	98 – 172	134,4	40			

The capillary samples were collected after finger puncture with the device Autolet® equipped with the "Neonatal Unilet Lancet"® supplied by Mölnlycke A/S, Division Medical, Oslo 6, Norway. The first drop of blood was discarded. The next drops were collected in Microtainer® tubes with dry EDTA. The tube was filled by freely flowing blood to the 250 µL mark.

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 %. Their reported difference should therefore be corrected to +2,0 %.

In another report [7] by Hersleth and Daae the mean of the capillary results was -0,6 % compared to the venous results. This calculation is based on values from 20 persons only. The capillary samples were collected from the 3:rd or 4:th drop of blood after finger puncture with "Minilancet" manufactured by C C S, Clean Chemical Sweden AB, Tunavägen 277 B, 781 73 Borlänge, Sweden. The type of collection tube for the venous samples is not stated.

D.W. Pi et al [8] have measured both capillary and venous samples on 174 female blood donors in childbearing age. They show that capillary samples are more likely to have higher haemoglobin concentration than venous samples, on average +3,2 g/L, but with large individual differences. The standard deviation of the differences was as high as 7,8 g/L.
The 95 % confidence interval for the mean difference was +2,0 to +4,4 g/L.
The type of collection tube for the venous samples is not stated.
The capillary samples were collected after a fingerprick done with a spring-loaded lancet device (Microcontainer, Becton Dickinson, Rutherford, NJ, USA).

Sfez M et al have found [9] that capillary results on average were -2,7 g/L compared to the venous results with the 95 % confidence interval -5,7 to +0,3 g/L. The type of lancet used for the capillary punctures and the type of collection tube for the venous samples was not stated.

B–Haemoglobin determinations are expected to have higher imprecision with capillary samples than with venous samples [7] [10]. Regardless of the analytical method used, it requires more skill of the sample collector to obtain reproducible results from capillary samples than from venous.

RESULTS AND DISCUSSION

During the evaluation period the Biotest Testers were checked daily with special control cuvettes with target values assigned by the manufacturer. All the values were within the specified limits.

As internal controls for Biotest the same Streck Para 4 as for the GenS comparison method were measured daily at two levels. All controls (except one outlier commented later) were within the acceptance limits specified by the manufacturer.

Evaluation in a department of clinical chemistry using venous samples

Internal quality control results

The results from internal quality control with Biotest are shown in table 6. One outlier was excluded in the calculations because of obvious reasons – the control container was almost empty and the content was dried.
The raw data are given in Attachment 2.

Table 6. Internal quality control results obtained with Biotest in the Department of Clinical Chemistry

Control name	B–Haemoglobin (g/L)		n	CV (%) (95 % confidence interval)
	Assigned value	Average result		
Streck, Para 4, Low	64 ±6	66,5	19	2,5 (1,9 – 3,7)
Streck, Para 4, High	164 ±7	164,8	19	1,0 (0,8 – 1,5)
Control cuvette	115 ±3	114,7	20	0,4 (0,3 – 0,6)

The results from internal quality control with Biotest are satisfying.

Within-series imprecision

Biotest within-series imprecision was calculated from the differences between duplicate determinations of venous EDTA samples drawn from 105 patients at Malmö University Hospital. Before the calculation the values were divided into three level groups according to the haemoglobin concentration. The differences were tested for outliers in each group according to Burnett [11]. This excludes duplicate values with differences higher than 0 ±3,02 SD when n = 20, and higher than 0 ±3,33 SD when n = 60. No outliers were found in this case.
The same calculation was made for the comparison method and one outlier was excluded.
The within-series imprecision for the GenS comparison method was, expressed as CV-values, 0,7 %, 0,4 % and 0,5 % in the low, medium and high level group.
The raw data are given in Attachment 3.

Table 7. Within-series imprecision.
Venous samples in the Department of Clinical Chemistry

Level group	B–Haemoglobin Interval (g/L)	B–Haemoglobin Mean value (g/L)	n	CV (%) (95 % confidence interval)
Low	37 – 99	75,7	21	0,7 (0,5 – 1,0)
Medium	100 – 149	127,3	59	0,7 (0,6 – 0,8)
High	150 – 229	178,3	25	0,6 (0,5 – 0,9)
All	37 – 229	129,1	105	0,7 (0,6 – 0,8)

Between-day imprecision
The between-day imprecision for Biotest has been calculated from the results of duplicate determinations of venous EDTA samples drawn from 20 patients at Malmö University Hospital. The between-day imprecision in table 8 includes the within-series imprecision.
Raw data are given in Attachment 4.

Table 8. Between-day imprecision in the Department of Clinical Chemistry

B–Haemoglobin Interval (g/L)	B–Haemoglobin Mean value (g/L)	n	CV (%) (95 % confidence interval)
68 – 167	132,1	20	1,2 (0,9 – 1,7)

Linear agreement
The linear agreement is calculated with the mean values of duplicate results obtained with Biotest and the comparison method on 100 venous EDTA samples. The samples were routine samples sent to the Department of Clinical Chemistry.

If the difference between Biotest and GenS had been more than 6 g/L a complete printout of the measurement was planned to be done from the Coulter GenS. This could make further investigation of deviating results possible. However, no such results occurred.

Before the calculation of the linear agreement a test according to Burnett was done, whether any value showed a residual higher than 0 ±3,47 SD. No such outlier was found. Mean values from duplicate Biotest measurements were compared with mean values from duplicate measurements with the comparison method. Only values below 200 g/L were included in this calculation as the specification from the manufacturer for linearity is more tolerant above 200 g/L.
Raw data are given in Attachment 3.

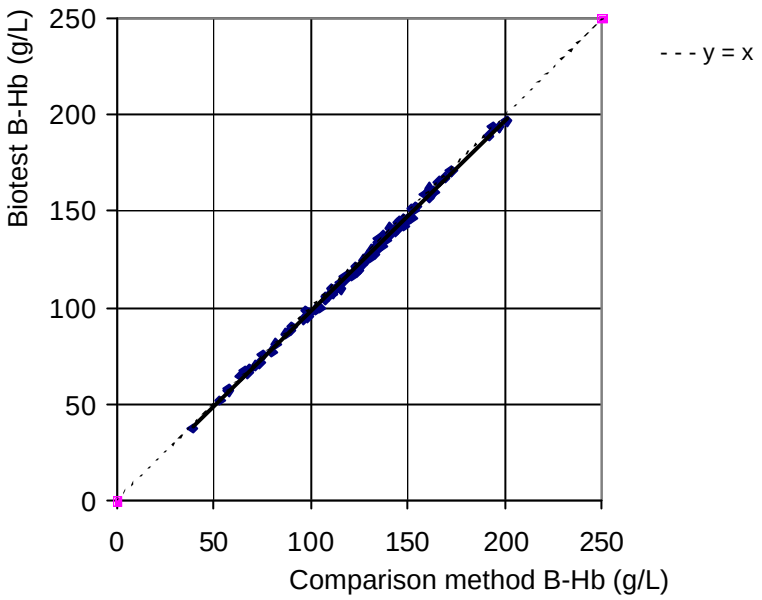

Figure 3. Biotest results from the Department of Clinical Chemistry compared with GenS comparison method results. Both methods with venous samples. Mean values from duplicates. n = 100

Table 9. Calculation of linear agreement.
Venous samples in the Department of Clinical Chemistry.

Parameter	**Calculation**
Equation	y = 0,989x - 1,1
Determination coefficient r^2 (95 % confidence interval)	1,00 (1,00 – 1,00)
Standard error, SE for residuals	1,6
Standard error in calculation of the slope of the regression line, SEa	0,005
Standard error in calculation of the intercept, SEb	0,6
Slope of the regression line (95 % confidence interval)	0,989 (0,980 – 0,999)
Intercept (95 % confidence interval)	-1,1 (-2,3 – +0,2)

Bias

The bias for Biotest compared to the comparison method, for venous EDTA samples, was calculated from 105 duplicate results from 105 patients. The samples were selected among samples sent to the Department of Clinical Chemistry. Mean values from duplicate measurements with Biotest were compared with the mean values from duplicate measurements with the comparison method. In table 10 the values are grouped together into three level groups according to the B–Haemoglobin concentration.

Raw data are given in Attachment 3.

Table 10. Bias. Venous blood in the Department of Clinical Chemistry

Level group	B–Haemoglobin Interval (g/L)	B–Haemoglobin Mean value (g/L)	n	Mean difference Biotest – Comparison method (95 % confidence interval) (g/L)
Low	37 – 99	75,7	21	-1,2 (-1,8 – -0,6)
Medium	100 – 149	127,3	59	-3,0 (-3,4 – -2,7)
High	150 – 229	178,3	25	-2,6 (-3,5 – -1,8)
All	37 – 229	129,1	105	-2,6 (-2,9 – -2,2)

The effect of both imprecision and bias, from the comparison method, on the results from Biotest in the Clinical Chemistry Department is illustrated with the diagram in figure 4.

Venous samples were measured with both Biotest and the GenS comparison method. The x-axis shows the average value of duplicates with the comparison method. The y-axis shows the deviations in percent between the first single value with Biotest and the average value of duplicates with the comparison method. The negative bias expressed in percent is about the same for low and high values. In such a cases it is preferable to let the y-axis in the diagram show the deviations in percent. The tolerance limits, ±5 %, are derived from biological variation as described earlier. The limits are shown as dotted lines in the diagram.
Raw data are given in Attachment 3.

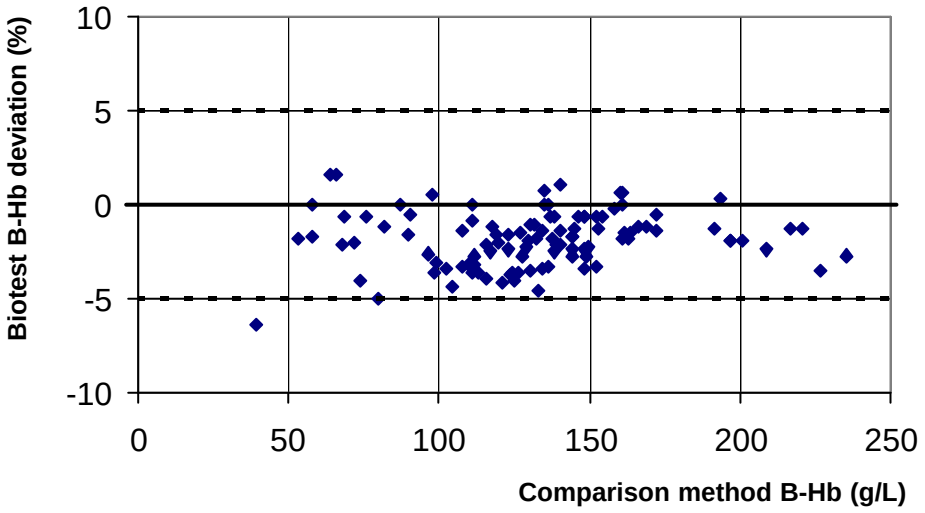

Figure 4. Diagram of deviations.
The y-axis shows the deviation of the Biotest result from the GenS comparison method, expressed in percent of the comparison method result.
Venous samples in the Department of Clinical Chemistry. n = 105
The tolerance limits, ±5 %, are shown as dotted lines.

Valuation of the testing with venous samples in the Department of Clinical Chemistry.

Table 7 shows that the within-series imprecision for Biotest with venous EDTA samples in the Clinical Chemistry Department was good. The CV was around 0,7 % in all level groups of results.

Table 8 shows that the between-day imprecision for Biotest with venous samples in the Clinical Chemistry Department was good. The CV was 1,2 % in our limited series of result. This between-day imprecision includes the within-series imprecision. There was almost no difference in B–Haemoglobin level of the results measured on the first day, mean 131,8 g/L and on following days, mean 131,7 g/L.

The xy-diagram in figure 3 and the linear agreement calculations in table 9 show that the linear correlation between Biotest and the comparison method is good with $r^2 = 1,00$.
The slope of the regression line, 0,989, is significantly different from 1, as 1 is not included in the confidence interval. Note that this small difference from 1 is statistically significant entirely because of low imprecision and high linear correlation.
The intercept, -1,1 g/L, is not significantly different from 0 g/L.

Table 10 also shows that the Biotest results with venous samples on average are 2,6 g/L lower than those obtained with the comparison method. This tendency holds at all B–Haemoglobin concentrations, as shown in the table where the patient values are grouped according to B–Haemoglobin concentrations.

The diagram of deviations in figure 4 confirms that, using the venous samples, the Biotest results were systematically, around 1,9 % lower than those of the comparison method. The negative bias expressed in percent is about the same for low and high values. This is supported by the fact that the slope of a trend line (not drawn in the figure), through the measurement points in the graph, is not significantly different from 0. In cases when the deviation is proportional to the concentration it is preferable to show the deviations in the diagrams in percent.

Together, our findings indicate that the Biotest results, with venous samples, are somewhat lower than those with the comparison method but these small differences have no importance in clinical use.

As described earlier, our analytical quality goals are derived from biological variation to a total error of less than ±5 %. These tolerance limits are shown as stippled lines in the deviation diagrams. Only one out of 105 results were outside these tolerance limits. 104 of 105, or 99 % of the results were within the tolerance limits, which means that the Biotest results obtained with venous samples in the Clinical Chemistry Department fulfil our quality goals.

Evaluation in primary care centres using venous samples

Imprecision

Within-series variation was calculated from the results of duplicate determinations with venous samples from 40 patients in each of the two Primary Care Centres.
In the calculation the values were divided into two level groups according to the B–Haemoglobin concentration. The differences were tested for outliers in each level group according to Burnett. In this case it means that duplicate values with differences higher than 0 ±3,02 SD would have been excluded. However, no outliers were found.
Raw data are given in Attachments 5 and 6.

Table 11. Within-series imprecision. Venous samples in Primary Care Centre A and B.

Level group	**B–Haemoglobin Interval (g/L)**	**B–Haemoglobin Mean value (g/L)**	**n**	**CV (%) (95 % confidence interval)**
Primary care centre A:				
Low	112 –132	124,6	20	0,8 (0,6 – 1,1)
High	133 –170	149,4	20	0,6 (0,4 – 0,8)
All	112 –170	137,0	40	0,7 (0,5 – 0,9)
Primary care centre B:				
Low	95 –134	120,8	20	0,3 (0,2 – 0,4)
High	135 –165	144,3	20	0,4 (0,3 – 0,6)
All	95 –165	132,6	40	0,4 (0,3 – 0,5)

Linear agreement

The linear agreement between Biotest and the comparison method both using venous samples from the Primary Care Centres A and B was calculated in the same way as described for the Department of Clinical Chemistry. The samples were collected and analysed with Biotest in the Primary Care Centres and subsequently sent to the Department of Clinical Chemistry for measurements with the comparison method.

If the difference between Biotest and the GenS comparison method exceeded 6 g/L a printout of all data from the measurement was taken from the Coulter GenS in order to seek the cause. Six venous samples from the Primary Care Centres exceeded this limit. However, the printout from the GenS could not explain any of these deviating results.

In this case the outlier test according to Burnett would have excluded any value with a residual higher than 0 ±3,22 SD. However, no such outlier was found.
Raw data are given in Attachments 5 and 6.

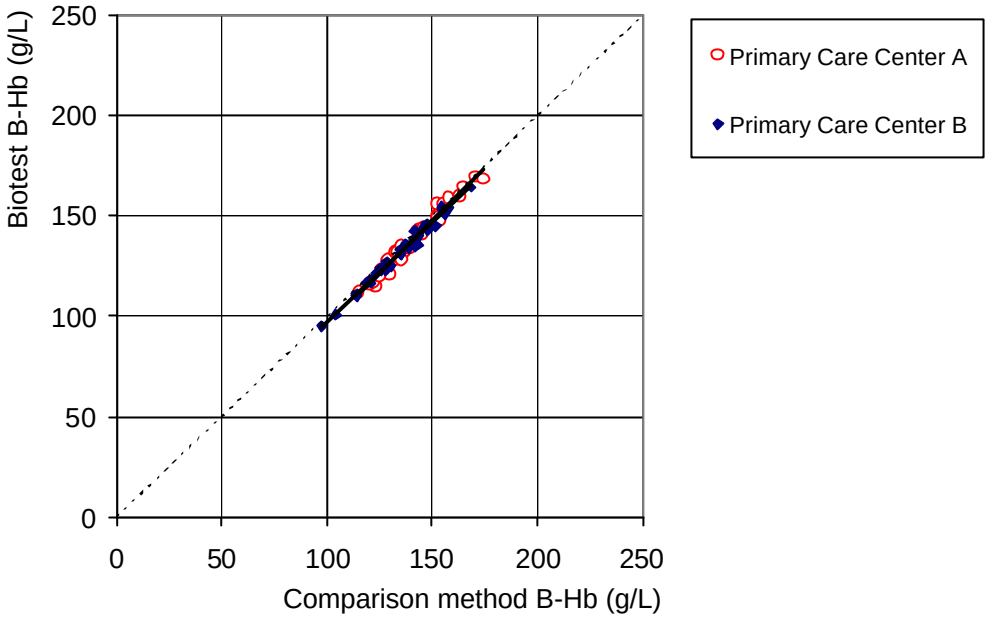

Figure 5. Biotest venous sample results in Primary Care Centre A and B are compared with GenS comparison method venous sample results. Mean values from duplicates. n = 80

Table 12. Calculation of linear agreement.
Venous samples in the two primary care centres.

Parameter	**Calculation**	
	Primary care centre A	Primary care centre B
Equation	y = 1,057 x - 10,7	y = 0,984 x - 1,2
Determination coefficient r^2 (95 % confidence interval)	0,98 (0,96 – 0,99)	0,99 (0,97 – 0,99)
Standard error, SE for residuals	2,36	1,83
Standard error in calculation of the slope of the regression line, SEa	0,026	0,019
Standard error in calculation of the intercept, SEb	3,7	2,7
Slope of the regression line (95 % confidence interval)	1,057 (1,004 – 1,110)	0,984 (0,945 – 1,023)
Intercept (95 % confidence interval)	-10,7 (-18,1 – -3,2)	-1,2 (-6,6 – +4,2)

Bias

The bias for venous sample results obtained with Biotest compared to those obtained with the comparison method was calculated from mean values of duplicate determinations on venous samples. EDTA samples from 40 patients were collected in each of the two Primary Care Centres for this purpose.

The calculations were carried out in the same way as described for the Department of Clinical Chemistry.

Raw data are given in Attachments 5 and 6.

Table 13. Bias. Venous blood in Primary Care Centre A and B.

Level group	**B–Haemoglobin Interval (g/L)**	**B–Haemoglobin Mean value (g/L)**	**n**	**Mean difference** **Biotest - Comparison method (95 % confidence interval) (g/L)**
Primary care centre A:				
Low	112 –132	124,6	20	-3,7 (-4,7 – -2,7)
High	133 –170	149,4	20	-1,7 (-2,9 – -0,6)
All	112 –170	137,0	40	-2,7 (-3,5 – -1,9)
Primary care centre B:				
Low	95 –134	120,8	20	-3,2 (-3,7 – -2,6)
High	135 –165	144,3	20	-3,5 (-4,6 – -2,4)
All	95 –165	132,6	40	-3,4 (-3,9 – -2,8)
Primary care centre A and B:				
All	95 –170	134,8	80	-3,0 (-3,5 – -2,6)

The deviation diagram below shows the effect of both bias and imprecision for the results obtained with Biotest versus those obtained with the comparison method using 80 venous samples from the two Primary Centres. The diagram is drawn according to the same principles as described for results from the Department of Clinical Chemistry.
Raw data are given in Attachments 5 and 6

.

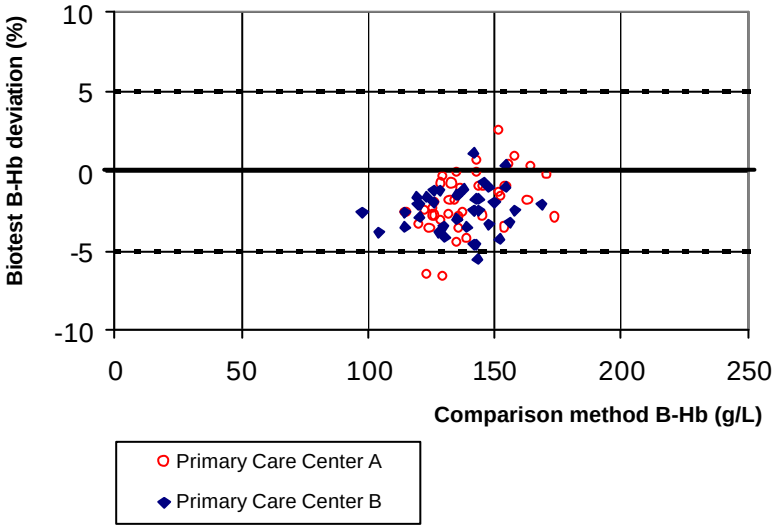

Figure 6. Diagram of deviations. Biotest venous sample results in Primary Care Centre A and B are compared with GenS comparison method venous sample results. n = 80.
The y-axis shows the deviation of the Biotest result from the comparison method, expressed in percent of the comparison method result.
The tolerance limits are shown as dotted lines.

Valuation of the testing with venous samples in Primary Care Centre A and B.
The Biotest results obtained with venous samples in Primary Care Centre A and B are similar to the results in the Department of Clinical Chemistry.

Table 11 shows that using venous samples the within-series imprecision for Biotest in Primary Care Centre A was as good as, and in Primary Care Centre B even better, than, that in the Department of Clinical Chemistry. The CVs were 0,7 % and 0,4 % in Primary Care Centres A in and B, respectively. The CV-values were about the same in different level groups of results.

The xy-diagram in figure 5 and the linear agreement calculations in table 12 show that the linear correlation between Biotest and the comparison method was good with r^2 = 0,98 and 0,99.
The slopes of the regression lines are 1,057 and 0,984. The first is significantly different from 1 but not the second.
The intercepts are -10,7 and -1,2 g/L. The first is significantly different from 0 g/L but not the second.
The figures indicate that using venous samples Biotest results are somewhat lower than those of the comparison method.
The regression line for Primary Care Centre A differs somewhat from that for the Clinical Chemistry department. It has a bigger negative intercept together with a steeper slope. The reason is not clear but one explanation could be that the values from the Primary Care Centres – in contrast to the Clinical Chemistry Department – have a narrow range with most of the values inside the reference interval for B–Haemoglobin. This causes a big uncertainty in the calculation of slope and intercept and this uncertainty becomes visible as large confidence intervals. Expressed otherwise, the slope of regression lines can easily be affected by just a few results deviating in the same direction.

Table 13 confirms that using venous samples Biotest results in Primary Care Centres A and B were on the average 3,0 g/L lower, than those obtained with the comparison method. There were similar differences at both haemoglobin levels when the patient values were grouped according to B–Haemoglobin concentrations.

The deviation diagram figure 6, shows that using venous samples the Biotest results in Primary Care Centres A and B were systematically lower, on the average 2,2 % than those of the comparison method. The negative bias expressed in percent was about the same for low and high values. This was supported by the fact that the slope of a trend line (not drawn in the figure), through the measurement points in the graph, is not significantly different from 0.
As described earlier in the present report, our analytical quality goals are derived from biological variation and estimated to a total error of less than ±5 %. These tolerance limits are shown as stippled lines in the deviation diagrams. Only three out of 80 results were outside these tolerance limits. 96 % of the results were within the tolerance limits, which means that the Biotest results obtained with venous samples fulfil our quality goals in the two Primary Care Centres.

Evaluation in primary care centres using capillary samples

In this part of the report, capillary B–Haemoglobin results obtained with Biotest are compared with venous results from the comparison method. During the evaluation, it became clear that this comparison would be highly affected by the preanalytical error in capillary samples. These errors are not depending on which instrument the measurements are done with. Please keep this in mind when reading the results from capillary samples in this report. The general problems with capillary samples are sorted out in next chapter of this report.

Imprecision

Within-series variation was calculated from duplicate determinations of capillary samples from 40 patients in each of the two Primary Care Centres. Each duplicate was collected from the same capillary puncture. Before the calculation the values from each Primary Care Centre were divided into two level groups according to the haemoglobin concentration. The differences were tested for outliers in each level group according to Burnett. This would exclude duplicate values with differences higher than 0 ±3,02 SD. However, none such outlier was found. Raw data are given in Attachments 5 and 6.

Table 14. Within-series imprecision. Capillary samples in Primary Care Centre A and B.

Level group	**B–Haemoglobin Interval (g/L)**	**B–Haemoglobin Mean value (g/L)**	**N**	**CV (%)** **(95 % confidence interval)**
Primary care centre A:				
Low	110 – 139	130,0	20	3,8 (2,9 – 5,5)
High	140 – 175	156,6	20	2,3 (1,7 – 3,3)
All	110 – 175	143,3	40	3,0 (2,4 – 3,8)
Primary care centre B:				
Low	99 – 141	124,6	20	1,1 (0,9 – 1,7)
High	142 – 171	150,0	20	0,7 (0,5 – 1,0)
All	99 – 171	137,3	40	0,9 (0,7 – 1,2)

Linear agreement

The linear agreement data on the results from capillary samples in Primary Care Centre A and B was calculated in the same way as described for the Department of Clinical Chemistry. Raw data are given in Attachments 5 and 6. In this case the outlier test according to Burnett excludes any values with a residual higher than 0 ±3,22 SD. No such outlier was found. Before comparison the raw values for venous blood obtained with the comparison method were corrected for dilution in the collection tubes. The tubes contain liquid EDTA, which causes a dilution of 3,8 %.

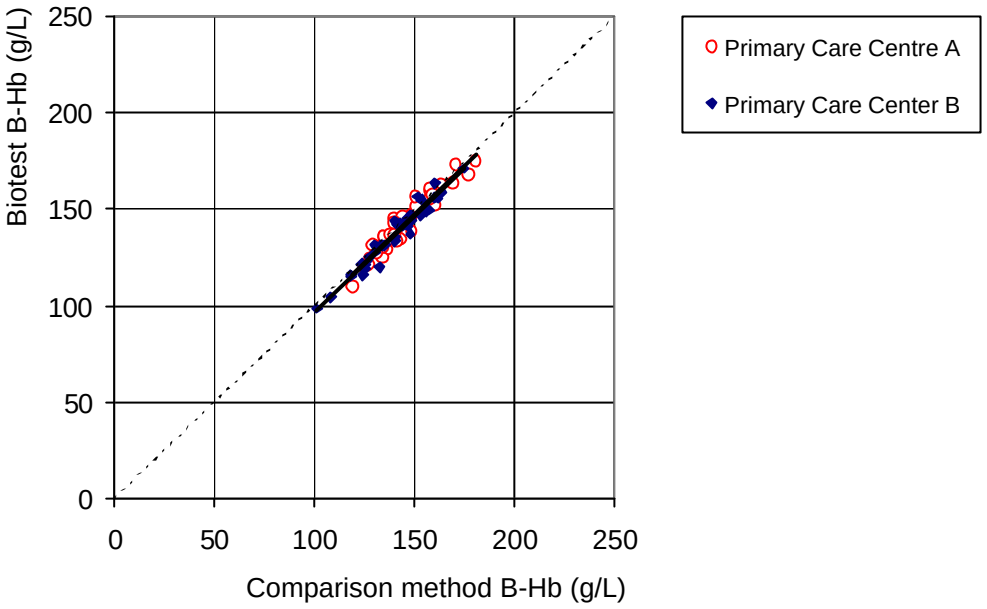

Figure 7. Capillary Biotest results in Primary Care Centres A and B compared with venous GenS comparison method results. Mean values from duplicates. n = 80

Table 15. Calculation of linear agreement. Capillary samples in Primary Care Centre A and B.

Parameter	**Calculation**	
	Primary care centre A	Primary care centre B
Equation	y = 1,017 x - 5,0	y = 0,999 x - 3,2
Determination coefficient r^2 (95 % confidence interval)	0,93 (0,86 – 0,96)	0,95 (0,91 – 0,97)
Standard error, SE for residuals	4,36	3,58
Standard error in calculation of the slope of the regression line, SEa	0,047	0,037
Standard error in calculation of the intercept, SEb	6,8	5,2
Slope of the regression line (95 % confidence interval)	1,017 (0,923 – 1,112)	0,999 (0,925 – 1,073)
Intercept (95 % confidence interval)	-5,0 (-18,8 – +8,7)	-3,2 (-13,7 – +7,3)

Bias

The bias for Biotest results with capillary samples, compared to those of the comparison method with venous samples, was calculated from mean values of duplicate determinations. Samples from 40 patients were collected in each of the two Primary Care Centres for this purpose. Before comparison the raw values for venous blood with the comparison method were corrected for dilution in the collection tubes. The tubes contain liquid EDTA, which causes a dilution of 3,8 %. The further calculations were done in the same way as described for the Department of Clinical Chemistry.

Table 16. Bias. Capillary blood in Primary Care Centre A and B.

Level group	**B–Haemoglobin Interval (g/L)**	**B–Haemoglobin Mean value (g/L)**	**n**	**Mean difference** **Biotest - Comparison method (95 % confidence interval) (g/L)**
Primary care centre A:				
Low	110 – 139	130,0	20	-4,3 (-6,1 – -2,6)
High	140 – 175	156,6	20	-0,8 (-2,7 – +1,2)
All	110 – 175	143,3	40	-2,6 (-3,9 – -1,2)
Primary care centre B:				
Low	99 – 141	124,6	20	-4,5 (-6,0 – -3,0)
High	142 – 171	150,0	20	-2,3 (-4,0 – -0,7)
All	99 – 171	137,3	40	-3,4 (-4,5 – -2,3)
Primary care centre A and B:				
All	99 – 175	140,3	80	-3,0 (-3,9 – -2,1)

The effect of both imprecision and bias on the results from Biotest in the two Primary Care Centres is illustrated in figure 8. Biotest measured capillary samples and the comparison method venous samples. The diagram is drawn according to the same principles as described for results from the Department of Clinical Chemistry.
Raw data are given in Attachments 5 and 6.

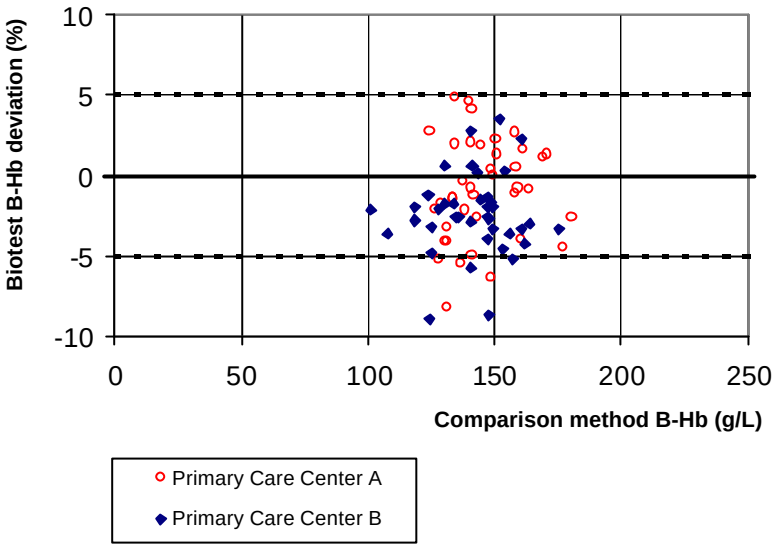

Figure 8. Diagram of deviations. Biotest results from capillary samples in Primary Care Centres A and B are compared with GenS comparison method results from venous samples. n = 80.
The y-axis shows the deviation of the Biotest result from the comparison method, expressed in percent of the comparison method result.
The tolerance limits are shown as dotted lines.

Valuation of the testing with capillary samples in Primary Care Centre A and B.

The within-series imprecision is calculated from duplicate values. The samples in each duplicate were collected from the same capillary puncture. It could be assumed that these CV-values would be lower than if the samples had been collected from two different collection sites.

Table 14 shows that using capillary samples the Biotest within-series imprecision in Primary Care Centres A and B was acceptable. The CV-values were on average 3,0 % for Primary Care Centre A and 0,9 % for Primary Care Centre B. The CV-values tended to be higher in the low level range of results. This shows that proper capillary sampling is difficult and that the imprecision can vary depending on the type of lancet used, sampling technique and the skill of the sample collector. The type of lancet used is the most probable explanation for our different imprecision results. Primary Care Centre A used a "low flow" lancet, which normally is used for collection of B-Glucose samples while Primary Care Centre B used a "normal flow" lancet normally used for collection of B-Haemoglobin samples.

The xy-diagram in figure 7 and the linear agreement calculations in table 15 show that the linear correlation between Biotest and the comparison method was less good than with venous samples, with $r^2 = 0,93$ and 0,95. Notice that the imprecision in Primary Care Centre B was low, as when using venous samples, but the linear correlation to the comparison method was less good, $r^2 = 0,95$ instead of 0,99. The explanation is given under the headline "Experience 2" in the next chapter of this report.
The slopes of the regression lines, 1,017 and 0,999, were not significantly different from 1. The intercepts, -5,0 and -3,2 g/L were not significantly different from 0 g/L.

Table 16 shows that the Biotest results from capillary samples were lower than those obtained with the comparison method using venous samples. The difference was on the average 3,0 g/L. Grouping according to B–Haemoglobin concentrations shows a tendency toward lower bias at the high level.

The deviation diagram, figure 8, shows that the Biotest results obtained with capillary samples in Primary Care Centre A and B were scattered when compared with venous sample results from the comparison method. There is a large individual variation. The capillary results are for some individuals higher and for others lower than the corresponding venous results.
The causes of these deviations are examined and explained in the next chapter of this report.

The diagram also shows that the Biotest results obtained with capillary samples were lower than those of the comparison method with venous samples. The difference was on average 1,9 %. The negative bias expressed in percent was about the same for low and high values. This was supported by the fact that the slope of a trend line (not drawn in the figure), through the measurement points in the graph, was not significantly different from 0.

As described earlier in the present report the analytical quality goals derived from biological variation were set to a total error of less than ±5 %. These tolerance limits are shown as stippled lines in the deviation diagrams. 11 out of 80 results or 14 % of the results were outside the tolerance limits. All these were below the low limit and no results were above the high limit. As the tolerance limits are calculated with 95 % confidence interval these results do not fulfil our analytical quality goals.

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 %. 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 (g/L)**	**(%)**	**Range of differences capillary - venous (%)**
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			

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.
For example 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 +3,2 , +2,0 , -0,6 and -2,7 % respectively. Only in one case, +2,0 %, we know that the difference have been calculated after correction for the dilution of the venous samples. The other references are 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 even when the capillary samples are collected from the same puncture. The CV 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.

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.

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.

PRACTICAL POINTS OF VIEW

During this premarketing evaluation only an English manual was available. The manual contains too much text, which at places makes it difficult to understand.

Points of view expressed during the practical use of Biotest are shown below in positive and negative comments.

Positive comments

- The system is easy to use.
- Results are quickly shown and easy to read.
- The small size of the instrument is space-saving in the laboratory.
- Hygienic.
- A minimum of maintenance is needed.
- All the laboratory staff summarised their judgement of Biotest and concluded that the system is quick and easy to use.

Negative comments

- It was sometimes difficult to fill the cuvette.*
- Annoying signal from the Tester.

* This comment comes from Primary Care Centre A only, where they used the “low flow” lancets to do the capillary puncture. The difficulties to fill the cuvette were noticed just a few times. It looked like the cuvettes were not completely filled. These cuvettes were read in the Biotest Tester and the results from those measurements are included in this report.

REFERENCES

1 "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).

2 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

3 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

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

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.

11 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.

Comment from Biotest Medizintechnik GmbH on the report
"PREMARKETING EVALUATION OF THE BIOTEST HEMOGLOBIN MEASURING SYSTEM"

Mail to SKUP dated 2002-02-06:

Biotest Medizintechnik GmbH has adjusted the routine for calibration of the Biotest photometers. The new procedure was implemented before the launch of the system in Scandinavia. The adjustment corresponds to 1,5 % higher haemoglobin values, which is in accordance with the SKUP evaluation results.

Alexander Velther
Quality Manager
Biotest Medizintechnik GmbH