

Comparison of an electrochemical biosensor with optical devices for hemoglobin measurement in human whole blood samples

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ABSTRACT

Background: An electrochemical based biosensor for hemoglobin measurement was developed as an alternative to the traditional optical method, and underwent testing for use in professional settings.

Methods: The affects of samples' freshness, hemolysis, bilirubin on the electrochemical method, as well as the repeatability, precision and accuracy were studied, using optical method devices as references.

Results: Samples were stored at room temperature or in a cold environment for 7 days, partially or completely hemolyzed samples, and samples containing bilirubin with a concentration of up to 150 mg/l were investigated with no effects for interfering studies. Repeatability of finger blood testings was verified with six consecutive tests on nine volunteers, results ranged from 3% to 8% variation. The test results of BeneCheck were correlated with Sysmex, Beckman Coulters, Cell-Dyn and HemoCue methods, the results have shown similar and 95% of test results were within a $\pm 15\%$ bias.

Conclusions: BeneCheck hemoglobin test system performed well and accurately, while requiring 1 μ l of blood sample and 10 s detection time. Based on the cost, accuracy, sample volume, measuring time, ease of viewing and portability, BeneCheck deliver the best characteristics for these purposes.

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1. Introduction

Many centralized instruments such as Beckman Coulters (Beckman Coulter, Inc., Brea, CA), Cell-Dyn (Abbott Laboratory, IL) and Sysmex KX-21N (Kobe, Hyogo, Japan), as well as portable meters such as HemoCue (HemoCue, Inc., Lake Forest, CA) have been on the market for years providing the measurement of hemoglobin and are all based on the colorimetric measuring method.

Centralized instruments using the cyanmethemoglobin measurement method are considered the gold standard for assessing hemoglobin concentration [1]. Although there are portable meters based on the cyanmethemoglobin principle [2], their reaction

products are measured by a photoelectric colorimeter as well. In general, colorimetric instruments or portable devices used for hemoglobin measurement all required high blood volume (usually $\geq 10 \mu$ l); long measuring time (45 s to several min); and are expensive or are complicated to use. The large volume of blood sample utilized and the long measurement time needed make repeated measurements from the same finger extremely difficult. Many literatures [1,3–5] have reported that the variations in results between finger blood and venous blood when measuring hemoglobin was either due to physiologic variation or hemodilution caused by excess pressure on the finger. Although using finger blood provides simplicity, venous blood is still the preferred sample for accurate judgment of hemoglobin.

A biosensor for hemoglobin measurement based on electrochemical technology was developed and distributed with a brand name of BeneCheck (General Life Biotechnology Co., Ltd, Taipei, Taiwan). The BeneCheck claims to use 1 μ l of sample and <10 s testing time, providing superior advantages compared to existing colorimetric based products available on the market. However, in realistic conditions, products will be challenged by different sample conditions

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such as freshness of blood sample, varying storage conditions, hemolysis, interference caused by bilirubin, etc.

2. Materials and methods

2.1. Instruments

BeneCheck hemoglobin test system contains strips and a meter. Hemoglobin strip was constructed by screen printing technique illustrated as in Fig. 1. On a polyethylene terephthalate (PET) surface (401), a silver layer (402') was screen printed as the conductive line. Carbon paste covered on the top of the silver layer and with an extra area as the working electrode (402''). Silver/Silver Chloride (402''') was printed as the reference electrode. A T-shape layer (403) confined a reaction channel which crosses the working and reference electrodes. Reaction layer (405) was immobilized in the reaction channel. A top layer (407) was placed on the top of a T-shape layer and created a capillary channel, while channel on both sides (403b) were used as the air vents. The theoretical volume of the capillary channel calculated with its dimension was about 0.67 μl . During a test, the tip of an electrode touched a sample and sample was sucked into the strip by the capillary action through the capillary channel. A mediator, GLBM, was developed by General Life Biotechnology Co., Ltd. GLBM bears a property of easily dissolved in an aqueous solution. A typical cycle voltammetry response of GLBM was shown previously by us [6], with a strong peak appeared at 0.7 V against a Ag/AgCl reference electrode. The measuring principle behind BeneCheck hemoglobin test system is described in our previous publication [6]. Simplifying the assay principle is the electron mediator on the working electrode surface of a strip, or GLBM. The GLBM can be oxidized instantly by a hemoglobin molecule, or by a voltage higher than 0.7 V. Electrons transfer from the mediator to the Ferric ion in the hemoglobin molecule, and reduce Fe (III) to Fe (II) when the mediator reacts to hemoglobin in the sample. After a set reaction time, starting from when a whole blood sample is introduced, a potential >0.7 V is provided by a hemoglobin meter. This oxidizes the remaining mediator and generates a current. The intensity of the current is proportional to the remaining mediator concentration on the electrode surface and inversely proportional to the hemoglobin concentration in the tested whole blood sample.

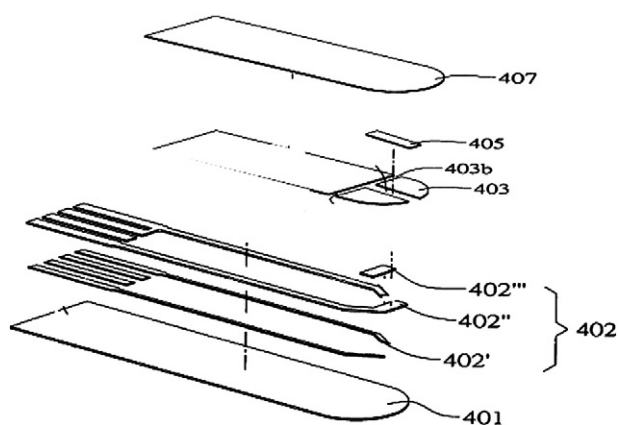


Fig. 1. A hemoglobin strip structure. Here, 401 is an insulated layer on which a conductive layer, 402', made by silver was screen printed on 401. Carbon layer was covered on the top of the conductive layer and with an extra area as the working electrode, 402''. A T-shape layer, 403, on the electrodes and confined a reaction zone. Reagent layer, 405, was coated in the reaction zone. A capillary channel, was constructed with a top layer, 407, covered on the T-shape layer and left 403b as the air vents.

3. Methods

3.1. Effects of blood sample freshness study

Whole blood samples drawn for hemoglobin measurement might not be utilized immediately in a laboratory. Ideally, the blood sample should be measured within a certain time period of 1–2 h, but sometimes blood samples are kept at room temperature or in a cold storage room for a longer period of time before hemoglobin measurement. In this study, venous blood samples were collected directly into vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing potassium EDTA as an anticoagulant. Concentrations of hemoglobin were adjusted to the desired sample levels of 110, 140 and 180 g/l. Each sample's hemoglobin concentration was determined by the Sysmex KX-21N, then divided into several aliquot vials and stored at room temperature or in a cold storage room with temperatures set between 2 and 8 °C. Hemoglobin concentration was then measured by both BeneCheck and Sysmex for those samples stored in different conditions following the planned amount of storage time. A total of 7 days' storage prior to evaluation of hemoglobin measurement was arranged.

3.2. Effects of hemolysis study

Venous blood samples were prepared as in the blood sample freshness study. From room temperature stored samples, 2 vials of each concentration were chosen for each evaluation. Four percent of Baso Hemolysis-WBC reagent (General Biological, Hsin Ju, Taiwan) was added to one vial of sample to cause hemolysis. The blood samples were then centrifuged to be certain that the red blood cells of the sample were lysed and hemoglobin was present in the plasma. The other vial with no hemolysis reagent added had no observable hemolysis. The effect of complete hemolysis was also studied by freezing followed by thawing and vortexing the blood samples. All samples were measured for hemoglobin concentration using BeneCheck and Sysmex.

3.3. Bilirubin interference study

A bilirubin (Sigma, St. Louis, MO) stock solution was prepared by dissolving bilirubin in a sodium hydroxide (Sigma) solution, 0.1 mol/l, to a concentration of 2.50 g/l. Venous blood was collected and divided into 2 sample tubes. The hemoglobin level was adjusted to 120 g/l in one sample tube and to 150 g/l in the other. Each venous sample was divided into five vials. Bilirubin from the stock solution was added into venous blood samples with different hemoglobin concentrations to the final bilirubin concentration of 0, 10, 50, 100 and 150 mg/l. Hemoglobin concentrations were measured by BeneCheck and Sysmex for samples with different bilirubin concentrations.

3.4. Repeatability of finger blood tests

Volunteers were asked to wash hands with soap and water, and then disinfect fingertips with isopropyl alcohol after their hands dried. Hands were to be completely dry before lancet piercing. Volunteers were also asked to rub their hands and fingers before lancet piercing. Each volunteer tested his/her hemoglobin concentration with one BeneCheck hemoglobin meter for at least 6 times. The first drop of blood was removed with a sterile cotton swab and disposed. A capillary blood sample was then taken and tested immediately with the BeneCheck hemoglobin test system. The used strip was disposed of and a new strip was inserted into the meter after the first test reading displayed on the meter. The remaining blood on the fingertip from the first test was removed with a sterile cotton swab, and then a fresh capillary blood sample was taken by applying pressure on the pierced finger for the

second test. Because BeneCheck hemoglobin test strip only requires 1 μ l of blood for a single test, hopefully hemodilution can be prevented due to the small amount of pressure necessary on the finger to obtain an adequate capillary sample.

3.5. Precision study of venous blood tests

Venous blood samples were collected directly into vacutainer tubes containing potassium EDTA as an anticoagulant. Twenty-five tests were performed with 10 BeneCheck hemoglobin meters. The mean of the average of test results, SD and the CV were calculated. Sysmex was also used as a reference for hemoglobin concentration confirmation.

3.6. Accuracy study

Studies had been performed at several hospitals for the comparison of BeneCheck to different instruments. Portable devices from BeneCheck and HemoCue 201 were compared using both capillary blood and venous blood. Two hundred patients were involved in the study of the comparison between BeneCheck and HemoCue 201 systems for the measurement of capillary blood samples. Capillary blood samples were measured by both devices in parallel. A total of 102 venous blood samples were collected for the comparison between BeneCheck and HemoCue 201. Sysmex and BeneCheck comparisons were also performed by testing patients' capillary blood and venous blood. The patient's venous blood was drawn within 5 min after his finger's blood had been tested. Capillary blood sample measurements on BeneCheck were comparable to venous blood samples measured by Sysmex and consisted of 84 samples. The comparison of venous blood sample measurement between BeneCheck and Beckman Coulter was also studied. The study to compare venous blood measurement between BeneCheck and Beckman Coulter consisted of 110 samples. The blood donors were selected based on the recommendations for distribution of data made in Table 1a of the Article EP9-A2, NCCLS, 2002 [7], with a slight adjustment of the percentages to reflect the realistic situations that were encountered. These studies were performed in Taipei Medical University—Shuang-Ho Hospital, Taipei Medical University, New Taipei City, Taiwan.

Samples with a hemoglobin concentration higher than 170 g/l (based on BeneCheck results) were prepared by adjusting the venous blood to the desired hemoglobin levels. No samples were taken from the Intensive Care Unit (ICU). Appropriate informed consents were obtained from all patients and control subjects in the study if necessary. These studies were approved by the ethics committees of Taipei Medical University-Joint Institutional Review Board.

Separate studies comparing the results of hemoglobin concentration measurement from venous blood of blood donors using BeneCheck and Cell-Dyn 3700 also have been performed in Baskent University Hospital (Ankara, Turkey) and Trust Hospital (Ankara, Turkey). A total of 79 venous samples were collected (37 from Baskent and 42 from Trust) and tested in parallel for these studies.

The correlation coefficients (r) were determined for each pair of comparisons except for the comparison to Cell-Dyn. Data were analyzed with the linear regression method. Bias was calculated for samples by subtracting the BeneCheck tested number to the comparison method tested number. Percentage of bias was calculated by dividing the bias with the testing results from the comparison method then multiplying by 100%.

4. Results

4.1. Blood sample freshness effect study

In this study, 6 samples with differing hemoglobin concentrations were divided into small vials and stored at room temperature.

Table 1a

Sample age effect study. Venous samples were stored in room temperature.

Room temperature storage		Test day (Hb, g/l)						
Sample #	Device	0	1	3	7	AVG	SD	CV%
1	Sysmex	117	117	118	117	117	0.5	0.43
	BeneCheck	118	123	119	121	120	2.2	1.83
2	Sysmex	123	119	114	117	118	3.8	3.2
	BeneCheck	119	116	120	126	120	4.2	3.5
3	Sysmex	135	148	139	143	141	5.6	4
	BeneCheck	136	150	134	146	142	7.7	5.44
4	Sysmex	145	141	148	150	146	3.9	2.67
	BeneCheck	147	145	141	153	147	5	3.41
5	Sysmex	185	188	185	181	185	2.9	1.57
	BeneCheck	183	192	178	183	184	5.8	3.15
6	Sysmex	184	186	185	193	187	4.1	2.2
	BeneCheck	170	169	176	189	176	9.2	5.23

Hemoglobin was measured with BeneCheck and Sysmex following a planned storage schedule. New vials of samples were chosen for each day of testing. The test results were shown in Table 1a. Blood samples were stored at room temperature for zero to 7 days and hemoglobin concentration was tested on days 0, 1, 3 and 7. The percentages of coefficient variation for Sysmex testing results ranged from 0.43% to 4.0%, while those of the BeneCheck test results ranged from 1.83% to 5.44%. Based on these results, we concluded that when stored at room temperature, the freshness of blood samples had no effect on hemoglobin measurement if tested within a few days. Samples stored at temperatures between 2 and 8 °C also showed similar results when tested as those stored at room temperature (data not shown).

4.2. Hemolytic effect study

Incomplete hemolysis of blood samples was achieved by the addition of 4% hemolysis solution. The hemoglobin concentration present in the plasma of the blood sample after hemolyzing, which was separated from the remaining sample with centrifugation, was about 10 g/l. Table 1b contains the results of the study of the effects of hemolysis. For those samples stored from 0 to 7 days and hemolyzed on the day of testing, the percentage of coefficient variation for the Sysmex test results ranged from 1.14% to 4.1%, while that of the BeneCheck test results ranged from 2.09% to 4.82%. The bias of the hemoglobin concentration between hemolyzed and non-hemolyzed samples ranged from −0.32% to +1.44% for Sysmex and −4.15% to +3.17% for BeneCheck, after adjusting for the dilution factor of the

Table 1b

Sample age effect study. Venous samples were stored in room temperature and partially lysed before testing.

(Room temperature storage)		Test day (Hb, g/l)						
With partial lysing								
Sample #	Device	0	1	3	7	AVG	SD	CV%
1	Sysmex	114	116	114	113	114	1.3	1.14
	BeneCheck	115	113	121	125	119	5.5	4.64
2	Sysmex	112	116	112	113	113	1.9	1.68
	BeneCheck	119	115	115	126	119	5.2	4.4
3	Sysmex	133	145	135	134	137	5.6	4.1
	BeneCheck	130	136	134	136	134	2.8	2.09
4	Sysmex	143	137	143	142	141	2.9	2.06
	BeneCheck	140	131	141	144	139	5.6	4.03
5	Sysmex	178	181	174	176	177	3	1.69
	BeneCheck	170	166	175	166	169	4.3	2.54
6	Sysmex	177	183	182	179	180	2.8	1.56
	BeneCheck	169	155	160	172	164	7.9	4.82

added hemolysis reagent. Results based on the above data show no significant effect on testing from hemolysis with the hemolytic level up to 10 g/l.

Complete hemolysis of blood samples was also studied. Blood samples with hemoglobin concentrations of 130 g/l and 165 g/l were frozen then thawed by hand without the addition of the hemolysis reagent. Vortexing the samples caused complete lyses of red blood cells. The bias of the hemoglobin concentration (original 130 and 165 g/l) between hemolyzed and non-hemolyzed samples ranged from −3.88% to −5.49% for Sysmex and +1.5% to +1.8% for BeneCheck.

4.3. Bilirubin concentration interference study

Bilirubin was added into venous samples with differing hemoglobin concentrations to the target bilirubin concentrations of 0, 10, 50, 100 and 150 mg/l. The hemoglobin concentration of each sample was measured at least 5 times. The pooled CV of all hemoglobin test results for samples ($n=33$) of differing bilirubin concentrations, with an average hemoglobin level of 134 g/l, was 7%. The pooled coefficient variation of all hemoglobin test results for samples ($n=29$) of differing bilirubin concentrations, with an average hemoglobin level of 156 g/l, was 4.4%.

4.4. Repeatability of finger blood tests

Nine volunteers participated in this study. Six consecutive tests were performed by lancing a single finger, and finger blood samples were obtained from a single lanced site. Because the measuring time of one testing takes only 8 s, thus the lanced hole made on the finger would not have enough time to coagulate before the completion of the tests. The CVs of the 6 tests from 9 volunteers ranged from 3.2% to 8.1%. Four of them were <5%.

4.5. Precision study of venous blood tests

Venous blood was drawn from 3 volunteers for the precision study. Every sample was continuously measured for 25 times. The CV was 4.7% for the sample from the volunteer who had a hemoglobin concentration of 108 g/l, 2.4% for the sample from the volunteer who had a hemoglobin concentration of 139 g/l, and 2.6% for the sample from the volunteer who had a hemoglobin concentration of 148 g/l.

4.6. Accuracy study

Capillary blood samples were compared between BeneCheck and HemoCue 201. Fig. 2a shows the testing comparison of capillary blood samples between BeneCheck and HemoCue 201. Range of hemoglobin values, according to test results done with HemoCue 201, was from 67 to 251 g/l. Results of the regression line equation for a total of 200 samples show a slope of 0.955 with a positive intercept of 6.4336 g/l and a CV of 0.955, $R^2=0.912$. In reaching the desired high hemoglobin levels, 8 samples with a hemoglobin concentration >170 g/l were prepared from venous blood to be tested by BeneCheck. The result of regression line equation (Fig. 2b) after removing those 8 high venous blood samples shows a slope of 0.8915 with an intercept of 14.699 g/l and a correlation coefficient of 0.9249 (R^2 is 0.8554). The range of hemoglobin values was also changed from 67 to 176 g/l. The hemoglobin concentrations of 18 samples were ≤100 g/l. Among those 18 samples, 10 (55.6%) were within a 5 g/l bias, 16 out of 18 tests (88.9%) were within a 10 g/l bias and 100% were within a 15 g/l bias. For those samples with a hemoglobin concentration >100 g/l, the number of tested results that remained within a 5% bias between BeneCheck and HemoCue was 112 tests out of a total of 182 tests (61.5%), 169 tests out of 182 tests (92.9%) were within a 10% of

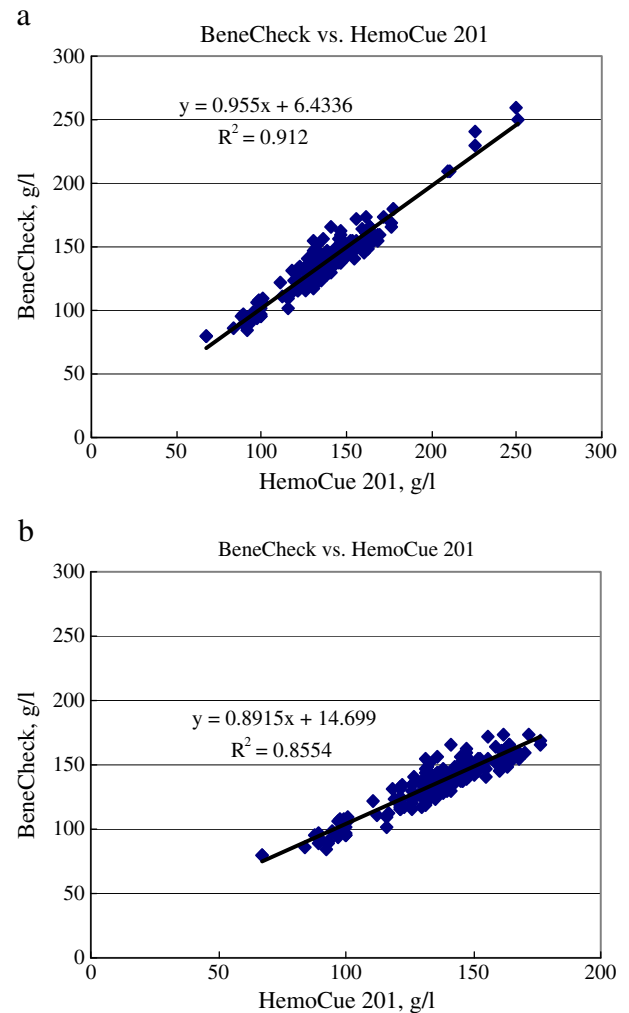


Fig. 2. a. Correlation between BeneCheck and HemoCue. There were 200 tests with capillary blood samples. b. Correlation between BeneCheck and HemoCue removed the prepared high hemoglobin concentration venous samples.

bias and 178 tests (97.8%) were within a 15% bias. The bias was between 15 and 20% for 2 tests (Table 2a).

A total of 102 samples of original venous blood without any concentration adjustment for high hemoglobin levels were used in the comparison of BeneCheck and HemoCue 201 for reading venous blood

Table 1c

Sample age effect study. Venous samples stored in room temperature. Hct were tested without partially lysed and partially lysed.

Sample #	Device	Test day; Hct %				Variation, %		
		0	1	3	7	0–1	0–3	0–7
1	Sysmex	36.5	39	42.9	43.8	6.9	17.5	20
	Lysing	36.8	39.6	41.4	40.9	7.6	12.5	11.1
2	Sysmex	33	40.2	40.8	42.8	21.8	23.6	29.7
	Lysing	34.6	40.3	39.3	37.9	16.5	13.6	9.5
3	Sysmex	42.2	50.6	50.6	53.2	19.9	19.9	26.1
	Lysing	43.3	49.1	49.9	47.9	13.4	15.2	10.6
4	Sysmex	45.8	48.2	52	53.6	5.2	13.5	17.0
	Lysing	44.3	47.6	51.1	47.4	7.4	15.3	7.0
5	Sysmex	59.8	61.9	65.6	65.1	3.5	9.7	8.9
	Lysing	57.2	61.5	61.9	64.2	7.5	8.2	12.2
6	Sysmex	57.6	63.9	62.5	68.1	10.9	8.5	18.2
	Lysing	55.6	64	62.6	58.7	15.1	12.6	5.6

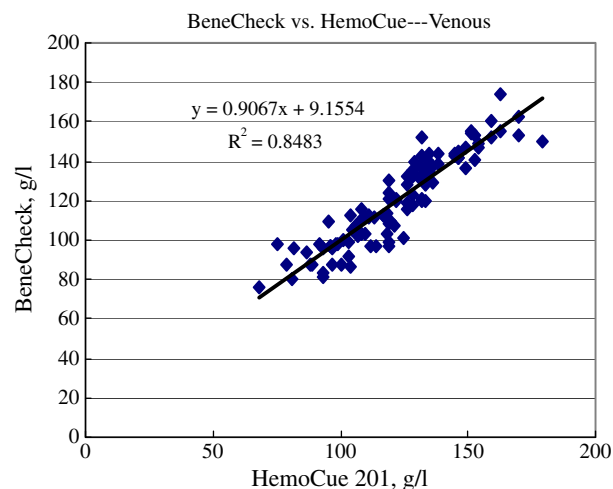
Table 2

Bias calculation for hemoglobin measurement between BeneCheck and compared devices. a, Capillary blood tested for BeneCheck and Hemocue. b, Venous blood tested for BeneCheck and Hemocue. c, Capillary blood tested for BeneCheck and venous blood tested for Sysmex. d, Venous blood tested for BeneCheck and Beckman Coulter. e, Venous blood tested for BeneCheck and Cell-Dyn.

a			
BeneCheck Hb hemoglobin test system to HemoCue201			
Bias % calculation; total 200 tests (capillary blood for both)			
≤ 100 g/l		> 100 g/l	
Within ± 5 g/l	10/18 (55.6%)	Within $\pm 5\%$	112/182 (61.5%)
Within ± 10 g/l	16/18 (88.9%)	Within $\pm 10\%$	169/182 (92.9%)
Within ± 15 g/l	18/18 (100%)	Within $\pm 15\%$	178/182 (97.8%)
Within ± 20 g/l	18/18 (100%)	Within $\pm 20\%$	182/182 (100%)
b			
BeneCheck Hb hemoglobin test system to HemoCue201			
Bias % calculation; total 102 tests (venous blood for both)			
≤ 100 g/l		> 100 g/l	
Within ± 5 g/l	13/22 (45.5%)	Within $\pm 5\%$	47/80 (58.8%)
Within ± 10 g/l	19/22 (86.4%)	Within $\pm 10\%$	71/80 (88.8%)
Within ± 15 g/l	21/22 (95.5%)	Within $\pm 15\%$	78/80 (97.5%)
Within ± 20 g/l	22/22 (100%)	Within $\pm 20\%$	80/80 (100%)
c			
BeneCheck Hb hemoglobin test system to Sysmex KX-21N			
Bias % calculation; total 84 tests (capillary to venous)			
≤ 100 g/l		> 100 g/l	
Within ± 5 g/l	6/14 (42.9%)	Within $\pm 5\%$	44/70 (62.9%)
Within ± 10 g/l	12/14 (85.7%)	Within $\pm 10\%$	66/70 (94.3%)
Within ± 15 g/l	14/14 (100%)	Within $\pm 15\%$	70/70 (100%)
d			
BeneCheck Hb hemoglobin test system to Beckman Coulter			
Bias % calculation; total 102 tests (venous blood for both)			
≤ 100 g/l		> 100 g/l	
Within ± 5 g/l	11/22 (55.0%)	Within $\pm 5\%$	47/80 (58.8%)
Within ± 10 g/l	16/22 (86.3%)	Within $\pm 10\%$	72/80 (90%)
Within ± 15 g/l	21/22 (95.5%)	Within $\pm 15\%$	80/80 (100%)
Within ± 20 g/l	22/22 (100%)	Within $\pm 20\%$	80/80 (100%)
e			
BeneCheck Hb hemoglobin test system to Cell-Dyn 3700			
Bias % calculation; total 79 tests (venous blood for both)			
≤ 100 g/l		> 100 g/l	
Within ± 5 g/l	0	Within $\pm 5\%$	47/79 (59.5%)
Within ± 10 g/l	0	Within $\pm 10\%$	68/79 (86.1%)
Within ± 15 g/l	0	Within $\pm 15\%$	77/79 (97.5%)
Within ± 20 g/l	0	Within $\pm 20\%$	79/79 (100%)

samples. The slope was 0.9067 and the intercept was 9.1554 g/l for the regression line equation (Fig. 3). The range of hemoglobin values was from 68 to 179 g/l, $R^2 = 0.8483$.

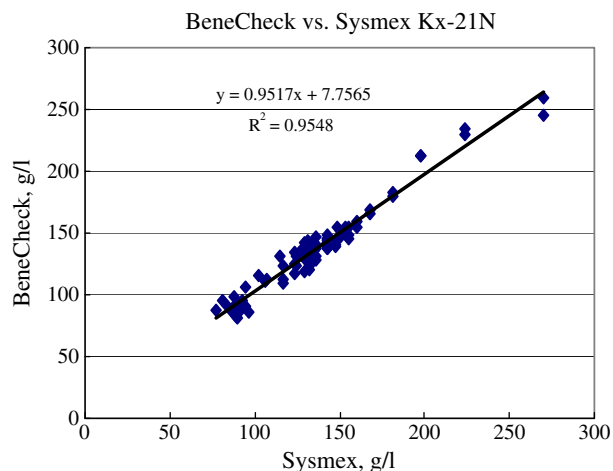
Venous blood comparison between BeneCheck and HemoCue 201 was performed and the biases between these 2 methods were calculated. This consisted of 22 samples with hemoglobin concentrations ≤ 100 g/l. Thirteen of 22 test results (45.5%) were within a 5 g/l bias, 19 out of 22 tests (86.4%) were within a 10 g/l bias, and 21 out of 22 tests (95.5%) were within a 15 g/l bias. For those with a hemoglobin concentration > 100 g/l, the number of tested results that ranged within a 5% bias between BeneCheck and HemoCue was 47 tests out of a total of 80 tests (58.8%). A total of 71 out of 80 tests (88.8%) were within 10% of bias and 78 tests (97.5%) were within a 15% bias. Two tests had a bias between 15 and 20% (Table 2b).

**Fig. 3.** Correlation between BeneCheck and HemoCue with venous blood samples.

Eighty-four samples were used in the study that compared the results of capillary blood samples tested by BeneCheck to that of venous blood samples from the same patients measured by Sysmex. According to the correlation curve plotted by BeneCheck vs. Sysmex, the results of regression line equation (Fig. 4) show a slope of 0.9517 with an intercept of 7.7565 g/l and an $R^2 = 0.9771$. The range of hemoglobin values was from 77 to 270 g/l based on the Sysmex test results. If the venous samples were removed, then the hemoglobin values ranged from 77 to 168 g/l. The slope and intercept of the new regression line equation became 0.9204 and 11.299 respectively and the R^2 became 0.95 (figure not shown).

A total of 84 samples, including venous blood samples, adjusted to high hemoglobin levels. There were 14 samples with a hemoglobin concentration ≤ 100 g/l, and 6 (42.9%), 12 (85.7%) and 14 (100%) samples were within a 5, 10 and 15 g/l bias respectively. Forty-four out of a total of 70 tests with a hemoglobin concentration > 100 g/l were within a 5% bias between the BeneCheck and Sysmex. A total of 66 out of 70 tests were within 10% of bias and 70 tests were within a 15% bias (Table 2c).

A comparison of venous blood sample testing results between BeneCheck and Beckman Coulter were studied using original venous blood from patients, excluding the venous blood samples that were adjusted to a high concentration. Fig. 5 shows the comparison results

**Fig. 4.** Correlation between BeneCheck and Sysmex KX-21N. Capillary samples were tested by BeneCheck and venous samples tested by Sysmex.

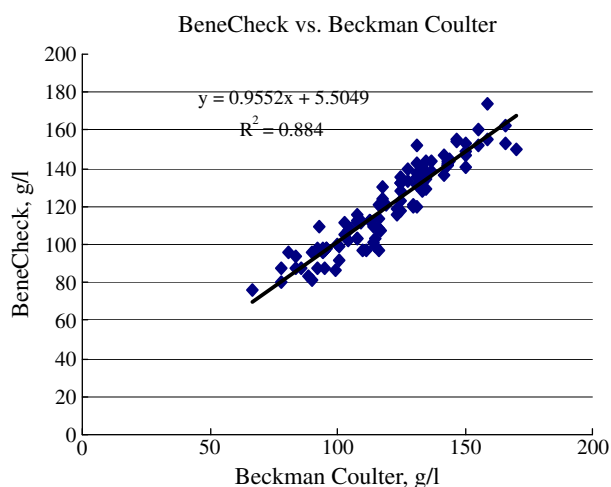


Fig. 5. Correlation between BeneCheck and Beckman Coulter. Venous blood samples were measured by both devices.

from 102 samples, showing a regression line equation with a slope of 0.9552, an intercept of 5.5049 g/l, $R^2 = 0.94$. Table 2d shows the mean of bias comparison between BeneCheck and Coulter. There were 22 samples with a hemoglobin concentration ≤ 100 g/l, and 11 (55%), 16 (86.3%) and 21 (95.5%) samples were within a 5, 10 and 15 g/l bias respectively. A total of 47 out of 80 tests (58.8%) tests with a hemoglobin concentration >100 g/l were within a 5% bias; a total of 72 tests out of 80 tests (90%) were within 10% of bias; and all tests were within a 15% bias.

The comparison of testing results between BeneCheck and Cell-Dyn was performed. Table 2e summarizes the test results. There were a total of 79 venous samples collected, with the range of hemoglobin levels between 125 and 179 g/l. There were 47 samples (59.5%) within a 5% bias to the reference method, 68 samples (86.1%) were within 10% of bias, and 77 samples (97.5%) were within 15% of bias.

5. Discussion and conclusion

Although BeneCheck claims that fresh blood taken from a fingertip is the recommended sample for testing, venous blood sample is commonly used in professional settings. The purpose of these studies was to evaluate the effect of venous blood freshness on the hemoglobin measurement. Based on the data collected, there were no effects on hemoglobin measurement if the blood samples were stored at room temperature or in a refrigerator for up to 7 days. When stored in a cold condition, blood samples should be rewarmed to room temperature before samples preparation in order to prevent hemolysis. According to Sysmex results shown in Table 1c, the hematocrit of blood samples stored at room temperature increased approximately 10% to 30% for non-hemolyzed samples. BeneCheck also claims to measure hematocrit, however, the hematocrit result is calculated by multiplying the measured hemoglobin concentration with a factor of 3. Therefore, the true hematocrit may be higher than the results shown by BeneCheck if the tested blood samples have been stored at room temperature for a few days. Fortunately, hematocrit does not change much if samples were stored in a cold environment (2 to 8 °C) when measured by Sysmex.

BeneCheck is able to produce more accurate hematocrit measurements if samples are stored in a cold environment. Sysmex showed better results than BeneCheck when taking hemoglobin measurements on partially hemolyzed samples. Sysmex testing results produced a -0.32% to $+1.44\%$ variation compared to -4.15% to $+3.17\%$ variations for BeneCheck. However, BeneCheck

showed better variation results, $+1.5\%$ to $+1.8\%$ for completely hemolyzed samples as compared to -3.88% to -5.49% for Sysmex. For clinical application, blood samples should be handled carefully to prevent hemolysis of red blood cells. However, sometimes slight hemolysis of samples is unavoidable, and when using BeneCheck, the effect of hemolysis on hemoglobin measurement can be ignored.

The normal range of bilirubin concentration in human plasma is from 2 to 12 mg/l [8]. In the effects of bilirubin study, a bilirubin concentration as high as 150 mg/l was tested. The interference of bilirubin in clinical chemistry laboratory tests is usually caused by the color of pigment absorption at certain wavelengths when using the optical method. However, when using the electrochemical method, the color of the sample will not interfere with the redox reaction unless the colored material plays a part in the reaction caused by the change in the electrical signal. Based on the test results, bilirubin concentration did not interfere with hemoglobin measurement on BeneCheck. When used for hemoglobin measurement, capillary blood generally gives a higher variation compared to venous blood [9] due to the inclusion of the extracellular fluid, which is difficult to control in the sampling procedure [10]. Technique is important for the person handling the finger prick, regardless of physiologic variation, a simple method is required for obtaining precise readings for capillary blood samples from the same pierced hole on a finger. Warming hands via a massage, capillary blood samples were then repeatedly squeezed out from the same pierced lesion and tested continuously by the same meter. The coefficient variation of the test results for nearly 50% of volunteers was lower than 5% of variability, which was either calculated from the first 3 tests or from a total of 6 test results. We expected better blood circulation and homogeneous of hemoglobin distribution in the blood vessels of the fingers after massaging hands and fingers.

According to Sari's study [1], using microcuvettes of HemoCue in a container whose seal had been opened for a longer period of time resulted in higher hemoglobin concentration results, which suggested that microcuvettes should be used within 2 months after the seal of the container is broken. BeneCheck hemoglobin strips were also studied for the effect of humidity on testing results, and surprisingly, BeneCheck hemoglobin strips showed no great change in testing results after being exposed (without a vial) in the laboratory for 15 h. The room temperature was within the range of 23 to 30 °C and humidity was within the range of 55 to 65%. Although we have confirmed that humidity does not have a great effect on the strips, strips should be kept in the strip vial and the cover of the strip vial be capped promptly after strip removal as suggested by the manufacturer, due to the fact that the humidity in the environment is difficult to control at different locations.

The results of the correlation study, done by comparing BeneCheck to various devices including large instruments and portable meters, were similar. Most of the devices showed that $>50\%$ of tests were within $\pm 5\%$ of bias, $>85\%$ were within $\pm 10\%$ of bias and $>95\%$ of tests were within $\pm 15\%$ of bias to the reference devices.

For the use of pre-screening hemoglobin levels of blood donors, the accuracy and cost of the compared reagents and devices had been debated [11,12]. Opinions differ concerning whether copper sulfate solution should or should not be used in blood banks for the pre-screening of donor hemoglobin. Based on the cost, accuracy, sample volume, measuring time and ease of viewing and portability, BeneCheck deliver the best characteristics for these purposes.

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