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## Evaluation of Three Methods for Hemoglobin Measurement in a Blood Donor Setting

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### ABSTRACT

### INTRODUCTION

**Context:** The hemoglobin (Hb) level is the most-used parameter for screening blood donors for the presence of anemia, one of the most-used methods for measuring Hb levels is based on photometric detection of cyanmethemoglobin, as an alternative to this technology, HemoCue has developed a photometric method based on the determination of azide methemoglobin.

**Objective:** To evaluate the performance of three methods for hemoglobin (Hb) determination in a blood bank setting.

**Design:** Prospective study utilizing blood samples to compare methods for Hb determination.

**Setting:** Hemotherapy Service of the Hospital Israelita Albert Einstein, a private institution in the tertiary health care system.

**Sample:** Serial blood samples were collected from 259 individuals during the period from March to June 1996.

**Main Measurements:** Test performances and their comparisons were assessed by the analysis of coefficients of variation (CV), linear regression and mean differences.

**Results:** The CV for the three methods were: Coulter 0.68%, Cobas 0.82% and HemoCue 0.69%. There was no difference between the mean Hb determination for the three methods ( $p > 0.05$ ). The Coulter and Cobas methods showed the best agreement and the HemoCue method gave a lower Hb determination when compared to both the Coulter and Cobas methods. However, pairs of methods involving the HemoCue seem to have narrower limits of agreement ( $\pm 0.78$  and  $\pm 1.02$ ) than the Coulter and Cobas combination ( $\pm 1.13$ ).

**Conclusion:** The three methods provide good agreement for hemoglobin determination.

**Key words:** Hemoglobin. Methods. Quantification. Equipment.

The hemoglobin (Hb) level is the most-used parameter for screening blood donors for the presence of anemia. This measurement is usually performed during the clinical-epidemiological interview that precedes blood donation.

Presently, one of the most-used methods for measuring Hb levels is based on photometric detection of cyanmethemoglobin, which is a stable compound derived from Hb. In addition, ready-to-use reagents, as well as cyanmethemoglobin standard solution for calibration, are commercially available.

As an alternative to this technology, HemoCue has developed a photometric method based on the determination of azide methemoglobin,<sup>1</sup> standardized against the International Committee for Standardization in Hematology (ICSH) method.<sup>2</sup> The azide methemoglobin is measured at 570 nm. To compensate for turbidity, e.g. due to lipids, a second measurement is taken at 880 nm.<sup>3</sup> The advantage of this technology is that it is simple, rapid and does not require sophisticated hematological equipment. The system is designed to use capillary, venous or arterial blood. Also, the instrument is small and portable

which allows its use in mobile blood collection units and physicians' offices.

Several studies performed on American blood donors have attested to the good reproducibility and accuracy of the HemoCue method.<sup>4,5</sup> However, comparability of the Hb level measured via the HemoCue method with other, more recently available hematological equipment has not been performed.

In this study we reviewed the performance of the HemoCue system in comparison with the Coulter and Cobas methods. Both of these methods are based on the detection of cyanmethemoglobin.

## METHODS

*Sample Selection.* A total of 259 blood samples were collected during the period from March to June 1996. Blood was collected with the Vacutainer system, containing EDTA-K<sub>3</sub> (Becton-Dickinson) to a total volume of 4.5 ml.

*Hemoglobin Levels.* All samples were evaluated by three methods: (a) Coulter STKS (Coulter Corporation, Hialeah, FL, USA), (b) Cobas Micros OT, 16 (Roche Diagnostic System, Montpellier, France) and (c) HemoCue, Inc., Mission Viejo, CA, USA).

*Hb Measurements.* For the 259 samples were performed by the three methods within an interval of 10-20 minutes to avoid variation during processing and measurement. For the reproducibility analysis of the Hb measurements, we used a single sample that was evaluated 10 times by each method. Based on these determinations we calculated the coefficient of variation (CV) defined as the ratio between the standard deviation and the mean of the Hb levels, multiplied by 100. The Coulter and Cobas were calibrated daily according to the manufacturer's recommendation. The HemoCue photometer is factory-calibrated and should not be recalibrated. The calibration was checked daily according to the manufacturer's recommendation. The calibration was stable during our study period.

*Statistical Methods.* All the statistical analyses were performed on the shareware

software EPIINFO, version 6 (linear regression analysis, Student "t" test, the calculation of the mean and standard deviation).<sup>6</sup> A "p" value less than 0.05 was considered as statistically significant.

## RESULTS

The reproducibility of each method was evaluated by measuring the Hb level 10 times from a single blood sample and determining the coefficient of variation (CV) for each assay. The CV for the Coulter, Cobas and HemoCue methods was 0.68%, 0.82%, and 0.69%, respectively (Table 1).

We first assessed the measurements of central tendency (mean and median) and variation (range and standard deviation) for the 259 Hb determinations from each method. As shown in Table 2, we could not find any statistical difference for these parameters. However, we observed that the HemoCue method showed the lowest mean (11.5 g/dl) and the lowest median (11.4 g/dl) when compared to both the Coulter and Cobas methods (mean = 11.6 and median = 11.7 and 11.6 g/dl, respectively).

We next studied the correlation coefficient for pairs of methods, from the linear regression analysis of the Hb determination for the three methods. Table 3 shows the parameters for this analysis and indicates that every pair had an excellent correlation coefficient (range 0.97 to 0.99). This result was somewhat expected since all three methods were designed to measure the same parameter (Hb level in g/dl). In this circumstance, the agreement between two variables is not correctly represented by measuring the strength of their relationship, or statistically speaking, by determining the coefficient of correlation of the linear regression.

Therefore, we decided to evaluate the agreement of the three methods of Hb determination by using the approach proposed by Bland and Altman.<sup>7</sup> Briefly, this approach assumes that if two methods are to agree then the mean of the difference between every paired

determination will not be statistically different from zero. By using this approach it is also possible to establish a limit of agreement (within a given confidence interval) between the two methods and to graphically visualize the dispersion of these differences across Hb levels.

**Table 1 - Determination of the coefficient of variation for the three methods of Hb measurement**

Method	Mean	SD	CV
Coulter	13.4	0.019	0.68
Cobas	12.8	0.10	0.82
HemoCue	13.0	0.09	0.69

*SD = Standard Deviation*

*CV = Coefficient of Variation*

For instance, this could indicate whether greater variability could be associated with a particular range of Hb determinations and thus suggest a lack of precision associated with that Hb range.

The application of the Bland and Altman approach to our data is shown in Table 4. It can be seen that the only pair of methods with a mean of the difference not different from zero is the Coulter/Cobas pair ( $p = 0.588$ ). Therefore, these are the methods which agree on Hb measurements. Pairs of methods that involved the HemoCue method gave a mean of the difference statistically different from zero ( $p < 0.001$ ).

The Bland and Altman approach allowed us to calculate the limit of agreement between

**Table 2 - Measurements of central tendency and variation for the three methods of Hb determination**

Method	n	Range <sup>a</sup>	Mean <sup>a,b</sup>	SD <sup>c</sup>	Median <sup>a</sup>
Coulter	259	7.2 to 18.3	11.6	2.1	11.7
HemoCue	259	6.7 to 18.5	11.5	2.3	11.4
Cobas	259	6.8 to 18.5	11.7	2.2	11.4

<sup>a</sup> Hemoglobin values in g/dl

<sup>b</sup> There was no statistically significant difference between the mean of the Hb determination for the three methods

<sup>c</sup> SD = Standard Deviation

**Table 3 - Linear Regression Analysis between pairs of the three methods used for Hb determination (n=259)**

Reference method	Testing method	Coefficient		
		Correlation	Slope	Y-Intercept
Coulter	HemoCue	0.99	1.05	-0.69
Coulter	Cobas	0.97	1.00	-0.02
Cobas	HemoCue	0.97	0.99	-0.11

**Table 4 - Use of the Bland and Altman approach for determining the agreement between pairs of the three methods used for Hb measurement**

Reference method	Testing method	Mean of the difference	Limits of agreement	p-value for the difference
Coulter	Cobas	-0.030	$\pm 1.13$	0.588
Coulter	HemoCue	0.100	$\pm 0.78$	<0.001
Cobas	HemoCue	0.129	$\pm 1.02$	<0.001

any two methods. These limits are also shown in Table 4. The pair Coulter/Cobas gave a limit of agreement of 1.13 g/dl, while pairs involving the HemoCue method show lower limits of agreement (Coulter/HemoCue = 0.78 g/dl and Cobas/HemoCue = 1.02 g/dl). The limit of agreement reflects the dispersion of the data around the mean of the difference (illustrated in Figure 1).

## DISCUSSION

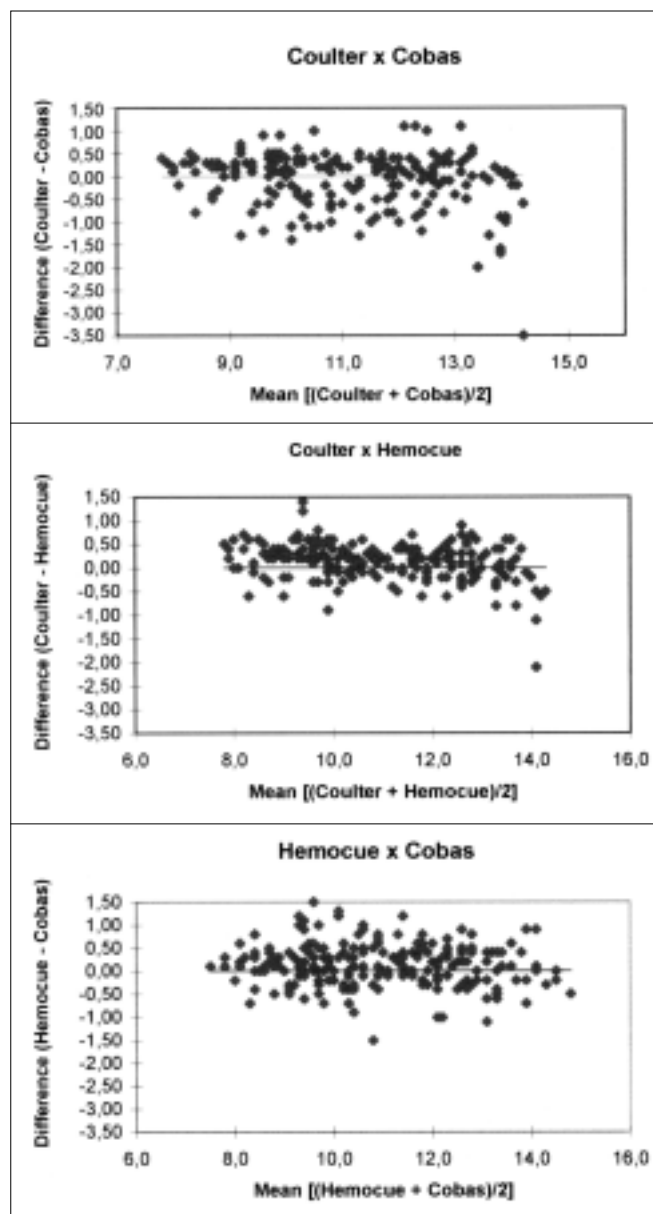
Taken as a whole, our data indicates that the Coulter and Cobas methods show the best agreement and that the HemoCue method gives a lower Hb determination when compared to both the Coulter and Cobas methods. This difference is 0.10 and 0.13 g/dl in relation to the Coulter and the Cobas methods, respectively. However, pairs of methods involving the HemoCue seem to have narrower limits of agreement than the Coulter and Cobas combination. This is in accordance with the CV for the three methods. Thus, although the HemoCue method shows lower measurement of Hb levels, these measurements seem to fluctuate less when compared with other methods.

In this study we used samples from venous puncture collected directly into Vacutainer tubes containing EDTA-K<sub>3</sub>. This was done to standardize the measurement of the Hb. Part of the 1% difference (0.10g/dl) between the HemoCue system and the Coulter STKS can be explained by the fact that the HemoCue system compensates for turbidity in the blood sample. Turbidity due to lipids, for example, will give falsely elevated readings by Coulter and other instruments measuring photometrically at only one wavelength.<sup>1,3</sup> The ICSH method<sup>2</sup> accepts a turbidity of 0.003 absorbance units, which corresponds to 0.11 g/dl hemoglobin. Higher turbidity is expected since blood donors are not fasting.

It is also possible that the biochemical method used for Hb measurement (cyanmethemoglobin versus hemoglobinazide) could

have influenced our result. It should be mentioned that the cyanmethemoglobin is the method recommended by the ICSH.

The advantages of the HemoCue system in terms of rapidity, simplicity and portability would recommend it as a screening method not only in a blood bank setting but also in the physician's office.



**Figure 1** - Individual differences between Hb values (n=259) were plotted against the average Hb value as determined by pairs of Hb measurement methods. Each figure shows plots of the following differences and averages: A, Coulter-Cobas; B, Coulter-HemoCue; and C, Cobas-HemoCue. The line indicates the null difference.

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## RESUMO

**Contexto:** A medida de hemoglobina (Hb) é o indicador mais utilizado para a verificação de anemia, na triagem de doadores de sangue. Atualmente o método fotométrico mais utilizado é o da ciano-metahemoglobina. Como alternativa a essa tecnologia, a HemoCue desenvolveu um método fotométrico baseado na determinação da azida metahemoglobina.

**Objetivo:** Avaliar o desempenho de três métodos de dosagem de hemoglobina (Hb) em doadores de sangue. **Tipo de Estudo:** Estudo prospectivo utilizando amostras de sangue para comparar testes de dosagem de Hb. **Local:** Serviço de Hemoterapia do Hospital Israelita Albert Einstein, instituição privada de atenção terciária de saúde. **Amostra:** Foram colhidas amostras seriadas de sangue de 259 indivíduos no período de março a junho de 1996. **Variáveis Estudadas:** O desempenho dos testes e suas comparações foram realizados pela análise do coeficiente de correlação, regressão linear e comparação das médias. **Resultados:** Os coeficientes de variação para os três métodos foram: Coulter, 0.68%, Cobas, 0.82%, e HemoCue, 0.69%. Não houve diferenças estatisticamente significativas entre as médias das determinações de hemoglobina pelos três métodos ( $p > 0,05$ ). Os métodos Coulter e Cobas mostraram melhor concordância enquanto o método HemoCue teve medidas de hemoglobina menores. Entretanto, os pares Coulter/HemoCue e Cobas/HemoCue tiveram limites de concordância mais estreitos ( $\pm 0,78$  e  $\pm 1,02$ ) daquele obtido com o par Coulter/Cobas ( $\pm 1,13$ ). **Conclusões:** Os três métodos demonstraram excelente concordância para a dosagem de hemoglobina.