Drops of capillary blood are not appropriate for hemoglobin measurement with HemoCue: A comparative study using drop capillary, pooled capillary, and venous blood samples

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Article

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Abstract

Background: Population-based surveys matched by time and in the same country, but which use different methodologies for determining hemoglobin (Hb) concentration, have shown inconsistencies in estimating anemia prevalence. This study aims to estimate measurement errors in Hb using the HemoCue 201+ in capillary blood, both in drops and pooled, compared with venous blood analyzed both with the HemoCue 201+ and clinical methodologies as references.

Methods: 149 participants (children, women, and older adults) interviewed in their homes in Jojutla, Morelos, Mexico, were randomly allocated to donate either a drop of capillary blood or 350 µL of pooled capillary blood. Each group also donated 4 mL of venous blood. Hb was analyzed in duplicate using the HemoCue 201+ and by a reference method using cyanmethemoglobin. We also compared the latter method with a clinical hemocounter. We performed Bland Altman and Lyn's concordance analyses. We calculated 95% confidence intervals (±95% CI).

Results: A positive bias was found in HemoCue 201+ (0.314 g/dL) which was used as an adjustment factor. After adjustment, both venous blood (0.000±0.33 95%CI -0.649, 0.649) and pooled capillary blood (-0.02± 0.36; 95%CI -0.75, 0.70) using HemoCue reported the same average Hb concentration as that estimated using venous blood with the cyanmethemoglobin method, and within a variation below 0.7 g/dL in the same individuals. Single drops of capillary blood produced a higher positive bias (0.425±0.81; 95%CI -1.176, 2.026) despite adjustment, and the variation between duplicate of hemoglobin was ±1.6 g/dL (95%CI -1.176, 2.026), and with some results as high as 2-3 g/dL. Comparatively, the variation of the cyanmethemoglobin method and the clinical hemocounter with venous blood was ±0.3 g/dL (95%CI -0.23, 0.38 ug/dL).

Conclusions: Random variation of results from drops of capillary blood cannot be corrected, affecting the accurate and precise measurement of Hb concentration from this source. Pooled capillary blood, in our hands, performed similarly to venous blood and may be useful as an alternative to venous blood when the latter is difficult to obtain. In any case, the popular use of drops of capillary blood for estimating Hb concentration both in individuals and in populations should be discontinued.

Background

Accurate anemia diagnosis among individuals and among population strata is essential to the design, monitoring, and evaluation of interventions and public policies associated with the reduction of this global health problem [1, 2].

Today, portable photometers (mostly HemoCue brand) are commonly used for quick and simple determinations of hemoglobin concentration. The model 201+ is factory calibrated against the hemoglobincyanide (HiCN) method [3], and has a stated accuracy of 1.5%. [4]. These devices are used extensively in population-based surveys to determine anemia prevalence using a drop of capillary blood (either from the heel for children younger than one year of age, or through finger prick for older individuals). However, values for anemia prevalence using this methodology have generally been much higher than those using venous blood in the same populations and in similar moments, as documented in Nepal, Jordan, Guatemala, Malawi, India, and other countries [5–9]. Recently, Hruschka et al [10] documented large inconsistencies in hemoglobin distribution and anemia estimation (from 9 to 31 percentage point differences) using capillary versus venous blood in two types of national representative surveys: the Demographic Health Survey (DHS) and the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project, as paired by country and time using HemoCue devices. Previously, other authors have discouraged the use of finger prick blood to determine hemoglobin concentration [11, 12], and some have evaluated the performance of Hb estimation from capillary pooled blood [11, 13], which may have practical applications in population-based surveys in the absence of venous blood. Nevertheless, finger prick blood is still commonly used for hemoglobin determination worldwide.

We found anemia trends with inconsistencies which are very difficult to interpret in the several national surveys (from 1999 to 2018-19) in Mexico [14–16]. Moreover, Mexican surveys matched by time and using same methodology for Hb estimation (Hemocue 201+, capillary drop blood) show different prevalence of anemia in children [17, 18].
In this study, we aimed to estimate measurement errors in Hb determination using HemoCue's in capillary blood, both drops and pooled, and compared against venous blood analyzed in the same HemoCue device as well as reference clinical blood analyzers (i.e., hemocounters). In this study, we used the cyanomethaemoglobin method as reference with venous blood collected from the same individuals, across groups including children 1–4 years, adult women (18–49 years), and the older adults (over 60 years).

Methods

Sampling design

This cross-sectional study was carried out between November and December 2020 in Jojutla, which is a small town at 970 meters above sea level in Morelos, Mexico. Samples from three different groups were collected: children aged 1-4 years, adult women (18-49 years old), and older adults (60 years and older). In each group, half of the individuals provided single drops of capillary blood by finger prick, while the other half provided pooled capillary blood (350-500 µL). At the same time, venous blood samples (4 mL) in vacutainer tubes containing K-EDTA were obtained from all individuals in the two groups. In total, we gathered samples from 49 children (1-4 years), 50 adult women, and 50 elderly persons. Inclusion criteria included willingness to participate, and we sought to include children of 1 to 4 years in similar age proportion and of both sexes, adult women and older adults of both sexes (25 men and 25 women).

Exclusion criteria included the presence of fever, cold, suspected exposure to or symptoms of COVID-19; being a woman who was pregnant, lactating, or with a history of cancer, chemotherapies, or mastectomy; or self-report of any disease related to hematological disorders.

Participants with hemoglobin measurement below 8 g/dL in the first HemoCue measurement using capillary blood (drops or pooled) were excluded and referred to a health care provider for further treatment.

Blood samples were collected by six trained and standardized personnel with previous experience collecting blood samples. Only one HemoCue apparatus per each surveyor was used for every aspect of the study. Personnel were instructed to collect blood samples in only one attempt, either through the finger (capillary blood) or the left arm (venous blood). If upon the first attempt insufficient capillary blood was collected (<350 µL), the individual was excluded from the study. In total, two children (8%) were excluded because of insufficient pooled capillary blood sampling, and two more children (8%) were excluded for venous blood sampling due to parental refusal for this blood collection after the capillary blood sample was already extracted. No adult female or elderly participant blood samples were excluded for these reasons.

Individuals were selected through a four-stage sampling design. In the first stage, basic geostatistical areas were identified using a proportional probability of the population under four years old. In the second stage, four street blocks were randomly selected; in the third stage, six houses in each block were selected by systematic sampling. Finally, in the fourth stage, one child of each year of age between one and four (12-23 months, 24-35m, 36-47m, and 48-59m), one adult woman, and one older adult were selected in each household.

Demographic information was collected using ad hoc questionnaires. All methods were performed in accordance with the approved protocol, relevant guidelines and regulations of our review board of the National Institute of Public Health.

Blood collection procedures and laboratory measurements

Capillary blood samples

Participants were randomly allocated into one of two groups according to the procedures and techniques summarized in Figure 1. In the group named "single drop", procedures were as follows: a finger prick was made on the left hand using high-flux BD lancets (pink for children - Mexico catalog key: 080.574.0032, code number: 366593, and blue for adults -Mexico catalog key: 080.574.0032, code number: 366594), and the first drop of blood was wiped away with a sterile 2×2 gauze. Then, the second and third capillary blood drops were placed on two microcuvettes (batch: 1903391) to measure two readings of hemoglobin in situ in HemoCue +201 devices. In the group called "pooled", capillary blood samples were obtained as follows: a finger prick was
made on the left hand with a lancet BD, as previously described. The first drop of capillary blood was wiped away with a sterile 2×2 gauze. The second drop and the subsequent drops were placed into the microtainer with K₂-EDTA (code number: 365974) to make 350–500 µL. Once sufficient capillary blood was collected, the microtainer was rotated and inverted 20 times to assure the mixture of the capillary pool with the anticoagulant (K₂-EDTA). A capillary tube (free mineral) was then inserted into the microtainers in a 45° angle to collect ≈40 µL of capillary blood and two drops of blood from the capillary tube were placed in two different microcuvettes to measure Hb in the HemoCue 201+ device. Then, the microtainer was capped, labelled, and stored at 5°C to be sent to the central laboratory in Cuernavaca, Morelos, for hemoglobin concentration measurement using the cyanmethemoglobin method.

**Venous Blood Sample**

All participants provided a sample of 4mL of venous blood from the left arm, collected in vacuum tubes with EDTA as anticoagulant (BD, code number 368171). Then, the vacuum was rotated and mixed 10 times to assure adequate mixture of the blood with the anticoagulant. A capillary tube was used to collect ≈ 40 µL of whole venous blood and two drops of blood were placed in two different microcuvettes (batch: 1903391) to measure hemoglobin in situ in HemoCue 201+ devices. The remaining venous blood was labelled and stored at 5°C to be sent to the central laboratory for hemoglobin concentration measurement using the cyanmethemoglobin method, as well as using the clinical commercial hemocounter ABX micros 60.

Figure 1 shows the diagram of measurements undergone by the participants.

**Laboratory measurements**

Samples of pooled capillary blood and venous blood were sent to the central laboratory the same day of sample collection. For both types of samples, hemoglobin concentration was determined using the cyanmethemoglobin method [19] in the equipment Beckman Coulter Ac•T 5diff® [20]. Hemoglobin concentration from venous samples was additionally quantified by a conventional method using a non-polluting generic lysing reagent (Diagon-Dya Lyse, code number: 20212) in the equipment ABX Micros 60® according to the manufacturer’s manual [21]. The handling of venous and pooled capillary blood samples in both hemocounters at the central laboratory was performed only by one experienced technician. The results of the cyanmethemoglobin method were considered as reference values (i.e., “gold standard”).

**Statistical Analysis**

**Exploratory analysis and data cleaning**

We decided as part of the design that any standardized differences between capillary blood Hb and venous blood Hb using cyanmethemoglobin ≥ 5 SD were eliminated from analysis as this may be reflective of a defective sample. This occurred in only one sample of pooled capillary blood, where the hemoglobin measurement was high as compared with venous blood and drop of capillary blood samples from the same individual. This may have been caused by insufficient mixing of the sample which favoring clotting.

The concordance correlation coefficient was used to evaluate the level of agreement between hemoglobin measurements of the different capillary blood source groups against venous blood analyzed using the gold standard. The Bland and Altman mean difference was used as an estimate of the absolute bias. The potential bias of venous Hb from HemoCue versus cyanmethemoglobin was used as correction factor for all Hb measurements obtained from HemoCue. The Pearson correlation coefficient was also used to determine the potential linear response of the methods used within the range of hemoglobin concentrations measured in the studied population.

A 95% distribution interval for the differences between duplicated Hb HemoCue measurements from drop and pooled capillary, and venous blood samples was used for comparison. The 95% distribution interval was obtained using the mean difference ± 1.96 times the standard deviation (SD).
A linear mixed model was used to study mean differences in Hb measurements. In the fixed component of the model, mean differences were estimated between Hb measurements using HemoCue 201+ as compared to the gold standard; both sampling methods of capillary blood were included in comparisons and the model was adjusted by age group and sex. In addition, the random component of the model allowed the estimation of variance components of the individual study subjects as natural variation, and the variability between the blood sample measurements in each subject as measurement error.

Statistical significance was set at alpha=0.05 for a 95% confidence. All analyses were performed using Stata v16.

Results

Table 1 shows participant characteristics by capillary blood source group. No differences were observed by age or sex (groups of children and elderly). No differences were observed among the mean of Hb concentration in venous blood analyzed using the cyanmethemoglobin method among the groups. Therefore, the capillary blood source group “drops” was comparable to the group “pooled”.

Table 1. Descriptive characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th><em>Drop</em> capillary blood</th>
<th>Pooled capillary blood</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>75</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Age (years, mean ±SD, [range])</td>
<td>35.5 ± 30.5 [1 to 90]</td>
<td>35.7 ± 28.7 [1 to 90]</td>
<td>0.974</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1 to 4y</td>
<td>33.3</td>
<td>32.4</td>
<td></td>
</tr>
<tr>
<td>18-45 y</td>
<td>33.3</td>
<td>33.8</td>
<td></td>
</tr>
<tr>
<td>≥60 y</td>
<td>33.3</td>
<td>33.8</td>
<td>0.993</td>
</tr>
<tr>
<td>Sex (female, %)</td>
<td>70.6</td>
<td>64.4</td>
<td>0.449</td>
</tr>
<tr>
<td>Hb* (g/dl) [range]</td>
<td></td>
<td>12.6 ± 1.3 [9.2 to 15.7]</td>
<td>0.768</td>
</tr>
</tbody>
</table>

*Measured in venous blood by cyanmethemoglobin

Comparative performance of cyanmethemoglobin method with the hemocounter ABX Micros 60 method

Figure 2 shows the correlation, and the Bland Altman concordance between the cyanmethemoglobin method and the Hemocounter ABX Micros 60 using all venous blood samples. The 95% confidence interval between results using these two methodologies was ±0.294 g/dL (95%CI -0.23, 0.38) in the range from 8 to 16 g/dL. Hemocounter ABX had a small positive and negligible bias (0.07 g/dL) when compared to the cyanmethemoglobin method. Only one value was outside that range, and it was below 1.0 g/dL. These results show the high accuracy, precision, and reproducibility of both these methods, which appear practically interchangeable.

Verification of the HemoCue versus cyanmethemoglobin method

In order to determine whether any combined factors (from HemoCue or from personnel) were yielding systematic error, we analyzed the results of each one of six HemoCue tests used against the results from the same venous samples using the cyanmethemoglobin method. Table S1 (supplementary material) shows the results, which reveal a greater bias of the HemoCue as compared to cyanmethemoglobin, with an average Hb concentration difference of 0.314 g/dL. Only the HemoCue apparatus used by one surveyor (#6) showed a smaller bias. This value was in the higher extreme of the 1.5% variation claimed for this equipment.
**Precision of methods**

*Figure 3* summarizes the variation of differences between duplicate measurements of the different blood source samples analyzed in HemoCues. Venous blood and pooled capillary blood produced results with similar variations. The 95% confidence interval for differences in the HemoCue using venous blood samples was ±0.5 g/dL (95%CI -0.565, 0.392), and ±0.6 g/dL (95%CI -0.599, 0.550) for pooled capillary blood. However, the variation of consecutive drops of blood from the same individual was much higher: ±1.2 g/dL (95 % CI -1.303, 1.005), meaning that the SD of the differences in paired blood drop samples were twice the SD for pooled capillary blood. This difference is relevant to ensuring accuracy, precision, and reliability, both in individuals and in populations, when only one drop of blood is analyzed.

**Comparing venous blood and pooled capillary blood**

We estimated the mean Hb difference of pooled capillary *versus* venous blood from the same individuals using the reference method (i.e., cyanmethemoglobin). Pooled capillary blood produced a slightly higher Hb concentration value (of »0.1 g/dl) than venous blood. This difference appears to be biological, with slightly higher concentration of hemoglobin present in pooled capillary blood. *Figure S1* (supplementary material) summarizes the variation between capillary and venous blood samples analyzed with the reference method. The 95%CI interval of differences between capillary blood and venous blood using the reference method was ±0.8 g/dL, 95%CI (-0.737, 0.931).

*Hemoglobin measurement with the HemoCue across different blood sample sources*

*Figure 4* shows the Bland-Altman plots of hemoglobin duplicate measurements using HemoCue in venous blood, pooled capillary blood, and drops of capillary blood against the measurements of the corresponding paired samples analyzed using the cyanmethemoglobin method, before and after adjustment for the HemoCue bias. After bias adjustment, both venous blood and pooled capillary blood in HemoCue showed the same hemoglobin average as that estimated using venous blood with the cyanmethemoglobin method, and which were higher before the adjustment. However, despite adjustment, the single drops of capillary blood maintained a higher positive bias. As expected, the variation of Hb concentration results increased from venous blood to pooled capillary blood and to drops of capillary blood. Thus, the 95% variation intervals were ±0.6 95%CI (-0.649, 0.649), ±0.7, 95%CI (-0.75, 0.70), and ±1.6 g/dL 95%CI (-1.176, 2.026), respectively, and for drops of capillary blood several samples were 2.0 to 3.0 g/dL away from the reference value. This shows that samples using single drops of capillary blood risk producing erroneous results as high as 20-30% of the real Hb value, and that those are randomly distributed (higher or lower) around the true value despite adjusting for the HemoCue bias. The variance ratio test for the dispersion of differences between drop capillary/venous blood was p<0.001; and for pooled capillary/venous blood was p=0.1291, respectively.

By age group, results were consistent. Higher dispersion were observed in the drop capillary blood in all age groups, being highest in children, followed by older adults and adult women. (*Table S2 and Figure S2* -supplementary material). In all age groups, the variance ratio test in drop based hb measurements were statistically different from the dispersion of differences in pool based hb measurements (p<0.05) as well as in venous (p<0.001). The variance ratio test in pooled capillary/venous blood was different in children (p=0.045), but not in adult women (p=0.4327) nor older adults (p=0.999).

Table 2 summarizes the comparative data of all Hb measurements using HemoCue *versus* cyanmethemoglobin, before and after bias adjustment. Pooled capillary blood shown a similar Hb mean difference to venous blood, and much higher precision than the drops of capillary blood group. Higher concordance was observed before and after bias adjustment for pooled capillary blood group (0.93 and 0.96) than drops of capillary blood group (0.76, 0.82), respectively. In both groups, as expected, venous blood analyzed with HemoCue yielded the highest correlation and concordance, and lowest Hb difference as compared to venous blood analyzed with the cyanmethemoglobin method. Results by age group are present in *Table S2* (supplementary material).

Table 2. Comparative results of blood sample sources analyzed using the HemoCue *versus* venous blood samples analyzed using the cyanmethemoglobin method
Comparison | Sampling method using HemoCue | Concordance | Pearson correlation | Relative bias | Hb mean difference (g/dL)
--- | --- | --- | --- | --- | ---
"Pooled" Capillary Blood |  |  |  |  |  
Before bias adjustment | Venous | 0.95 | 0.97 | 0.97 | 0.26
Pool (first and second lecture) | 0.93 | 0.96 | 0.97 | 0.29
Pool (first lecture) | 0.94 | 0.96 | 0.97 | 0.28
Pool (second lecture) | 0.93 | 0.95 | 0.97 | 0.3
After bias adjustment | Venous | 0.98 | 0.98 | 1.00 | -0.05
Pool (first and second lecture) | 0.96 | 0.96 | 1.00 | -0.02
Pool (first lecture) | 0.97 | 0.97 | 1.00 | -0.03
Pool (second lecture) | 0.95 | 0.95 | 1.00 | -0.1
"Drops" of Capillary Blood |  |  |  |  |  
Before bias adjustment | Venous | 0.94 | 0.97 | 0.97 | 0.36
Drop (second and third drop) | 0.77 | 0.86 | 0.89 | 0.74
Drop (second drop) | 0.79 | 0.87 | 0.91 | 0.66
Drop (third drop) | 0.75 | 0.86 | 0.87 | 0.81
After bias adjustment | Venous | 0.97 | 0.97 | 0.99 | 0.05
Drop (second and third drop) | 0.83 | 0.86 | 0.96 | 0.43
Drop (second drop) | 0.84 | 0.87 | 0.97 | 0.35
Drop (third drop) | 0.81 | 0.86 | 0.94 | 0.50

Figure S3 (supplementary material) shows the frequency distribution of differences around the mean, before and after adjustment, using venous blood, pooled capillary blood, and drops of capillary blood. Adjustment for the HemoCue bias was required independently of blood sample source, and results for drops of capillary blood continue being erroneous despite adjustment.

**Mixed linear regression model for differences in Hb measurements**

Results for the adjusted mixed linear regression model are presented in Table S3. Mean difference of Hb from the pooled capillary blood group did not vary significantly from venous blood HemoCue measurements ($b=0.02$, $p=1.00$). This means that, despite a larger variation of the results than those with venous blood, pooled capillary blood may be useful for determining hemoglobin concentration and is therefore a type of sample that may be collected in population-based surveys when collecting venous blood is not possible.

By age group, both adult women and older adults showed the highest mean difference through drops of capillary blood. A marginally significant difference was observed for both Hb in capillary blood using the cyanmethemoglobin method and Hb in venous blood using the ABX Micros 60 hemocounter, as compared to venous blood using cyanmethemoglobin ($b=0.09$, $p=0.09$ and $b=0.08$, $p=0.059$, respectively).

No differences in the variation of the results were observed in Hb concentration between the first and second measurements of the pooled capillary blood group ($b=0.02$, $p=0.911$), nor of the drops of capillary blood group ($b=0.14$, $p=0.571$), nor of the
venous blood samples \((b=0.06, p=0.778)\) using HemoCue. The two Hb measurements of drops of capillary blood were equally different from the reference value, as shown in the Bland Altman analysis (Table S4 - supplementary material). This is evidence that these measurements have higher random variation and, hence, produce less reliable results. By considering the average of the two Hb measurement, dispersion of Hb did not improve the estimation (Table S4 - supplementary material).

Random effects from the mixed model showed that natural variation (by subject) represented over 90% of total variance, and 82.5% in children. This implies that measurement error is higher in children (17.5%) than in other age groups analyzed (3% for adult women and 7.3% for older adults). This emphasizes the importance of proper sampling procedures and well-trained personnel for blood sample collection in children (Table S5 - supplementary material).

**Discussion**

Our results include several major findings: (a) pooled capillary blood samples, in our hands, show acceptable performance in Hb measurement using HemoCue 201+ when compared with venous blood samples; (b) drops of capillary blood showed the largest Hb measurement variations and inaccuracy than pooled capillary or venous blood measurements using HemoCue 201+; (c) variation of results with drops of capillary blood is random, and therefore not correctable; (d) the HemoCue 201+ device in our study showed a positive bias (that is a systematic error) which can be corrected by adjusting the Hb values of the same blood samples (reference samples with hemoglobin concentration within the range of hemoglobin concentrations of 8-16 g/dL) analyzed in HemoCue and by a laboratory reference method; and (e) the HemoCue measurements (considering the measurement error from personnel) contributed to low variation in the Hb measurement system performance as expected (7%), with the main source of variability being the individual heterogeneity of the same blood samples, especially in drops of capillary blood. Variation from measurement error was highest in children, which reflects the challenge of obtaining good quality blood samples in this population group.

Results of hemoglobin concentration values as estimated using drops of capillary blood in the HemoCue 201+ varied widely from the true values calculated using venous blood, such that they could not be considered reliable. In addition, the higher intra-variability in subsequent drops of capillary blood is additional evidence that this is not a suitable source for measuring hemoglobin concentration as the error can be as high as 20-30% of the true value. Bond [12] documented a coefficient variation 3.4 times for Hb for subsequent drops than in venous blood measured in a hematology analyzer.

Our results coincide with Conway et al [11], who discourage the finger prick for drops of capillary blood to estimate Hb values due to larger variation than when using pooled capillary blood samples and, logically, much higher than using venous blood. Bond et al [12] also concluded that hemoglobin measurement using several drops of blood may be necessary to reduce variation, but this is neither practical nor economically sensible. In our study, two measurements from capillary drops did not reduce the variation. Our results suggest that drops of capillary blood introduce considerable misinterpretation of the anemia status of individuals (under- or overestimation) and populations (overestimation of the prevalence, due to the use of a threshold value to estimate anemia prevalence).

Pooled capillary blood produced a slightly higher difference in Hb concentration of »0.1 g/dL than venous blood, but which we considered small enough to not necessitate corrections. The high concordance of Hb from pooled capillary versus venous blood found in our study highlights that pooled capillary blood may be considered an acceptable alternative for estimating Hb using a portable photometer in population-based field studies when the collection of venous blood is challenging. Dasi et al [13] reported a similar finding: a concordance of 0.97 from Hb from pooled capillary blood measured by a portable autoanalyzer versus venous blood measured using a clinical method. The reliability of Hb measurement from pooled capillary blood data may be explained by the low variability due to collection of a greater amount of blood as compared to single drops of blood. Nevertheless, pooled capillary blood variability might be affected by other factors, such as insufficient training and standardization of personnel who collect the blood sample (as discussed below), time duration in collecting the sample, and postural effect and a defective mixed sample [22]. In the Hb distribution in pooled samples, we found that six values were out of the expected range, which may suggest coagulation of blood samples during collection due to possible failure to adequately mix the blood with the anticoagulant. In order to narrow the ratio of EDTA to blood, our study collected 350-500 µL of blood. Previous
data analyzing Hb from venous blood with EDTA versus heparin did not find statistically significant differences in Hb determination (Méndez Gómez-Humarán I, in process of publication).

One study performed in Guatemala and Honduras showed HemoCue bias with the opposite tendency to that observed in our study, finding a systematic and negative bias (-0.13g/dL, \(p = 0.01\)) in Hb measurement with HemoCue 201+ models. Nevertheless, a positive bias (+0.24 g/dL, \(p < 0.001\)) was found with the HemoCue 301[23]. Therefore, the bias may depend on the apparatus and not the model, so the performance of each apparatus should be verified before use and its performance examined periodically. Calibration to manufacturer standards is insufficient, as it checks optical conditions of the apparatus but not accuracy of measurement against blood samples [3]. The latter is especially important if the apparatus is intended for use in population-based surveys where small changes may have important implications for the measured distribution of hemoglobin concentration in the population. Systematic bias can be easily corrected, either by adjusting the results of the apparatus using blood standards analyzing them with a reference method and in each portable photometer or by collecting additional blood samples in a subsample of the study population for analysis with a clinical hemocounter.

Our findings may explain the discrepancy in estimations of anemia prevalence among surveys [14–18] when verification of the equipment is uncommon, and the blood samples are frequently drops of capillary blood. Recently, Hruschka et al [10] documented wider discrepancies in Hb distribution and substantial differences (from 9 to 30%) in anemia estimation from two nationally representative survey matched by time and country when using the HemoCue device as compared to other methodologies. The DHS used capillary drop blood, while the BRINDA project used venous blood. The median difference in anemia prevalence was consistently lower in BRINDA surveys than in DHS surveys (+19% in children and +9% in women).

Some studies have documented higher Hb values (in the range of 0.3 to 0.8 g/dL) from HemoCue 201+ when using a hemocounter [24,25], while others have not found a bias in the equipment. Our data came from a field setting scenario, where participants were interviewed at home in conditions outside clinical settings, and without humidity and temperature control, which are factors that might affect the stability of the HemoCue cuvettes and electronic performance of the HemoCue 201+ device [26,27]. A previous study done in San Luis Potosí, Mexico using HemoCue 201+ reported failures in completing Hb readings in locations with high temperatures (>35°C) (Berenice Gaona, personal communication, data not published).

In summary, it is important to emphasize that the main reason for errors in the determination of hemoglobin using HemoCues is not the bias of the apparatus but the large and random variation if using single drops of blood obtained by finger prick. This measurement error was highest in children (17%) as compared to women (3%) and older adults (7.3%). Since this error is not amenable to correction, it may impact the anemia prevalence estimates, with the greatest affect in estimations for children.

Different sources of error may affect the accuracy and precision of the Hb measurements [27]. Well-trained and standardized personnel are a crucial factor which minimizes potential error in Hb determination in field studies. In the present study, personnel were instructed to puncture only once (in the finger for capillary blood or the arm for venous blood) to avoid participant exclusion, especially due to the refusal of mothers to allow their children to participate. Our success rate in collecting pooled capillary blood was very high (97.3%) in all samples in comparison with a study done in Honduras [23], where non-trained health or medical personnel were involved. In that study, pooled capillary blood samples could not be collected in a high proportion of children (38/46, 83%) or some women (3/24 = 12%). We attribute the success rate of our study, both in pooled capillary and venous blood samples in children and adults, to the time invested (around two weeks) in training, sensitization, and standardization of our personnel for collecting and handling blood samples in field settings. These are important skills that must be assured for the reliable human measurement of Hb, independently of the method. The assumption that drops of capillary blood are suitable for estimating Hb concentration is incorrect. Our results confirm previous recommendations to discourage the use of single drops of capillary blood collected by finger prick for Hb measurement [11,12,22].

Other sources of error may include at the time of loading blood into the HemoCue micro-cuvettes, which increases the magnitude of error using these devices [22]. This may be even worse if unexperienced personnel are involved.

Here, it is important to note that our results differ from one previous validation study using the HemoCue (B-Hemoglobin model) done in a hospital in Mexico [28]. The results of that study found a non-statistically significant bias from drops of capillary blood
in both children and adults (-0.14±1.05 and -0.12±0.87 \( p=0.025 \), respectively), as compared to pooled capillary (-0.29 ±0.78 in adults) and venous (-0.47±0.53 in adults) blood. Nevertheless, larger variation from drops of capillary blood, particularly in children, was consistent with our study [28]. Notably, the previous study was performed in clinical setting (hospital), while our study was performed in a field setting where external factors may have affected methods.

The following should also be considered when interpreting our results and comparing them to conflicting data reported in population-based surveys: we used high-flow BD-type lancets, which are characterized by a higher number of bezels (pentapoint) and allow high blood flux. Most national surveys use low-flow lancets that cause a shallow wound (one bezel), likely with the aim for less invasive methods. However, the one bezel lancet may restrict the flux of red blood cells and therefore increase inaccuracy and variation in hemoglobin concentrations estimated using single drops of capillary blood. Our study did not examine the effects of using low-flow lancets.

To our knowledge, this is the first study to evaluate the results of hemoglobin concentration measurements using the HemoCue in a field setting scenario with different blood sample sources: venous blood, pooled capillary blood, and drops of capillary blood. Furthermore, it is common that the performance of HemoCues is not verified using venous blood and a reference methodology; our results demonstrate the importance of estimating the bias of each HemoCue apparatus against venous blood which is further analyzed using a clinical hemocounter.

Finally, it is important to highlight that our validation study was done in a field setting, and therefore had marked discrepancies from those studies done in a clinical setting. Nonetheless, our main findings related to drop-to-drop variability as well as pooled capillary blood variation were consistent with previous studies in clinical settings. Therefore, verification and adjustment of the results of HemoCues should be done before their use in the field. This verification should also be periodically confirmed to maximize the reliability of the results [29].

Determination of anemia prevalence in both individuals and in populations requires the use of reliable procedures and methodologies, because producing erroneous results may cause serious negative consequences, and waste of resources and time, that using only clinical indicators.

**Conclusion**

Venous blood is the ideal type of blood sample to determine hemoglobin concentration using HemoCue and similar devices. However, when collection of venous blood is not possible, pooled capillary blood samples may be a valid alternative if collected by experienced personnel. Nevertheless, it is important to bear in mind that measuring hemoglobin concentration is insufficient for estimating anemia prevalence. It is also essential to identify the etiological factors of anemia, for which the collection of sufficient amounts of blood is needed to assess the associated biomarkers. Therefore, it is important to determine whether samples of pooled capillary blood are sufficient for satisfying these other measurements.

We also conclude, as previous authors have done, that reliance on samples using drops of capillary blood should be discontinued for the determination of hemoglobin concentration in both individuals and populations.

**Declarations**

**Ethics approval and participant consent**

The study protocol was approved by the Research, Research Ethics, and Biosecurity Committees in the National Institute of Public Health. An informed consent form was signed and submitted by all adult participants. Parents of children 1-4 years of age gave their informed consent on behalf of their children after a thorough explanation of study procedures. Approval number: 1652

**Consent for publication**
Availability of data and materials

The datasets used and/or analyzed throughout the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

VDG, TSL, IMGH and OD. designed the research; VDG, BGP and TS conducted the research; IMGH, OD and VDG. analyzed the data; and VDG, BGP, TSL, IMGH and OD wrote the manuscript. VDG had primary responsibility for the final content. All authors read and approved the final manuscript. The contents of this article do not necessarily reflect the views of USAID nor the United States Government where OD is affiliated.

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References


Figures

![Figure 1](image-url)

*One pooled capillary blood sample was excluded due to a large difference (> 5 SD) between Hb capillary blood as compared with Hb venous cyanmethemoglobin; this was likely caused by coagulation of the capillary blood.

Figure 1

Procedures and measurements
Correlation, Concordance and Bland-Altman plot for venous blood samples using the cyanmethemoglobin method and the Hemo-Counter ABX Micros 60.
Box plots of hemoglobin difference* (g/dL) between measurement duplicate analyzed using the HemoCue 201+

* Bland Altman Mean ± SD; (95%CI)

Figure 3

Box plots of hemoglobin difference* (g/dL) between measurement duplicate analyzed using the HemoCue 201+
**A) Before adjustment**

<table>
<thead>
<tr>
<th>Blood Sample Source</th>
<th>Mean $\delta$ Hb</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous blood</td>
<td>$0.314 \pm 0.33$</td>
<td>(-0.335, 0.963)</td>
</tr>
<tr>
<td>Pooled capillary blood</td>
<td>$0.292 \pm 0.36$</td>
<td>(-0.43, 1.014)</td>
</tr>
<tr>
<td>Drops of capillary blood</td>
<td>$0.739 \pm 0.81$</td>
<td>(-0.862, 2.34)</td>
</tr>
</tbody>
</table>

**B) After adjustment**

<table>
<thead>
<tr>
<th>Blood Sample Source</th>
<th>Mean $\delta$ Hb</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous blood</td>
<td>$0.000 \pm 0.33$</td>
<td>(-0.649, 0.649)</td>
</tr>
<tr>
<td>Pooled capillary blood</td>
<td>$-0.02 \pm 0.36$</td>
<td>(-0.75, 0.70)</td>
</tr>
<tr>
<td>Drops of capillary blood</td>
<td>$0.425 \pm 0.81$</td>
<td>(-1.176, 2.026)</td>
</tr>
</tbody>
</table>

*The values of the HemoCue were adjusted by subtracting the average bias of $0.314 \text{ g/dL}$.*

**Figure 4**

Bland-Altman plots of hemoglobin concentration determined using the HemoCue in different blood sample sources *versus* using the cyanmethemoglobin method with venous blood.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial190722.docx](#)