Validation of hemoglobin measurement in venous blood using HemoCue for the Ensanut 2022

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Abstract

Objective. To validate hemoglobin (Hb) concentration in venous blood (VB) using the HemoCue 201+ in a subsample of children and women from the Mexican National Health and Nutrition Survey 2022. **Materials and methods.** Prior to field work 50 HemoCue 201+ devices were verified using venous blood. During the field work 57 children (aged 1-11) and 62 women (aged 12-49) donated 3 mL of VB each. Hb was measured in each device of HemoCue 201+ and in a hematologic autoanalyzer for the validation. **Results.** No significant bias was found in most of the devices. An adjustment criterion was used for 22 devices. Hb mean difference results were -0.049±0.578 g/dL in children and -0.098±0.628 g/dL in women. **Conclusions.** The HemoCue 201+ is a valid tool for estimating Hb concentration to produce reliable estimates of anemia prevalence when using venous blood.

Keywords: hemoglobin; venous blood; HemoCue; validation

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Resumen

Objetivo. Validar la metodología de determinación de hemoglobina (Hb) en sangre venosa usando HemoCue 201+ en una muestra de niños y mujeres, de la Encuesta Nacional de Salud y Nutrición 2022. **Material y métodos.** Previo al trabajo de campo, 50 HemoCues fueron verificados usando sangre venosa. Durante el trabajo de campo, 57 niños (1-11 años) y 60 mujeres (12-49 años) donaron 3 mL de sangre venosa. La Hb se midió en el HemoCue 201+ y en un autoanalizador hematológico para la validación. **Resultados.** No se encontró un sesgo significativo en la mayoría de los HemoCue. Se usó un criterio de ajuste para 22 HemoCues. La diferencia promedio de Hb fue de -0.049±0.578 g/dL en niños y -0.098±0.628 g/dL en mujeres. **Conclusiones.** El HemoCue 201+ es un instrumento válido para estimar la concentración de Hb usando sangre venosa y produce resultados confiables de la prevalencia de anemia a nivel poblacional.

Palabras clave: hemoglobina; sangre venosa; HemoCue; validación

Reliable hemoglobin (Hb) measurements are essential to monitoring anemia and evaluating the effectiveness of interventions designed to address anemia and micronutrient deficiencies.[1] Since 1999, the *Encuesta Nacional de Salud y Nutrición* (Ensanut) has been carried out to provide monitoring of anemia and other nutritional conditions and inform the design of public health policies which target populations at higher risk.[2]

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In Mexico, anemia has been recognized as a public health problem which particularly affects infants, pregnant women, and older adults. For years, the Ensanut has used drops of capillary blood obtained through finger pricking to measure Hb with HemoCue devices (B-Hemoglobin and 201+).[3] The most recent estimates of the prevalence of anemia reported in the Ensanut 2018-19,[3] suggest an increase over the past six years across all population groups.*[4] This pattern is surprising given an overall improvement of living conditions and the recent introduction of multiple interventions to improve nutritional status in the country.

Notable inconsistencies in Hb determination and anemia estimation have been reported in previous studies using drop capillary vs. venous blood.[5,6] In surveys paired by country and time, a substantial difference from 9 to 31 percentage points was revealed in the prevalence of anemia measured through drop capillary vs. venous blood using HemoCue devices (201+ and 301+).[7] Previously, we reported increments in the magnitude of the random variation (imprecision) in the determination of Hb concentration from capillary blood drops in comparison to pooled capillary and venous blood using the HemoCue 201+ using an hemocounter as reference. At the population level, this has implications when estimating anemia prevalence and its severity, since larger random variation of the results from finger-pricked blood translates to increased anemia misclassification.[8]

Measurement of Hb concentration through venous blood using an hematological analyzer is considered the gold standard for assessing Hb concentration. However, this method brings challenges in field settings, notably due to the need to transport blood samples to laboratories within short windows of time. The integrity of blood samples can also be altered by environmental conditions such as exposure to humidity and extreme temperatures.[9]

In preparation for the Ensanut 2022, the objectives of this study were to: 1) evaluate the accuracy and precision of 50 HemoCue 201+ devices to be used in field work, and 2) validate the results of Hb concentration and estimation of anemia prevalence through venous blood using the HemoCue 201+ against the gold standard method in a subsample of children aged 1-11 and women aged 12-49 who participated in the Ensanut 2022.

* De la Cruz-Góngora V, Shamah-Levy T, Villalpando S. Overview of trends in anemia and iron deficiency in the Mexican population from 1999 to 2018-19. In press, 2023.

Materials and methods

Ensanut 2022 data was collected from July to December 2022. The procedures, indicators, and scope of the survey have been published elsewhere.[10]

Study 1. Precision and accuracy of the HemoCue 201+

In June 2022, performance (accuracy and precision) of 50 HemoCue 201+ devices was verified and further calibrated. Our methodology was based on the determination of Hb concentration in venous blood samples of five adults (≥20 y) participants, who each donated 8 mL of venous blood that was collected in two vacutainer tubes of 4 mL each with ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. Tubes were inverted 10 times until blood was well mixed with the EDTA. One tube was used for the determination of Hb in each of the 50 HemoCue 201+ devices, and the other was sent to a laboratory for the determination of Hb in a certified hematology analyzer (SYSMEX XS-1000i, Norderstedt, Germany).

A trained phlebotomist performed venipuncture and venous blood collection, as well as Hb measurement, in all five participants in each of the 50 HemoCues. For each HemoCue, a different cuvette was used with the blood sample. Pasteur pipettes were used to transfer venous blood from the vacutainers to the HemoCue cuvettes in batches of 10 each. The HemoCues were numbered from 1 to 50.

To control for the effect of time on Hb determination in the blood sample (i.e., coagulation) in addition to using different HemoCue 201+ devices, each participant's measurements were initiated in different order. Participant 1 samples were measured starting with HemoCue 1 and ending with HemoCue 50. Participant 2 samples were measured starting with HemoCue 11 and ending with HemoCue 10. Participant 3 samples were measured starting with HemoCue 21 and ending with HemoCue 20. Participant 4 samples were measured starting with HemoCue 31 and ending with HemoCue 30. Participant 5 samples were measured starting with HemoCue 41 and ending with HemoCue 40. An assistant helped record this information.

Study 2. HemoCue performance in the field

For the field validation study, a subsample from three states of Mexico was selected from the ongoing Ensanut survey to evaluate the performance of the HemoCue devices. States were selected to account for variability in altitude, temperature, and humidity, and included Nuevo

León, Morelos, and Yucatán. For this subsample, 3 mL of venous blood were collected in vacutainers containing EDTA for 57 children aged 1-11 and 60 women aged 12-49. The tube was inverted 10 times until the blood was homogenized with the anticoagulant. Subsequently, a Pasteur pipette was introduced to take a sample and place a drop (~15 mcL) in the microcuvette to be placed in one HemoCue 201+ device. The Hb reading was recorded in g/dL. The remaining venous blood in the vacutainer tube was stored at a temperature of 5-10º C until it was transferred to a state-certified laboratory for subsequent Hb analysis in a conventional hematology autoanalyzer on the same day of sample acquisition. Only eight HemoCues were used for this subsample.

As per prior agreement with the health laboratories of each state, samples were analyzed the same day of blood collection and the resulting Hb concentrations were considered reference values. In Nuevo Léon, the CELLDYN 1800 ABBOTT was used; in Morelos the *XN-10* Automated Hematology Analyzer, and in Yucatán the XS 1000i™ Automated Hematology Analyzer and WIENER LAB COUNTER 19.

Statistical analysis

Study 1. Precision and accuracy of the HemoCue 201+

A concordance analysis was performed to study the precision and accuracy of measurements based on 50 HemoCue devices against the gold standard. Bland Altman's analysis was used to study the observed differences between Hb determinations.[11]

Since the 50 HemoCues were to be used in the survey, the mean of the differences of the Hb concentration in each HemoCue represented the estimated bias of each device. A random intercept model was used, the fixed part was used to estimate the mean difference of each HemoCue device with respect to the gold standard. The random part separates the variability due to the subjects, called the random intercept, and the residual variability represents the measurement error.[12] The mathematical representation of the model is:

$$Hb_i = \underbrace{\beta_0 + \sum_{j=1}^{k} \beta_j Hc_j}_{\text{fixed part}} + \underbrace{\mu_{0i} + \mu_{ij}}_{\text{random part}}$$

Where:

β_0 is the intercept of the linear model representing the mean Hb for the gold standard

β_j is the mean difference of HemoCue with respect to the gold standard

Hc_j is the dummy variable for the HemoCue

μ_{0i} is the random intercept for the subject

μ_{ij} is the residual error

A Bonferroni's multiple comparison technique was used to identify whether any HemoCue had a significant deviation from the gold standard. Bias correction is evaluated as an alternative for those HemoCues that presented relatively large mean differences. Bias adjustment was tested using the estimated mean differences from the model, centering the mean only for HemoCues with absolute differences higher than 0.1 g/dL.

Study 2. HemoCue performance in the field

A concordance analysis was performed to study the precision and accuracy of measurements for the subsamples of 57 children (aged 1-11) and 60 women (aged 12-49). Bland Altman's analysis was used to compare uncorrected and corrected HemoCue measurements obtained in the field, according to the results of study 1. Simple regression models were used to identify any differences in temperature and humidity between subsample localities.

Ethical considerations

The Ensanut 2022 and its venous blood validation component were approved by the Committee of Ethics in Research and Biosafety commissions of the National Institute of Public Health of Mexico. Participants provided written informed consent and children's parents authorized their child's participation.

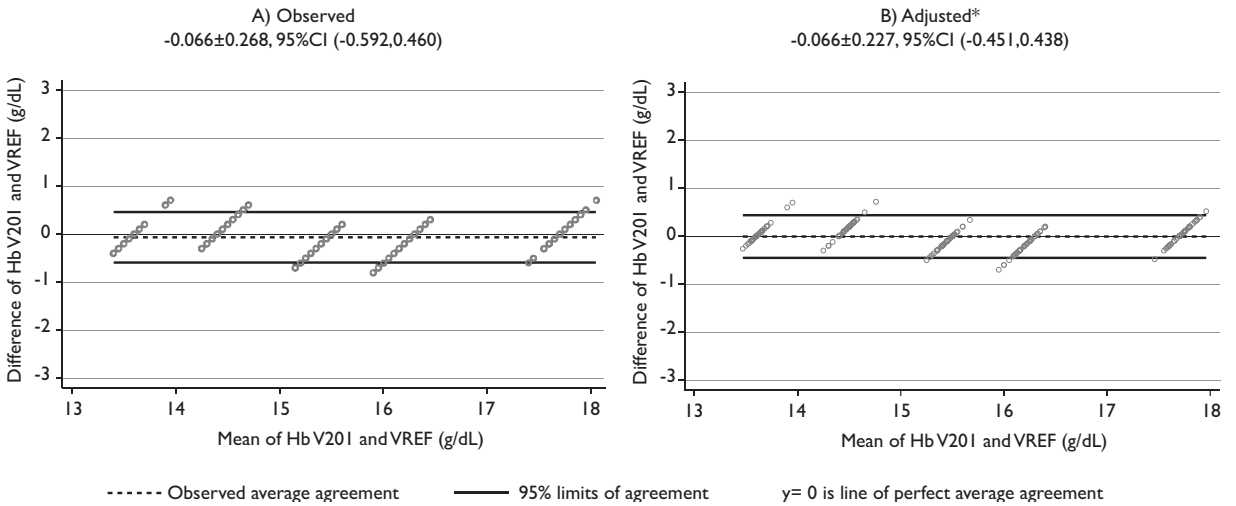
Results

Study 1. Precision and accuracy of the 50 HemoCue 201+

Hemoglobin concentration measurements using the HemoCue for the five participants showed a mean Hb concentration of g/dL, range 13.2 to 18.4 g/dL. Laboratory analysis showed a mean of g/dL, range 13.6 to 17.7 g/dL.

Figure 1 shows the mean Hb concentration of the five blood samples analyzed in the 50 HemoCues. These results suggest that the same blood sample for measuring Hb concentration using different HemoCue devices may show differences up to |0.5| g/dL, and in a few cases to an even larger magnitude. This implies that the results of some HemoCue devices should be adjusted to produce accurate results.

Bonferroni's multiple comparison test (not shown) only identified one device with a significant difference. In our study, we applied a bias adjustment if the mean difference was |0.1| g/dL or larger, based on the standard error of mean differences of 0.114 g/dL from the model. Twenty-two HemoCues (44%) were adjusted



* Adjustment was performed in 22 HemoCues of the 50 total.

Figure 1. Mean differences observed between measurements by 50 HemoCue 201+ devices among five participants prior to field work. Mexico, 2022

adding or subtracting the mean difference to the observed measurements.

Figure 2 shows the model mean Hb estimates for the five blood samples measured with the 50 HemoCues. Left image shows the unadjusted estimates, it is clear that the results depend on the device's performance. The right image shows the adjusted HemoCue results. In this case, the results are very consistent and homogeneous, which demonstrates that once the devices are verified and adjusted, they can produce similar results as the gold standard. The right panel of figures 1 and 2 show the adjusted results.

We decided to t-test as a cutoff point for device correction, one standard deviation (in this case, |0.11|) for the average difference of Hb concentration using HemoCue devices, based on the model-estimated standard error, as a strict criterion to determine when is necessary to introduce device adjustments.

Nontheless, HemoCues that produce mean differences greater than |0.3| g/dL (99th percentile) are considered influential and should not be used without bias correction, and devices with lower differences may still be useful without bias correction, depending on the chosen cut off point.

Study 2. HemoCue 201+ performance in the field

Study aimed to determine whether bias adjustment was necessary for population-level studies in the field setting.

Figure 3 shows Hb differences of HemoCue measurements in children and women, respectively. The variation in the difference of mean Hb was greater than that observed in study 1 in both population groups. The mean difference of Hb was -0.049±0.578 g/dL in children and -0.098±0.628 g/dL in women, which were reduced to -0.029±0.594 and -0.065±0.647, respectively, after applying the bias adjustments.

Figure 4 shows the same results using a correlation of concordance analysis. Although the results improved after adjustment, those were not very different because variation is attributable to the blood sample and the technical skills of our field personnel, and not to the performance of the HemoCue devices. As expected, the variation of the results was larger in the field than in the calibration that took place under more controlled conditions. The dispersion of Hb data in the field (CI95%) using HemoCue was around 1.2 g/dL against 0.5 g/dL under laboratory settings. This variation may be acceptable for population studies, but it may be too large for anemia diagnose in individuals. For the latter, a more intense training of the sampling personnel is needed to produce reliable determinations with a total variation of Hb 0.5 g/dL or lower.

Environmental conditions of our study did not contribute to the mean differences in Hb measurements. The humidity values were in the range 26 to 98%, and temperature were in the range 10 to 33ºC. In the regression model, changes in one unit of humidity (coef: -0.0024, p=0.462) or temperature (coef: -0.007, p= 0.566) were not associated with changes in the mean difference between Hb values (data not shown).

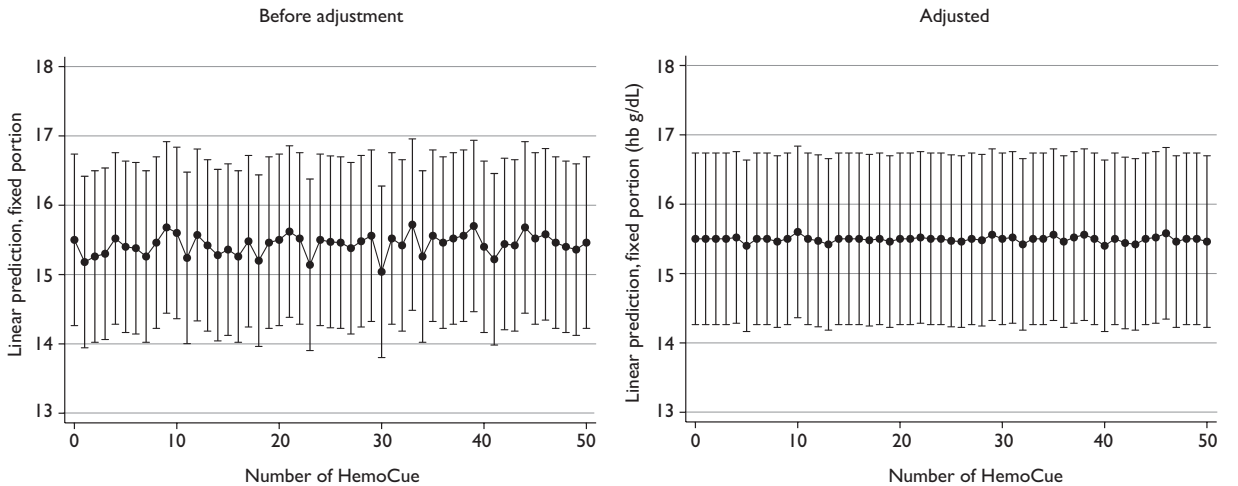


Figure 2. Model used to estimate mean differences between HemoCue 201+ devices. Mexico, 2022

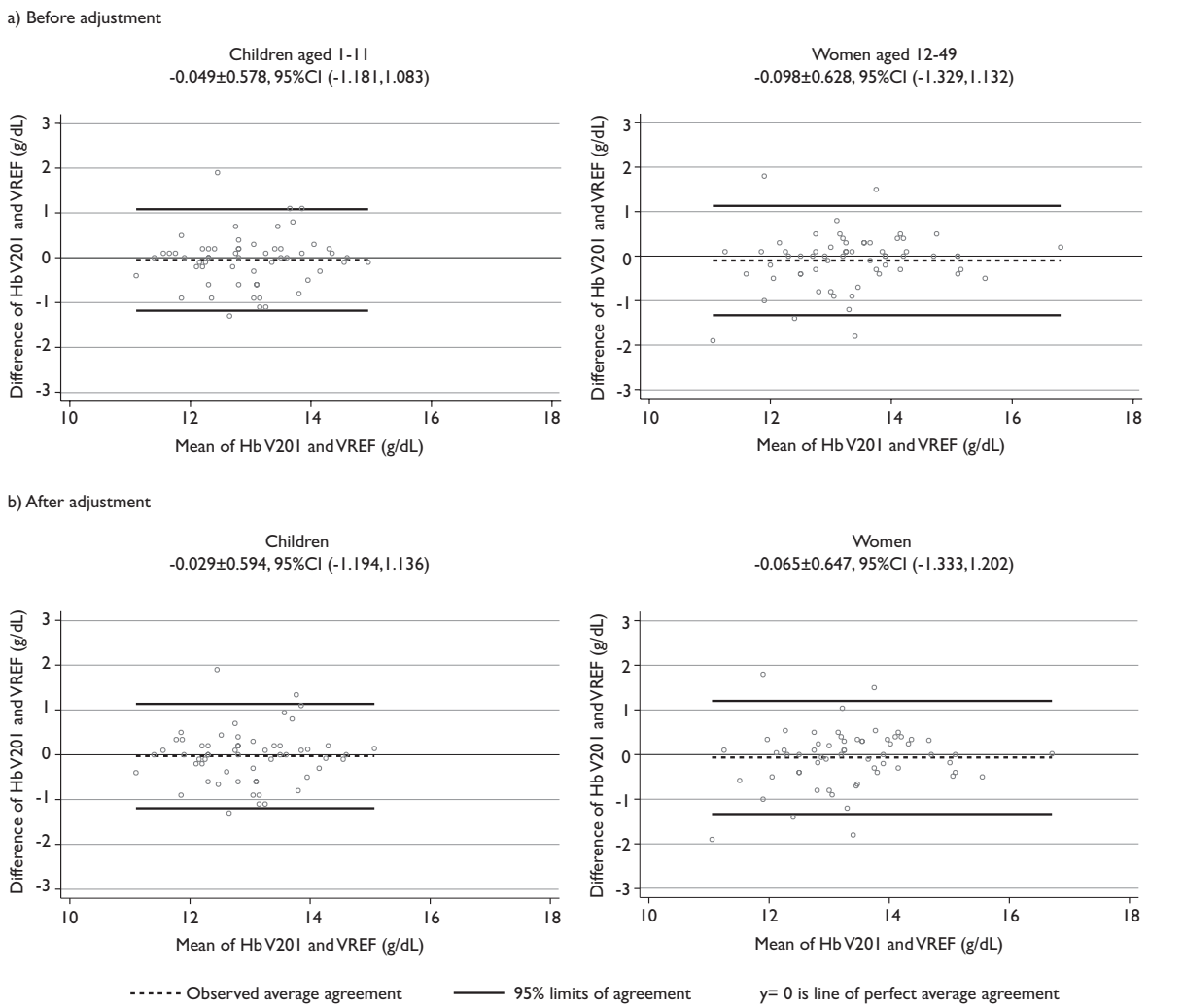


Figure 3. Bland Altman analysis of HemoCue 201+ performance in children and women. Mexico, 2022

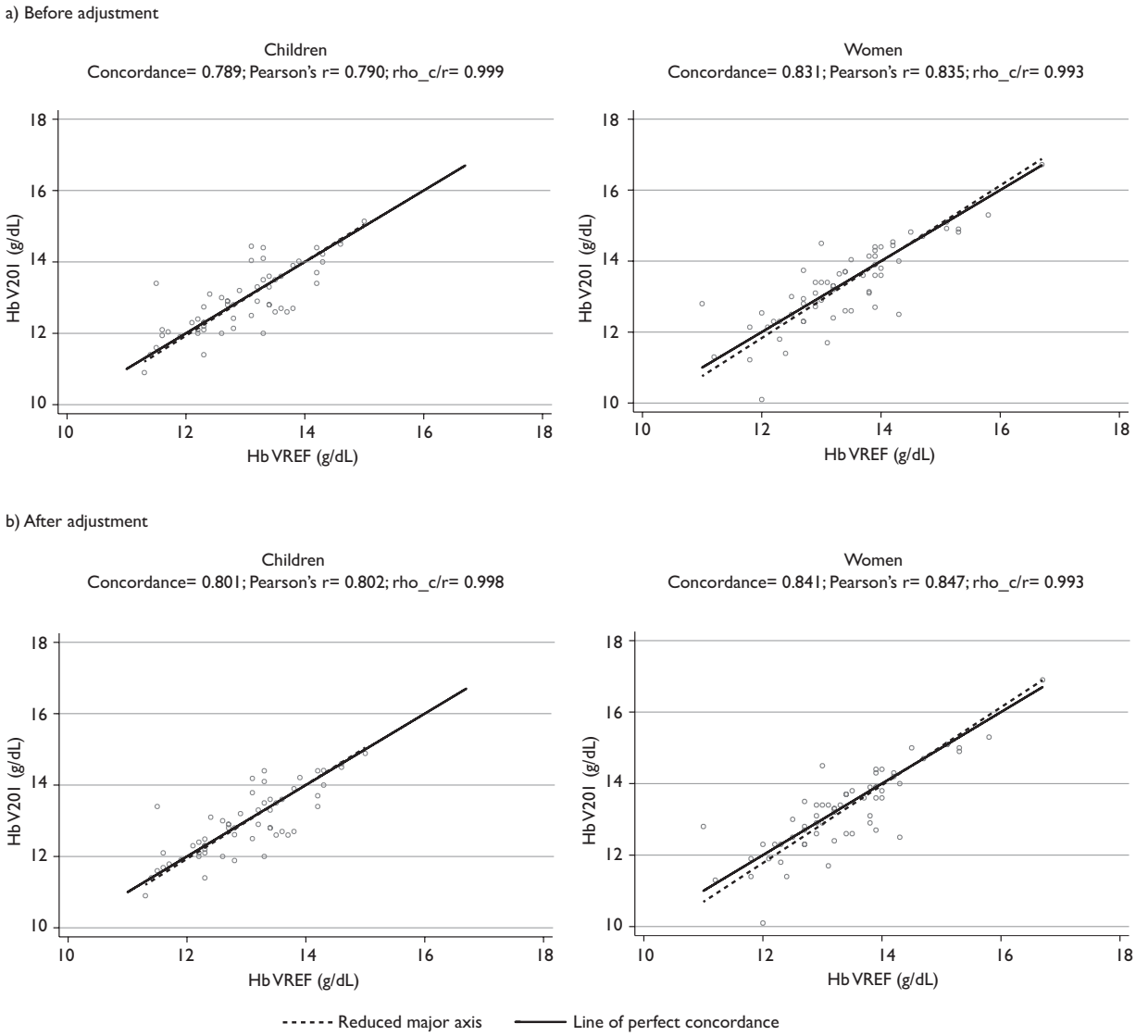


FIGURE 4. CONCORDANCE ANALYSIS OF HEMOCUE 201+ PERFORMANCE IN CHILDREN AND WOMEN. MEXICO, 2022

Due to limited sample size, zero children and four women had anemia according to the reference venous blood in this subsample. Using the HemoCue, anemia prevalence was identified in one child and eight women before bias adjustment, and one child and six women after bias adjustment.

Performance of the adjustment of Hb in the overall sample of Ensanut participants

The adjustment criteria calculated in the study 1 was applied to the Hb data in the overall Ensanut 2022 sample to assess potential differences in anemia classification. Through venous blood the values were not statistically significant ($p< 0.05$), although anemia prevalence was slightly lower after bias adjustment in 22 HemoCues (table I). These results suggest that for population-level studies, the use of venous blood when analyzed in point-of-use hemoglobinometers may perform acceptably in the classification of anemia. Bias adjustment may only be needed for those devices with larger differences (≥ |0.3| g/L) as compared to the gold standard.

Discussion

The present study offers two major findings: 1) performance of the HemoCue 201+ using venous blood in

the field setting is able to acceptably estimate anemia prevalence with no evidence of bias, and 2) adjustment of systematic errors may be needed for select HemoCue devices in cases when the difference of measured values against the gold standard is high (e.g, ≥|0.3| g/dL). In population-level studies, variability among Hb measurements often depends on the quality of the blood sample and the skills handling by the technician to collect and process the blood sample and the use of the method rather than the point-of-use device. Using venous blood, variations as large as 0.5 g/dL in laboratory setting, and as larger as 1.2 g/dL in the field may be expected. This variation may be acceptable at the population level, but is not recommendable for diagnosing anemia in individuals. Precision of Hb measurements using the HemoCue 201+ was good, and most devices (95% or more) showed strong accuracy in Hb measurement from venous blood as compared to the gold standard. Our results suggest that for population studies devices with an average difference above |0.3| g/dL (99th percentile) should either be discarded or adjusted for bias as previously described. For individuals, adjustment for bias may be needed if it is larger than |0.1| g/dL for a precise diagnose of anemia.

The Ensanut 2022 data used for this validation study was collected in real-time. Protocols required phlebotomists with previous experience, and new personnel were thoroughly trained. Standardization and training of personnel who measured Hb concentration using HemoCues were checked against the quality criteria established in the present study, where the total variation of results must be lower than |1.0| g/dL when compared against the results of a hematology autoanalyzer. However, differences between the HemoCue values and those of the gold standard were much larger (1.1-1.2 g/dL 95%CI) in the field than those obtained in the laboratory with the reference blood samples (0.5 g/dL 95%CI) and in our prior study,[4] the use of bias adjustment (for most devices between |0.1| and |0.2| g/dL, and for a few devices between |0.3| and |0.5| g/dL) had a small influence on population-level results. Most variation among results is dependent on the quality of blood samples and their handling by technicians.[13-15] These results suggest the need for more care towards reducing variation associated with the collection and processing of blood samples in the field and the use of the HemoCue's. Prior to start a field study, it is important to ensure that the variation of results using venous blood is lower than |1.0| g/dL against results obtained with the same blood samples in a hematology analyzer. If higher precision is required the hematology autoanalyzer should be considered as the superior measurement tool. Using capillary blood drops the variation of results can increase two-fold or more compared to venous blood.[8] Therefore, values measured through capillary blood drops have a higher probability of being erroneous.

Environmental factors such as temperature and humidity affect microcuvettes and, hence, Hb estimation. In the present study, temperature and humidity were not found to be associated with variations in Hb concentration.[9] The range of temperatures within study areas was 10 to 33ºC and humidity from 26 to 98%. Within this ranges, the HemoCue has demonstrated good performance when microcuvettes are kept closed in storage and until and the device is kept at a stable temperature (not exposed to sunlight).[16]

Even despite the limited sample size used for anemia classification, the low proportion of anemia found in this subsample was unexpected. When analyzing all population groups, anemia prevalence was low in comparison with previous surveys.[17] Anemia classification results using venous blood did not change significantly after the bias adjustment.

In the present Ensanut survey, some states of the Mexican Republic have population representativeness. In the study *Primera Infancia*, data on Hb concentration through pooled capillary blood was collected in 559 children, while in Ensanut, Hb was measured in 46 children using venous blood. Both samples were independent and representative of children aged 1-4 years from Nuevo León. Anemia, as measured through Hb in pooled capillary blood was classified in 5.6% (3.1, 10.1)[18] while in venous blood it was 5.3% (1.3,19.6). These results confirm, as reported previously,[8] that pooled capillary blood and venous blood yield similar Hb estimates and the results obtained in the present survey are dependable.

Table I

Anemia prevalence in the Mexican population by age group, by bias adjustment. Mexico, Ensanut 2022

Age group in years	*Before adjustment*		*After adjustment**		*Delta (pp)*
	%	*95%CI*	*%*	*95%CI*	
Children 1-4	6.93	(4.59,10.33)	6.79	(4.48,10.18)	0.14
Children 5-11	3.96	(2.15,7.19)	3.79	(2.01,7.04)	0.17
Adolescents 12-19	10.53	(6.8,16)	10.11	(6.8,14.7)	0.42
Women 20-49	16.04	(12.98,19.67)	15.98	(12.84,19.71)	0.06
Older adults ≥60	10.39	(7.29,14.60)	10.31	(7.2,14.55)	0.08

* Adjustment was performed for 22 HemoCue devices
(pp): Percentage points (pp)
Ensanut: *Encuesta Nacional de Salud y Nutrición.*

Regarding the subsample of participants who donated venous blood, one limitation is that results may be related to response propensity, biasing towards those who are more willing to participate. The Ensanut 2022 response rate (around 48%) was similar to previous Ensanut surveys.

To our knowledge, the present study is the first validation of Hb measurement in an Ensanut survey. Verification and, when needed, bias identification and adjustment of HemoCue devices is a useful procedure that should be done for each survey prior to field work. Systematic errors may occur using HemoCue devices and should be corrected for with bias adjustment to improve the accuracy of Hb measurements. The use of a random intercept model is an appropriated way to adjust the devices based on the estimated the mean differences.

The current validation study confirms that the HemoCue 201+ allows reliable Hb measurements when using venous blood for anemia classification. Further studies will allow a better understanding of the main causes, determinants, and drivers of anemia to target interventions and monitor progress for population groups at higher risk.[1]

Conclusions

The performance of the HemoCue 201+ using venous blood samples was acceptable to estimate Hb concentration in the Ensanut and produce reliable estimations of anemia prevalence. Measurement accuracy was improved after the adjustment of minor systematic error found in some HemoCue devices. In subsequent surveys, the performance of HemoCue devices should be routinely analyzed and calibrated.

Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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