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Effects of preanalytical factors on hemoglobin measurement: a comparison of two HemoCue® point-of-care analyzers

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Abstract

Background—In field studies, hemoglobin (Hb) is often measured using a battery-operated, portable HemoCue® hemoglobinometer.

Methods—We compared the performance of 2 HemoCue® models (Hb-201+ and Hb-301) and investigated effects of preanalytical factors on Hb results by simulating unfavorable field conditions.

Results—The Hb-301 produced 2.6% higher results compared to the Hb-201+. Hb had to be measured within 1 min of filling the Hb-301 cuvette to avoid artificially elevated concentrations (1.3% per min). The Hb-301 cuvettes withstood elevated temperature (37°C) and humidity (72%) for 3 wk, while the Hb-201+ cuvettes degraded within 10 min under those conditions. Both cuvette types withstood elevated temperature for 3 wk. Properly-collected venous and capillary blood produced comparable results. Pooled capillary blood produced comparable results to the second and third but not the fourth drop of blood (3.3% lower). Blood could be stored for ≤4 d at 10–30°C before Hb-201+ measurement, but only for 1 d at 10–23°C before Hb-301 measurement (≤1% change in Hb).

Conclusions—Higher Hb results obtained with the Hb-301 may influence the interpretation of anemia prevalence in health surveys. While the Hb-301 performed better in high humidity conditions, the Hb-201+ provided more user flexibility regarding delayed Hb reading.

Keywords

venous blood; capillary blood; storage stability; freeze-thawing; elevated temperature; humidity

1. Introduction

The World Health Organization reported in 2008 that anemia affects about 1.62 billion people worldwide, with the highest prevalence among preschool age children and women of

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childbearing age (1). Hemoglobin (Hb), a protein in red blood cells that carries oxygen to the tissues, is the most commonly used biomarker to assess the prevalence of anemia in a population and Hb data are usually collected as part of national nutrition surveys (2). The HemoCue® point-of-care analyzer is most commonly used in field studies to generate instant results (3). While several HemoCue® models have been available for nearly 30 y, two models, the Hb-201+ and Hb-301, have been predominantly used during the last decade in health and nutrition surveys in low- and middle-income countries.

Extreme environmental conditions such as high temperature and humidity as well as poor infrastructure resulting in inadequate specimen transportation and storage conditions are some of the challenges when working in a low-resource environment. These factors could negatively affect Hb measurements. A 2013 review by Sanchis-Gomar *et al.* provides a summary of 31 published articles that evaluated different HemoCue® models regarding their performance under various conditions and with different specimen types as well as their comparability to reference hematology analyzers (3). Many of the articles focused on the utility of the HemoCue® to screen potential blood donors or to assess Hb concentrations in hospitalized and critically ill patients. The literature is much more scant when it comes to evaluating various HemoCue® systems under field conditions (4,5) or evaluating the newer Hb-301 analyzer (4,6–8). Furthermore, most studies addressed either the diagnostic accuracy of the HemoCue® system as compared to reference hematology analyzers or the comparability of venous and capillary specimens, but few studies addressed questions of specimen or reagent stability under suboptimal conditions (6).

Our laboratory has provided technical assistance to health and nutrition surveys for over 15 y and we are often faced with questions that pertain to field logistics. Thus, the goal of this article was to summarize comprehensive information we generated over the years in different experiments that assessed the comparability of HemoCue® models (including supplies and operation), the robustness of HemoCue® analyzers and supplies, the comparability of venous and capillary specimens, and the effects of sample storage and freeze-thawing on Hb results.

2. Materials and Methods

2.1. Instruments and Supplies

We evaluated 2 models of HemoCue® analyzers, the Hb-201+ and Hb-301. Both are hand-held photometers that use microcuvette technology to provide instant Hb results from a capillary, venous, or arterial whole blood sample. Model-specific HemoCue® cuvettes are required for each analyzer. The Hb-201+ cuvettes contain a sodium deoxycholate reagent that leads to hemolysis of the erythrocyte membranes which releases Hb from red blood cells. The Hb iron is then converted by sodium nitrate from ferrous to ferric state to form methemoglobin, which then combines with azide to form a stable azidemethemoglobin which is detected at 570 nm and 880 nm (9). This reaction is similar to the formation of cyanmethemoglobin in the accepted reference method for the photometric determination of Hb (10). The Hb-301 cuvettes contain no active ingredients. Hb concentration is determined at 506 nm and 880 nm by measuring the absorbance at an Hb/oxyhemoglobin isosbestic point. For the Hb-201+ analyzer, the manufacturer-recommended operating temperature is

15–30°C and cuvette storage temperature is 15–30°C (11). For the Hb-301 analyzer, the manufacturer recommends a wider operating temperature range of 10–40°C and the recommended storage temperature for cuvettes is 10–40°C (12). Because of a built-in self-test system, neither model requires a control cuvette, which was used in the predecessor HemoCue® model B-Hemoglobin. The use of liquid Hb control materials is optional according to the manufacturer. HemoTrol® controls are available at 3 levels for the Hb-201+ analyzer and Hb-301 controls for the Hb-301 analyzer (Eurotrol, Inc., Burlington, MA). It is advisable to test these materials daily prior to using the analyzer and to confirm that the Hb results are within the pre-specified control limits.

2.2. Blood Specimens

This study was approved by the CDC Institutional Review Board. Venous and capillary EDTA blood specimens were obtained from 2 sources. Venous blood specimens from 25 blood donors (21 men and 4 women, age 20–59 y) from Tennessee Blood Services (Memphis, Tennessee) were collected in 10-mL purple top EDTA Vacutainers™ and aliquoted after receipt at CDC the next day into 9 500-μL K_2EDTA Microtainers™ per person. Baseline Hb concentrations were measured for each specimen upon arrival at CDC. Paired venous and capillary blood was obtained from 35 CDC volunteer donors (12 men and 23 women, age 25–61 y). Venous blood was collected into three 2-mL purple top EDTA Vacutainers™ and capillary blood was collected into a 500-μL K_2EDTA Microtainer™ or individual drops were used directly (sufficient volume was only available for 33 [experiment 6] and 32 [experiment 7] of the 35 donors). The baseline Hb concentrations were measured for each specimen directly after collection.

2.3 Experimentation

Only fully trained laboratory staff conducted experiments for this study (Supplemental Text 1). Details about each experiment with regards to design, specimens used, and laboratory analysis conducted are provided in Table 1. In short, we conducted experiments that can be grouped into 4 categories: assessing the comparability of 2 HemoCue® models, including supplies and operation (experiments 1–3); assessing the robustness of the 2 HemoCue® analyzers and their supplies (experiments 4–5); assessing the comparability of venous and capillary blood (experiments 6–7); and assessing the effects of sample storage and freeze-thawing in Hb concentrations (experiments 8–9). Because multiple readings provide a more accurate mean than a single measurement, we carried out multiple readings where possible (sufficient specimen volume or use of liquid controls). Hb measurements were performed at CDC following the HemoCue® product sheets and training videos except when we specified otherwise to test the effect of deviating from the recommended procedure. For example, HemoCue® does not specify the time within which Hb should be measured after filling the cuvette, however the implied understanding is that the measurement takes place immediately after the cuvette is filled. Hence, we tested the effect of delayed Hb reading in experiment 3. Reliable Hb results could not be obtained for the Hb-201+ in experiment 5 when cuvettes were exposed to up to 3 wk of elevated temperature and humidity in open boxes. The sodium deoxycholate reagent inside the Hb-201+ cuvettes disintegrated causing insufficient blood specimen to be collected into the cuvette. Thus, an additional short-term experiment (up to 1 h) was designed to assess how quickly the Hb-201+ cuvette reagent disintegrated.

When we compared Hb results obtained for individual drops of blood vs. pooled capillary blood in experiment 7, we wiped away the first drop of blood and used the second, third, and fourth drop of blood to compare to the pooled blood from the fifth to the 10th drop of blood. If blood was stored at lower or higher temperature than room temperature (experiments 8–9), specimens were allowed to reach room temperature before the Hb reading was carried out (frozen specimens were thawed at room temperature for 2 h).

2.4. Statistical analysis

Descriptive statistics, including mean, standard deviation (SD) and coefficient of variation (CV) were calculated for each experiment. If multiple readings and/or multiple analyzers were used for an experiment, the mean is reported. Due to the different experiment designs, we used either one-factor or two-factor fixed or random effect analysis of variance (ANOVA), as appropriate using SAS software (version 9.2; SAS Institute, Cary, NC), and reported mean [95% confidence interval (CI)] to make statistical inferences. P values reported in this paper are either the relevant pairwise comparisons or overall effects from the appropriate ANOVA model. All statistical comparisons were evaluated at a significance level of $\alpha = 0.05$. We did not adjust for multiple comparisons because we were more concerned with missing the opportunity to identify factors that impact Hb results than with over-identifying factors.

3 Results and Discussion

3.1. Comparability of HemoCue® models, including supplies and operation

3.1.1. Comparison of HemoCue® models (experiment 1)—The within- and between-model comparability was assessed using 25 venous blood samples that covered an approximate Hb concentration range of 80–170 g/L. The within-model variability (2 Hb-201+ and 12 Hb-301 analyzers) was excellent, with <1% CV among analyzers of the same model. The between-model comparability showed a significant model difference (p <0.0001), with the Hb-301 model [mean (95% CI): 131 (123 to 140) g/L] measuring 3.4 (−2.6 to 4.3) g/L higher than the Hb-201+ model [128 (119 to 136) g/L]. This corresponded to a relative difference of 2.6%. The correlation between the two models was very high (Pearson $r = 0.996$) and the least-squares linear regression equation (Hb-201+ used as the reference) had a slope (95% CI) of 0.96 (0.923 to 0.997; different from 1) and intercept of 8.6 (3.8 to 13.3; different from 0) g/L (Supplemental Fig. 1, panel A). The Bland-Altman mean difference (95% CI) was 3.5 (2.6 to 4.3) g/L and 95% of all Hb-301 results were between 0.4 g/L lower and 7.4 g/L higher (limits of agreement) than the Hb-201+ results (Supplemental Fig. 1, panel B). When we used 5 freshly collected capillary blood samples to compare the 2 models, we received similar results, with the Hb-301 model producing 3.3% higher results than the Hb-201+ model. This confirms that the model difference observed in the main experiment was not because the venous samples were analyzed 1 d after collection.

The Clinical Laboratory Improvement Amendments of 1988 (CLIA) specify an allowable total error for Hb of ±7% (13), while a smaller total error based on biologic variation of 4% has been suggested (14). Tayou *et al.* found a high correlation between the Hb-201+ and Hb-301 models ($r = 0.98$) and slightly higher Hb results with the Hb-301 model (limits of

agreement of 0.6 to 5.2 g/L) (n = 236 pregnant women) (7). A few studies compared the Hb-301 to reference hematology analyzers and found no bias ($n \approx 300$), with 90% of Hb-301 results being within ±4% of the reference values (6), a small bias of 3 g/L (limits of agreement of ±13 g/L, n = 471) (7), or a small bias of −5.4 g/L (limits of agreement of −12.8 to 2.0 g/L, n = 60) (4). Several studies presented in the Sanchis-Gomar *et al.* review (3) including a few more recent studies (15,16) assessed the comparability of the Hb-201+ with reference hematology analyzers. Generally, investigators found good correspondence, at least with venous blood samples, but the Hb-201+ produced approximately 3 g/L (15) or 8 g/L (16) higher Hb results.

3.1.2. Cuvette lot comparison (experiment 2)—The cuvette lot-to-lot variability was assessed for both instrument models using the same 25 venous blood samples as in experiment 1. We found a small but significant lot-to-lot variability for both analyzer models of about 1%: Hb-201+ (2 lots): CV = 1.3% [mean difference (95% CI) lot 1 *vs.* lot 2: −2.3 (−3.1 to −1.5) g/L, p <0.0001]; Hb-301 (3 lots): CV = 1.2% [lot 1 *vs.* lot 2: −2.8 (−3.7 to −1.8) g/L, p <0.0001; lot 1 *vs.* lot 3: −2.9 (−4.1 to −1.7) g/L, p <0.0001; lot 2 *vs.* lot 3: −0.1 (−1.1 to 0.08) g/L, p = 0.7444). This small variability is acceptable.

3.1.3. Delayed Hb reading (experiment 3)—We assessed whether delayed reading of whole blood for up to 6 min after adding venous blood to the cuvette changed the Hb concentration for either of the two analyzer models using 10 venous blood samples that covered an approximate concentration range of 80–170 g/L. The Hb-201+ model showed comparable mean Hb results (p = 0.1238) from baseline (135 g/L) to 6 min (136 g/L), while the Hb-301 model showed a significant linear trend of increasing mean results (139 g/L at baseline and 151 g/L at 6 min, p <0.0001), corresponding to a 1.3% increase per minute. Users of the Hb-301 model need to be aware of this and it may be prudent if the manufacturer mentioned this in the product sheets and training videos.

3.2. Robustness of HemoCue® analyzers and supplies

3.2.1. Exposure of instruments or supplies to elevated temperature (experiment 4)—The robustness of Hb measurements was tested after exposing either HemoCue® analyzers or supplies to elevated temperature for extended periods of time. Keeping both models (2 analyzers each and 2 readings per analyzer) for up to 3 wk at 37°C, did not significantly change the mean Hb concentrations obtained for properly-stored control materials (Table 2). Keeping the cuvettes for both models (1 analyzer each and 2 readings per analyzer) for up to 3 wk at 37°C, led to small but significant changes (±1%) in mean Hb concentrations for 24 venous blood samples with the Hb-201+ model (wk 1: p = 0.0002; wk 2: p = 0.0195; wk 3: p = 0.0433), but no significant changes with the Hb-301 model (wk 1: p = 0.38; wk 2: p = 0.09; wk 3: p = 0.07). Lastly, keeping the controls for the Hb-301 model (2 analyzers) for up to 3 wk at 32°C resulted in comparable results at 1 wk (p = 0.23). However, at 2 wk (p = 0.0224) and 3 wk (p = 0.0057), Hb concentrations were slightly but significantly increased (1.3 g/L, corresponding to 1%).

Morris *et al.* found no significant deviations from the initial Hb results when keeping Hb-301 cuvettes at 4°C or 23°C for up to 24 h (6). We are not aware of other reports that

evaluated the robustness of the HemoCue® system for longer periods and higher temperatures similar to our experiment.

3.2.2. Exposure of cuvettes to elevated temperature and humidity (experiment 5)—Independent of the storage condition for Hb-301 cuvettes (cuvettes stored in a closed box at room temperature in a climate controlled laboratory; cuvettes stored in a closed box at 37°C and 72% humidity; cuvettes stored in an open box at 37°C and 72% humidity), Hb results did not change significantly with time over 3 wk except for level 3 ($p = 0.0355$) (Table 3). However, storage of Hb-201+ cuvettes for up to 3 wk either in a closed box at room temperature in a climate controlled laboratory or in a closed box at 37°C and 72% humidity led to significantly higher Hb results with time for level 2 ($p = 0.0079$) and level 3 ($p = 0.0101$) (Table 3). When Hb-201+ cuvettes were stored in an open box and thus exposed to a combination of elevated temperature and humidity, a rapid degradation of the cuvettes was observed within less than 1 h; already after 20 min, Hb results for level 1 ($p = 0.0343$) and level 3 ($p = 0.0267$) were significantly lower than results obtained at time 0 (Table 4).

The Hb-301 model is advertised as offering “robust testing within a wide range of temperatures and humidity” (12) and our data confirm this. The sensitivity of the Hb-201+ cuvettes against humidity has been reported before (3,17,18), but those studies assessed the storage of cuvettes in their original container with the moisture absorbing material in the lid for a few days in a tropical environment. Our study shows to what extent the cuvettes degrade within 1 h when kept open in a high temperature and high humidity environment.

3.3. Comparability of venous and capillary blood

3.3.1. Comparison of venous vs. capillary blood (experiment 6)—Using a Hb-201+ analyzer, we measured 33 paired venous and capillary blood samples (3 readings each) that covered an approximate concentration range of 100–170 g/L. The 3 replicate readings produced comparable mean results (venous blood: 142, 143, and 143 g/L; capillary blood: 143, 144, and 144 g/L) and we found no significant difference between venous [mean (95% CI): 143 (138 to 148) g/L] and capillary [144 (139 to 149) g/L] blood samples ($p = 0.0711$), although Hb results in capillary blood samples were slightly higher (1 g/L).

A number of studies compared Hb concentrations in venous and capillary blood samples and several of these studies are addressed in the Sanchis-Gomar *et al.* review (3). Possibly the largest study compared these 2 specimen types in close to 9,000 blood donors (19). The capillary Hb concentration was slightly higher than the venous Hb concentration (1.5 ± 6.8 g/L), but this small difference is acceptable because it is not of clinical relevance. In the vast majority of donors, the 2 specimen types differed less than 10 g/L. Furthermore, the categorization of Hb for blood donation was concordant between the 2 specimen types in over 90% of donors.

3.3.2. Comparison of individual drops of blood vs. pooled capillary blood (experiment 7)—Using a Hb-201+ analyzer, Hb concentrations were compared from individual drops of blood to those obtained with pooled blood from a Microtainer™ using 32 capillary blood samples covering an approximate concentration range of 100–170 g/L. We

found no significant difference in the mean (95% CI) Hb concentration for the second [147 (142 to 152) g/L, $p = 0.67$] or third [148 (143 to 153) g/L, $p = 0.28$] drop relative to pooled blood [147 (142 to 152) g/L]. However, the fourth drop [142 (137 to 147) g/L, $p < 0.0001$] produced significantly lower Hb concentrations by 4.8 g/L, corresponding to −3.2%.

Conway *et al.* presented HemoCue® Hb data from single drops of blood (first and fourth drop) *vs.* pooled blood (about 20 drops of blood after the fourth drop) collected via finger sticks by trained biomedical scientists and trained health visitors (20). They showed that pooling drops of blood improved precision and allowed more novice users to achieve results comparable to those obtained by experienced laboratory staff.

3.4. Effects of sample storage and freeze-thawing on Hb results

3.4.1. Storage of blood at 10°C, room temperature, and 30°C for up to 4 d (experiment 8)—We measured Hb concentrations in 24 venous blood samples that were stored at 10°C, room temperature, and 30°C for up to 4 d using 2 Hb-201+ and Hb-301 analyzers each and compared the results to those obtained with freshly collected blood at baseline. The Hb-201+ model showed comparable mean (95% CI) Hb results from baseline [129 (120 to 137) g/L] to 4 d [129 (121 to 137), 129 (120 to 137), and 127 (119 to 135) g/L at 10°C, RT, and 30°C, respectively] with small changes in the Hb concentration (≤1%), while the Hb-301 model showed a significant linear trend of increasing results at each storage temperature [133 (125 to 141) g/L at baseline and 137 (128 to 145), 139 (131 to 147), and 167 (156 to 178) g/L after 4 d at 10°C, room temperature (RT), and 30°C, respectively] (Table 5). The proportional increase was significant at each time point and temperature, but was <1% after 1 d at 10°C and room temperature. After 4 d, the proportional increase was 2.8%, 4.8%, and 25.5% at 10°C, RT, and 30°C, respectively.

Morris *et al.* conducted a short stability study where they kept whole blood samples ($n = 8$) at 4°C and 23°C for up to 24 h (6). Similar to our data (<1% change during the first day), they also found no significant deviations from the initial Hb result and the largest difference was for samples stored for 24 h at 23°C (≤6%).We are not aware of other reports that exposed samples for several days to different temperatures similar to our experiment. Given that the Hb-301 analyzer measures the absorbance at an Hb/oxyhemoglobin isosbestic point, it may be expected that prolonged sample storage changes the ratio of Hb/oxyhemoglobin and thus the Hb result.

Effect of freeze-thawing on Hb results (experiment 9)—While it is unusual to conduct Hb measurements on previously frozen whole blood samples, it is helpful to have freeze-thaw stability information (Supplemental Text 2). In short, the Hb-201+ produced slightly lower results after 1 (1.8%) and 2 (1.6%) freeze-thawing cycles, while the Hb-301 produced slightly higher results after 1 (1.6%) and 2 (2.1%) freeze-thawing cycles compared to fresh blood samples.

4 Conclusion

This paper contains a comprehensive series of experiments designed and carried out over a few years that address issues of field logistics pertaining to the measurement of Hb with the

HemoCue® Hb-201+ and/or Hb-301 models. The results will be useful to researchers, public health scientists, and international organizations who support national nutrition surveys and appreciate the challenges of collecting Hb data in field settings. Table 1 provides a succinct summary of our findings and conclusions for each experiment. Both HemoCue® models showed acceptable variability among analyzers of the same type and among cuvette lots, and the robustness of the analyzers and supplies generally matched with manufacturer statements. However, the 2 models did not produce interchangeable Hb results, which may influence the interpretation of anemia prevalence in health surveys. It may be advisable to use the same HemoCue® model for baseline and follow-up investigations to assess the impact of an intervention, if possible.

We confirmed that the Hb-301 model performed better in high temperature and high humidity conditions, while the Hb-201+ model provided more user flexibility regarding measuring Hb in samples that were stored for a few days after collection. While it is not recommended to freeze whole blood samples prior to measuring Hb, our data show that the average Hb difference between thawed and fresh whole blood samples was relatively small (<2%). The majority of evidence indicates that Hb results in capillary blood samples are higher than in venous blood samples, which is also what we observed. However, the extent of the difference between these 2 specimen types differs across studies and is likely influenced by the health status of the population in which Hb is measured and by the proficiency of the technician collecting the capillary blood specimen. Proper training and standardization exercises are of utmost importance to obtain valid capillary blood specimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CV	Coefficient of variation
Hb	Hemoglobin
RT	Room temperature
SD	Standard deviation

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Table 1

Experimental design, specimens used, laboratory analysis conducted, findings, and conclusions from the measurement of hemoglobin using the HemoCue® Hb-201+ and/or Hb-301 analyzers[1]

Experiment	Design	Specimens	Laboratory analysis	Finding	Conclusion
Comparability of HemoCue® models, including supplies and operation					
1. HemoCue® model comparability	Test multiple analyzers of 2 HemoCue® models	Venous EDTA blood (n = 25) from TNBS donors	Hb-201+ (2 analyzers, 1 reading/analyzer) Hb-301 (12 analyzers, 1 reading/analyzer)	Hb-301 measured 2.6% higher than Hb-201+	Results from Hb-201+ and Hb-301 are not comparable
2. Cuvette lot-to-lot variability	Test multiple cuvette lots for 2 HemoCue® models	Venous EDTA blood (n = 25) from TNBS donors	Hb-201+ (2 lots, 1 reading/lot) Hb-301 (3 lots, 1 reading/lot)	Low variability for both HemoCue® models (CV = 1%)	Cuvette lot variability is acceptable
3. Delayed Hb reading	Read Hb at 0 (reference), 1, 2, 3, 4, 5, and 6 min after filling the cuvette	Venous EDTA blood (n = 10) from TNBS donors	Hb-201+ (1 reading) Hb-301 (1 reading)	Hb-201+ showed comparable results up to 6 min Hb-301 showed increasing results (1.3% per min)	Reading on Hb-301 must be carried out within 1 min of filling the cuvette
Robustness of HemoCue® analyzers and supplies					
4. Exposure of analyzers or supplies to elevated temperature	a. Keep analyzers at 37°C (incubator) for up to 3 wk and test weekly (reference: 0 wk)	Controls (level 1, 2, and 3) for each model	Hb-201+ (2 analyzers, 2 readings/analyzer) Hb-301 (2 analyzers, 2 readings/analyzer)	Keeping analyzers (both models) at 37°C for up to 3 wk did not affect results by more than ±1%	Analyzers for both HemoCue® models can withstand elevated temperature
	b. Keep cuvettes at 37°C (incubator) for up to 3 wk and test weekly (reference: 0 wk)	Venous EDTA blood (n = 24) from TNBS donors	Hb-201+ (2 readings) Hb-301 (2 readings)	Keeping cuvettes (both models) at 37°C for up to 3 wk did not affect results by more than ±1%	Cuvettes for both HemoCue® models can withstand elevated temperature
	c. Keep Hb-301 controls at 32°C (incubator) for up to 3 wk and test weekly (reference: 0 wk)	Controls (level 1, 2, and 3)	Hb-301 (2 analyzers, 1 reading/analyzer)	Keeping controls (Hb-301) at 32°C for up to 3 wk did not affect results by more than ±1%	Controls for the Hb-301 can withstand elevated temperature
5. Exposure of cuvettes to elevated temperature and humidity	a. Keep cuvettes (Hb-301) at 37°C/72% humidity (incubator) in either closed or open boxes for up to 3 wk and test at 0 (reference), 1, 8, 15, and 21 d	Controls (level 1, 2, and 3)	Hb-301 (2 readings)	Keeping cuvettes for Hb-301 (closed or open box) at 37°C and 72% humidity for up to 3 wk did not affect results	Cuvettes for Hb-301 can withstand elevated temperature and humidity for several weeks
	b. Keep cuvettes (Hb-201+) at 37°C/72% humidity (incubator) in closed boxes for up to 3 wk and test at 0 (reference), 1, 8, 15, and 21 d	Controls (level 1, 2, and 3)	Hb-201+ (2 readings)	Keeping cuvettes for Hb-201+ (closed box) at 37°C and 72% humidity for up to 3 wk did not affect results	Cuvettes for Hb-201+ must be stored in the tightly closed box
	c. Keep cuvettes (Hb-201+) at 37°C/72% humidity in open boxes for up to 1 h and test at 0 (reference), 10, 20, 25, 30, 40, and 60 min	Controls (level 1, 2, and 3)	Hb-201+ (2 readings)	Keeping cuvettes for Hb-201+ (open box) at 37°C and 72% humidity for up to 1 h produced significantly lower results after 10 min compared to 0 min	Cuvettes for Hb-201+, when exposed to humidity, they degrade within 10 min
Venous vs. capillary blood samples					

Experiment	Design	Specimens	Laboratory analysis	Finding	Conclusion
6. Comparability of venous and capillary blood	Measure paired venous and capillary blood samples	Venous and capillary EDTA blood (n = 33) from CDC donors	Hb-201+ (3 readings)	Hb-201+ produced slightly higher results for capillary blood samples, but the difference was not significant	In apparently healthy individuals, venous and capillary blood samples produce comparable results when sampled by an experienced technician
7. Comparability of individual drops vs. pooled capillary blood	Measure 2nd, 3rd, and 4th drop capillary blood and compare to pooled capillary blood from Microtainer™	Capillary EDTA blood (n = 32) from CDC donors	Hb-201+ (1 reading)	Hb-201 produced comparable results for second or third drop relative to pooled blood; however, fourth drop produced significantly lower results by 3.2%	Individual drops of blood (second and third) and pooled blood produce comparable results when sampled by an experienced technician
Sample storage and freeze-thawing					
8. Storing blood at different temperatures for up to 4 d	Store venous blood at 10°C, room temperature, and 30°C (incubator) for up to 4 d and test daily (reference: 0 d)	Venous EDTA blood (n = 24) from TNBS donors	Hb-201+ (2 analyzers) Hb-301 (2 analyzers)	Hb-201+ produced comparable results at each temperature and day Hb-301 produced increasing results with time and temperature; storage of blood for more than 1 d at higher than ambient temperature resulted in >1% increase	When using Hb-201+, blood can be stored for a few days prior to measurement at a wide range of temperatures (10–30°C) When using Hb-301, blood can to be stored at ambient or refrigerated temperature for maximum 1 d prior to measurement
9. Freeze-thawing	a. Freeze capillary blood at −70°C for 4 d and compare to result obtained with fresh blood	Capillary EDTA blood (n = 32) from CDC donors	Hb-201+ (2 readings)	Hb-201+ produced slightly lower results (1.7%) in thawed compared to fresh blood samples	Hb should be measured in fresh samples; Hb measured in thawed samples is no longer accurate
	b. Freeze venous blood at −70°C, conduct 1 freeze-thawing cycle on day 1 and a second freeze-thawing cycle on day 2 and compare to result obtained with fresh blood	Venous EDTA blood (n = 25) from TNBS donors	Hb-201+ (2 analyzers) Hb-301 (2 analyzers)	Hb-201+ produced slightly lower results after 1 (1.8%) and 2 (1.6%) freeze-thawing cycles compared to fresh blood samples Hb-301 produced slightly higher results after 1 (1.6%) and 2 (2.1%) freeze-thawing cycles compared to fresh blood samples	Hb should be measured in fresh samples; Hb measured in thawed samples is no longer accurate

[1] Hb, hemoglobin; TNBS, Tennessee Blood Services

Table 2

Exposure of instruments or supplies to elevated temperature for up to 3 wk and effect on measured hemoglobin concentrations (g/L): mean (SD)

		HemoCue® model							
		Hb-201+				Hb-301			
Condition	Specimen(s)	Time, wk				Time, wk			
		0	1	2	3	0	1	2	3
Analyzers at 37°C[a]	Level 1	73.5 (2.4)	72.5 (1.3)	73.5 (1.7)	75.0 (2.8)	79.8 (0.5)	80.3 (1.0)	80.8 (2.1)	79.8 (0.5)
	Level 2	132 (2.4)	133 (2.7)	131 (0.5)	133 (3.2)	119 (1.3)	118 (0.6)	118 (1.0)	118 (1.3)
	Level 3	174 (3.2)	175 (2.9)	175 (3.0)	173 (2.8)	160 (1.3)	159 (1.2)	159 (1.8)	158 (1.3)
Cuvettes at 37°C[b]	Venous blood	143 (12)	141 (11)	142 (11)	144 (12)	147 (11)	147 (11)	148 (11)	148 (11)
Control materials at 32°C[c]	Level 1	n.m.	n.m.	n.m.	n.m.	71.0 (2.8)	70.5 (2.1)	71.5 (3.5)	72.0 (2.8)
	Level 2	n.m.	n.m.	n.m.	n.m.	132 (4.2)	133 (1.4)	134 (3.5)	134 (2.8)
	Level 3	n.m.	n.m.	n.m.	n.m.	170 (1.4)	172 (2.8)	172 (4.2)	172 (3.5)

[a] Analysis of 3 HemoCue® controls by 2 analyzers per model and 2 readings per analyzer (n=4)

[b] Analysis of 24 venous blood specimens by 1 analyzer per model and 2 readings per analyzer (n=2)

[c] Analysis of 3 HemoCue® controls by 2 analyzers for model Hb-301 and 1 reading per analyzer (n=2); n.m., not measured for model Hb-201+

Table 3

Effect of suboptimal cuvette storage conditions for up to 21 d on measured hemoglobin concentrations (g/L): mean (SD) from 2 readings each

HemoCue® model	HemoCue® controls	Cuvette condition	Time, d				
			0	1	8	15	21
Hb-301	Level 1	Reference[a]	71.5 (0.7)	72.0 (1.4)	72.5 (0.7)	72.5 (0.7)	72.5 (0.7)
		37°C/humid, closed[b]	71.5 (0.7)	72.5 (0.7)	71.0 (0)	71.0 (0)	71.5 (0.7)
		37°C/humid, open[c]	71.5 (0.7)	72.0 (0)	72.5 (0.7)	72.5 (0.7)	73.0 (0)
	Level 2	Reference[a]	130 (2.1)	131 (0)	132 (0.7)	130 (0.7)	131 (0)
		37°C/humid, closed[b]	130 (2.1)	131 (0)	130 (0.7)	130 (0)	130 (0.7)
		37°C/humid, open[c]	130 (2.1)	131 (0)	132 (0.7)	132 (0.7)	132 (0)
	Level 3	Reference[a]	171 (0)	172 (0)	175 (0)	174 (0)	175 (0)
		37°C/humid, closed[b]	171 (0)	174 (2.1)	172 (2.1)	171 (1.4)	170 (0.7)
		37°C/humid, open[c]	171 (0)	174 (0.7)	173 (0)	172 (0)	174 (0.7)
Hb-201+	Level 1	Reference[a]	79.5 (2.1)	78.0 (0)	79.5 (0.7)	79.0 (0)	80.0 (1.4)
		37°C/humid, closed[b]	79.5 (2.1)	80.0 (1.4)	80.0 (1.4)	80.0 (0)	80.2 (1.6)
	Level 2	Reference[a]	116 (0.7)	116 (0)	116 (0.7)	116 (1.4)	120 (0)
		37°C/humid, closed[b]	116 (0.7)	117 (0)	118 (0)	118 (0.7)	120 (3.5)
	Level 3	Reference[a]	158 (0.7)	157 (0.7)	158 (0)	158 (0.7)	160 (2.1)
		37°C/humid, closed[b]	158 (0.7)	157 (0)	159 (0.7)	159 (1.4)	159 (0)

[a] Cuvettes were stored in their original box at room temperature in a climate controlled laboratory

[b] Cuvettes were stored in their original box in an incubator set to 37°C and 72% humidity

[c] Cuvettes were stored in an open container in an incubator set to 37°C and 72% humidity

Table 4

Effect of suboptimal cuvette storage condition for up to 1 h on measured hemoglobin concentrations (g/L) using the Hb-201+ HemoCue® model: mean (SD) from 2 readings each

HemoCue® controls	Cuvette condition	Time, min						
		0	10	20	25	30	40	60
Level 1	37°C/humid, open[a]	81 (0)	79.5 (0.7)	74.5 (3.5)	79.5 (2.1)	77.5 (0.7)	72.5 (3.5)	74.5 (3.5)
Level 2	37°C/humid, open[a]	119 (0)	118 (3.5)	102 (4.2)	112 (10.6)	112 (19.1)	110 (21.9)	112 (10.6)
Level 3	37°C/humid, open[a]	161 (0)	162 (6.4)	145 (2.8)	141 (12.7)	118 (2.1)	113 (1.4)	122 (3.5)

[a]Cuvettes were stored in an open container in an incubator set to 37°C and 72% humidity

Table 5

Geometric mean Hb concentration and change relative to baseline after storing venous EDTA blood samples (n = 24) at different temperatures for up to 4 d: mean from 2 analyzers per HemoCue® model

Temperature	Time (d)	Hb-201+			Hb-301		
		Mean (SD), g/L	Change, %	*p*	Mean (SD), g/L	Change, %	*p*
10°C	0	129 (21.3)			133 (20.3)		
	1	129 (21.3)	0.26	0.3744	134 (20.1)	0.66	0.0005
	2	130 (21.3)	0.88	0.0167	135 (20.1)	1.32	<0.0001
	3	129 (21.0)	0.71	0.0299	135 (20.4)	1.68	<0.0001
	4	129 (21.3)	0.35	0.2376	137 (20.9)	2.78	<0.0001
Room temperature	0						
	1	127 (21.5)	−1.11	0.0061	134 (20.3)	0.73	0.0248
	2	129 (21.3)	0.03	0.9176	136 (20.1)	2.65	<0.0001
	3	129 (21.2)	0.35	0.2889	138 (20.3)	4.31	<0.0001
	4	129 (21.5)	0.03	0.8889	140 (20.1)	4.84	<0.0001
30°C	0						
	1	128 (21.2)	−0.07	0.8230	138 (20.0)	3.76	<0.0001
	2	127 (20.1)	−0.78	0.0910	141 (20.4)	6.20	<0.0001
	3	127 (20.2)	−0.76	0.0704	160 (27.5)	20.0	<0.0001
	4	127 (20.4)	−1.26	0.0036	167 (27.3)	25.5	<0.0001