Protocol for Comparative Evaluation of Blood Sampling Methods and Analytical Devices in the Measurement of Hemoglobin in Population Surveys – A Laboratory Study

Developed by the HEmoglobin MEasurement (HEME) Working Group
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Introduction

Anemia is diagnosed by measuring hemoglobin (Hb) concentration in blood. Anemia prevalence is determined by the percent of cases below a pre-determined reference value of the Hb concentration. When necessary, the threshold is adjusted for altitude and smoking status, as these have been shown to influence Hb concentration (WHO 2001). Automated hematology analyzers used with venous blood are considered an appropriate standard for hemoglobin measurement (Karakochuk et al. 2019). However, for field studies, portable devices have been used. The HemoCue device (HemoCue®, Angelholm, Sweden) with its 201+ and 301 models are commonly used (Whitehead et al. 2019). HemoCue measures Hb concentration using either reagent-driven changes (HemoCue 201+) or physical properties associated to hemoglobin (HemoCue 301). The amount of blood is small, and is loaded into a holding accessory called a microcuvette.

Either a venous or capillary blood sample is used for Hb determination. Studies have shown that the mode of blood collection can result in different Hb measurements, especially in field settings where quality of the blood collection can be difficult to ensure (Karakochuk et al. 2015; Neufeld et al. 2002; Gwetu and Chhagan 2015; Boghani et al. 2017; Conway et al. 1998). Other factors that can influence measurement of Hb concentration include deterioration of the microcuvette’s reagent due to humidity (especially seen in HemoCue 201+ model) and operational factors such as long lapse of time between loading the blood into the microcuvette and taking a reading with the equipment (seen with HemoCue 301 model). Several studies describe discrepancies in Hb concentration that are mainly due to factors such as use of capillary versus venous blood (Chen et al. 1992; Patel et al. 2013; Karakochuk et al. 2015; Neufeld et al. 2002; Adam et al. 2012; Sari et al. 2001; de A. Paiva et al. 2004; Wendt et al. 2020) or use of different HemoCue device models (Hinnouho et al. 2017). However, the studies have not been able to clearly determine if the different results are from differences of capillary and venous blood, or errors in the blood-collection process, or environmental factors like heat and humidity, or the type of the field instrument.

These discrepancies found in the determination of hemoglobin concentration could have a substantial effect in the estimation of anemia prevalence, especially in population surveys. In low- and middle-income countries, anemia data are most commonly obtained from field surveys. The DHS program determines Hb concentration on a drop of blood obtained through finger prick, and using a HemoCue 201+ (ICF International 2012), while micronutrient surveys have collected either a venous or capillary blood sample and used HemoCue 201+ or the 301 model. If capillary blood is used, it may be collected from a single blood drop or a pooling of several drops from finger pricking. The venous or the pooled capillary blood samples can be analyzed immediately using the HemoCue device or stored for assessment in an automated hematology analyzer.

Whitehead et al. 2019 reported inconsistencies in Hb concentrations estimated in single-drop capillary samples when compared to pooled capillary samples, as well as variations in Hb values coming from HemoCue devices when compared to autoanalyzers. In studies that include use of HemoCue devices, internal testing is conducted in a controlled setting as part of standardized operating procedures before the devices are used in the field. However, despite the use of these standardized procedures, differences in the field setting have been reported. A comparative study that examined hemoglobin distributions between surveys done by the DHS program and the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia project reported lower prevalence in the latter (by 2 to 31 percentage points for children and 1 to 16 percentage points for women) as compared to DHS. We also see a trend of decreasing anemia prevalence as the age of the children increases (comparing children aged 6-11, 12-23, and 24-59 months) (Hruschka et al. 2020); while this is due to metabolic differences, the challenges in obtaining a blood sample from the children less than two years of age cannot be understated. Therefore, there is a need to make a systematic comparison on the
determination of Hb concentration using different types of blood samples from the same individuals and analyzed using different instruments (either the laboratory or the field). This is needed for establishing the proper conditions to attain reasonable accurate results in population surveys.

**Aim of the Study**

The aim of the study is to identify the best procedures and methods for determining Hb concentration in a population survey.

**Research Questions**

We identified several questions to examine the differences in Hb concentration in two settings: a laboratory validation of the methodology; and field validation that refers to a comparison of methods under field settings. This protocol refers to the laboratory component (questions A1 and A2, below). A protocol for a follow-up study, complementary to this one, will consider the results of the laboratory study.

**A1. HemoCue Validation:** What is the performance of three HemoCue device models (201+, 301 and, if possible, 801) in a controlled laboratory setting compared to a certified autoanalyzer when measuring Hb concentration using venous blood from women of reproductive age (WRA) and children 12-59 months of age?

**A2. Sample Validation:** What is the performance of three HemoCue device models (201+, 301 and, if possible, 801) in a controlled laboratory setting compared to a certified autoanalyzer when measuring Hb concentration using pooled capillary or capillary blood drop (third drop) against venous blood in samples from WRA and children 12-59 months of age?

**Materials and Methods**

In the methods section, we describe the inclusion criteria for the study population, the epidemiological and biological data sampling, and the analysis plan.

**Setting**

The study will be conducted in at least three different countries. Each country will follow the same process of recruitment, collection, and analysis and test, at a minimum, the HemoCue 201+ and 301, with the option to also test the 801. The selection of the country will depend on the presence of an accredited laboratory with a certified autoanalyzer that participates in an external quality assurance program for the determination of hemoglobin. These laboratories will serve as the reference laboratory in the follow up study in the field, to conduct Hb measurements in venous blood using the autoanalyzer.

**Population**

We will conduct the study in two groups of apparently healthy volunteers of WRA that may include pregnant and non-pregnant women, and children 12-59 months of age. We will exclude volunteers exhibiting symptoms of any underlying pathology (and who have not been febrile or sick in the past week). We will also exclude volunteers with a previous diagnosis of hypertension, diabetes, or blood disorders. The screening will include a broad questionnaire (draft version enclosed in Appendix A to be refined by the bidders on the request for applications) to identify the study population. Among children aged 12-59 months, we will recruit equal number of participants from two age strata - 12-23 months and 24-59 months. This is to ensure that there is equal contribution of these two groups to the variability in Hb measurement.
Participants will be selected from a population in a clinical setting. Upon screening, parents of children with Hb below 7 g/dL and WRA with Hb below 8 g/dL, as per the venous blood measurement from the autoanalyzer, will be referred for follow-up care as per national guidelines. All subjects will have consented to participate in the study (we will take consent from parents for children 12-59 months of age), willing to provide both a venous blood and either a capillary single-drop or pooled sample. Information on anemia will be provided, describing its importance, and the need for accurate diagnosis and management.

Separate cohorts of individuals will be recruited for single-drop and pooled capillary samples, with each cohort also providing venous blood – we will not obtain two types of capillary samples from the same individual. The reference comparison group for all these studies is the Hb estimated from venous blood samples in an autoanalyzer in an accredited laboratory.

**Laboratory Studies**

**Study A1 (HemoCue Validation) and Study A2 (Sample Validation)**

These studies will compare Hb measurements on blood samples collected in a laboratory setting from volunteer subjects (WRA and children) and measured by laboratory staff on autoanalyzers and HemoCue machines. As seen in Figure 1, there will be four cohorts of volunteers. Each person in cohort 1 will provide a single venous blood sample and a pooled capillary sample; each person in cohort 2, 3, and 4 will provide a single venous blood sample and a single-drop capillary sample. Venous and capillary blood samples will be extracted by experienced phlebotomists. The determination of hemoglobin in the autoanalyzer will be done by following the usual laboratory procedure and as per the device manufacturer’s instructions. HemoCue devices will be used by the same personnel who is going to extract the venous and capillary blood samples.

In Cohort 1, the venous sample will result in up to four sets of Hb measurements— one on the autoanalyzer and one each on the HemoCue devices (201+, 301, and 801). This will allow us to compare the performance of the HemoCue devices against the autoanalyzer (Study A1).

The pooled capillary sample will result in three sets of Hb measurements—one on each of the HemoCue devices (201+, 301, and 801) (Study A2 with pooled capillary blood). These will be pair matched and compared against the Hb measurement using venous blood on the autoanalyzer (as measured in Study A1). This study will allow us to compare the performance of pooled capillary blood in the different HemoCue devices against venous blood in autoanalyzer.

In Cohort 2, 3, and 4, the Hb from a single-drop (the third) of capillary blood will compared against the Hb measurement on the autoanalyzer using venous blood from the same person in each cohort. This will allow us to compare the performance of single-drop capillary blood in the different HemoCue devices against venous blood in autoanalyzer (Study A2 with a drop of capillary blood). The phlebotomists are going to keep an aliquot of the venous samples, place in microtubes, and determine the concentration of hemoglobin in the corresponding HemoCue. The phlebotomists are also going to determine the concentration of hemoglobin in the single-drop of capillary blood samples using the corresponding HemoCue. This will allow to compare the performance of single-drop capillary blood against venous blood both in the same HemoCue devices, and comparing with the results of the autoanalyzer using venous blood. The reason for three separate cohorts is to promote more participation by ensuring that each volunteer undergoes a single finger prick and one phlebotomy for venous blood sampling.

We will include 18 WRA and 18 children (Cohort1, of which we will recruit 9 children each from the 6-23 and 24-59 month age groups), and 54 WRA and 54 children in Study A2 (Cohorts 2, 3, and 4, and in each cohort, we will recruit 9 children each from the 6-23 and 24-59 month age groups). Study A2 also includes Cohort 1 as the sample of pooled capillary blood will come from that Cohort. A total of 72 WRA and 72 children (36 children each from the 6-23 and 24-59 month age group) will be studied.
If a sample is lost or insufficient for the cases of venous and pooled capillary samples, another individual will be recruited until completed the required number of results. The number of people who were dropped from the study due to failure of sample collection, and the reason for that failure, will be documented for all cohorts.

For all cohorts, the venous, pooled capillary, and single-drop capillary samples are going to be collected by the same phlebotomist.

For both cohorts, it would be ideal if the measurement on the autoanalyzer and HemoCue devices are separated by no more than two hours. Results for each type of blood sample analyzed in each HemoCue device will be compared per average and variation values, receiver operating characteristic (ROC) curves, and area under the curve (AUC), as well as correlation and concordance against the results of the autoanalyzer.

Figure 1. Studies A1 and A2 – Study A1 (HemoCue validation) and Study A2 (Sample validation)

**Sample Size Calculations**

We calculated sample sizes for a comparison of two instrument types—Autoanalyzer and HemoCue—using venous blood samples; and for a comparison of venous blood with capillary blood, using a HemoCue device. Since the source of the blood sample is an individual in the study, we indicate the total number of individuals in the sample size table. We also include the number of results that will be conducted per individual in each one of the four studies (Table 1). Hemoglobin concentration is a continuous numerical variable, therefore to test agreement on the results coming from the different methods, we used statistical tools that measure concordance or agreement. The sample size calculation was based on measures of concordance correlation coefficient. (CCC) (L. I. Lin 1989; L. I. Lin et al. 2002; L. Lin 1992) (see Appendix B for details of the calculations).
Table 1. Sample Size Requirements for Each Comparison in Children and WRA (using three HemoCue device models).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Children 12-59 months</th>
<th>WRA</th>
<th>Total participants (results)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HemoCue Validation: Study A1 (venous blood from cohort 1)</td>
<td>18 children (4 results: 3 for each HemoCue and 1 for the Autoanalyzer) for a total of 72 results.</td>
<td>18 WRA (4 results each: 3 for each HemoCue and 1 for the Autoanalyzer) for a total of 72 results.</td>
<td>18 children and 18 WRA (108 HemoCue and 36 autoanalyzer results) for a total of 144 results</td>
</tr>
<tr>
<td>Sample validation – Study A2- pooled capillary (comparison of pooled capillary with venous blood in cohort 1)</td>
<td>18 children (determination of Hb in the venous sample has been done in Study A1)</td>
<td>18 WRA (determination of Hb in the venous sample has been done in Study A1)</td>
<td>18 WRA, for a total of 108 HemoCue results.</td>
</tr>
<tr>
<td>Sample validation – Study A2-single-drop capillary (comparison of single-drop capillary with venous blood in cohort 2, 3, and 4)</td>
<td>54 children (for one result for the Autoanalyzer and one each for different HemoCue machines) for a total of 108 results.</td>
<td>54 WRA (for one result with single-drop capillary blood in single HemoCue devices) for a total of 54 results.</td>
<td>54 children and 54 WRA (108 autoanalyzer results and 216 HemoCue results) for a total of 324 results</td>
</tr>
<tr>
<td>Total laboratory validation</td>
<td>144 participants- 72 children and 72 WRA for a total of 576 results (144 autoanalyzer results and 432 HemoCue results)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WRA=Women of reproductive age
Analytical Devices

We will evaluate three models of HemoCue devices (201+, 301, and 801) against a laboratory autoanalyzer. The study sites will procure the HemoCue machines to be used in the study.

HemoCue Models

We will use the Hb201+, Hb 301, and the newly released Hb 801 models of HemoCue. Researchers will decide to use either one, two, or the three HemoCue models. We will follow standardized protocols for minimizing errors associated to improper practices.

Quality Control and Assurance

Study sites will perform daily quality control by measuring and recording the results from the use of HemoTrol (liquid controls for HemoCue) at the low, normal and high range for the device. This will be done at the beginning of a day before blood collection and at the end of the day, after blood collection is complete.

Hematology Autoanalyzer

Hematology autoanalyzers are considered reference standards for measuring Hb and we will use venous blood samples to measure Hb. The technology underpinning the measurement of Hb by these devices varies by manufacturing company. The samples are run in batches in these devices.

Internal quality control samples of two levels will be run with each batch of samples. Details of the internal quality assessment are provided in Appendix C.

Central Laboratory Requirements

We will select a laboratory, whose hemoglobin determination by autoanalyzer has been certified, and that participates in an external quality assurance program, with pre-specified criteria established in the Micronutrient Survey Toolkit. Briefly, it will include the following:

1) Testing of quality control samples several times a year, with results sent to a reference laboratory.
2) Documentation of equipment monitoring.
3) Documentation of policies and procedures.
4) Training and evaluation of field staff.
5) Method validation and verification.
6) Laboratory audits.

Please see Appendix D for details of the external quality assurance program.

The autoanalyzers and HemoCue device models will be standardized using quality control and assurance procedures in each setting.

Biological Sampling

Data collection methods will be standardized across sites and will incorporate demographic information. We will follow the guidelines laid down by the Centers for Disease Control and Prevention (CDC) in its Micronutrient Survey Toolkit (Centers for Disease Control and Prevention (CDC) 2020).

We will use three methods for collecting the biological samples (i.e., venous, single-drop capillary, and pooled capillary). We will follow the steps in the CDC toolkit referenced above. The procedure for collection of the blood samples are briefly described below. Details for collection of blood samples and
the materials that will be needed for the procedures are available in Appendix E. All samples will be collected with an observance of universal precautions for handling and disposing of biological materials or in contact with biological materials.

**Venous Sample**

Venous samples will be collected by an authorized phlebotomist. In the laboratory, the venous samples will be stored under appropriate conditions and run in batches in the autoanalyzer. Three to four milliliters of the venous sample will be collected in a Dipotassium Ethylenediaminetetraacetic acid (K₂-EDTA) containing vacutainer tube. For the hemoglobin determination in the HemoCue devices: Transfer a few drops of blood onto special wax paper using a small plastic transfer pipette and the appropriate amount of the sample will be loaded into the HemoCue microcuvette.

**Single-Drop Capillary Sample**

Single-drop capillary samples will be collected by finger prick from WRA and children under 59 months of age with the type of lancet that allows good flow of capillary blood. HemoCue manual instructions will be followed. Briefly, the first and second drops of capillary blood are wiped away, and a sample from the third drop is collected by capillary action into a microcuvette without touching the finger.

**Pooled Capillary Sample**

The pooled capillary sample will be collected by a finger stick puncture similar to the single-drop capillary using a high-flow lancet. The first drop of blood will be wiped away. The phlebotomist will collect subsequent blood drops (approximately 8-15 drops) to obtain a volume of at least 250 µL up to 500 µL in a 250-500 µL K₂-EDTA microtube. The number of blood drops will be recorded in each case. After mixing the pooled sample, a few drops of blood will be placed onto special wax paper using a small plastic pipette, and the sample will be drawn into the HemoCue microcuvette.

**Data Management**

The study protocol will be registered in the ClinicalTrials.gov database.

**Ethical Clearance**

Ethical clearance will be obtained from the JSI institutional review board and from a recognized institutional review board in countries where the study will be conducted.

**Data Analysis**

For each information coming from each country, the laboratory validation, all blood samples would be compared with the gold standard (hemoglobin determination in an autoanalyzer using venous blood), to estimate concordance correlation coefficient (CCC), which is the agreement and concordance between the a new test and a gold standard or reference test that are measuring the same variable. Agreement among the methods will also be evaluated. We will use Hb as a continuous variable in assessment of agreement; we will also carry out receiver operating characteristics curve analysis (graphical ROC curves and AUC) using Hb concentration in venous blood measured on an autoanalyzer to assess the optimal cutoff to use for comparing the various groups.
Laboratory Validation

There are currently 10 methods that combine both device and type of blood sampling that will be used in the analysis, as follows:

1) Venous autoanalyzer
2) Venous 201+
3) Venous 301
4) Venous 801
5) Pooled capillary 201+
6) Pooled capillary 301
7) Pooled capillary 801
8) Capillary 3rd drop 201+
9) Capillary 3rd drop 301
10) Capillary 3rd drop 801

The estimation of CCC and Kappa statistic would be done for the following pairs of methods:

A. Agreement with reference standard
   1) “1” vs “2”
   2) “1” vs “3”
   3) “1” vs “4”
   4) “1” vs “5”
   5) “1” vs “6”
   6) “1” vs “7”
   7) “1” vs “8”
   8) “1” vs “9”
   9) “1” vs “10”

B. Agreement among methods (not reference standard)
   10) “2” vs “3”
   11) “2” vs “4”
   12) “2” vs “5”
   13) “2” vs “6”
   14) “2” vs “7”
   15) “2” vs “8”
   16) “3” vs “4”
   17) “3” vs “5”
   18) “3” vs “6”
   19) “3” vs “7”
   20) “3” vs “9”
   21) “4” vs “5”
   22) “4” vs “6”
   23) “4” vs “7”
   24) “4” vs “10”
   25) “5” vs “6”
   26) “5” vs “7”
   27) “6” vs “7”

Also, Overall CCC could be estimated using the 7 methods (1 to 7) from cohort 1. All contrasts mentioned above for each country could be pooled in a meta-analysis using information from the three or more countries participating in the study. This could give us agreement values to be used as a
reference. In the case of the children, while the 6-23 and 24 to 59 months age groups are equally represented, the results are going to be pooled together for both age groups for analysis.

Furthermore, bias (location and scale shifts) with respect to the reference standard will be estimated. In this regard, we will perform a Bland-Altman analysis (i.e., display the differences between the two methods versus the average of each pair of measurements [Bland and Altman 1999]). This will show how large the differences are and whether they vary by the ‘true’ values (as, for example, represented by the average). The Bland-Altman approach assumes constant variance. The assumption of constant variance will be checked by visual inspection of the Bland–Altman plot. In our graphical ROC curves and AUC calculation, we will use the Hb concentration in venous blood measured on an autoanalyzer and consider various cutoffs that maximize the AUC, including those cutoffs that are currently being used to determine whether an individual is anemic (WHO 2001). The latter will be done only for determining sensitivity and sensibility, and comparative predictive value of the different methods, and not for diagnosis of anemia.

**Outcomes**

Study results will inform use of standardized sampling procedures and appropriate field devices in population surveys.
References


Appendix A. Questionnaire for Identifying the Subjects for the HEME Study and a sample consent form

Consent obtained from caregiver of child?
Consent obtained from non-pregnant WRA?

Elements to be included:
1) Sex
2) Date of birth
3) Location (by GPS)
4) Altitude (smart phone or public databases)
5) Temperature and humidity
6) Date of sample collection
7) Time of sample collection
8) Pregnancy status
9) (For WRA): What date was the first day of your last menstrual period?
10) (for children): Has your child been suffering from any febrile illness in the past week (yes/no)
11) Do you currently smoke cigarettes every day, some days, or not at all? (every day/some days/not at all)
12) On average how many cigarettes do you currently smoke each day? _

Other elements in study questionnaire
1) Venous Hb (which arm)
2) Capillary Hb (which hand and finger)

We also include a Sample consent form that was adapted from the template provided in the Micronutrient Survey Toolkit (Centers for Disease Control and Prevention (CDC) 2020)]

HEME Validation Study – Consent for participation
(Please note that the consent form should be an independent document and it needs to be modified to meet the minimum GCP requirements. This is provided as a sample)

<table>
<thead>
<tr>
<th>Household Consent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you agree that a woman of reproductive age of the same household can participate in this study?</td>
</tr>
<tr>
<td>Do you agree that a child 12-59 months of age of the same household can participate in this study?</td>
</tr>
</tbody>
</table>
RECORD WHETHER OR NOT CONSENT IS PROVIDED ON THE QUESTIONNAIRE

| Consent for primary caregivers of children 12-59 months are selected |
| (Read consent information to the primary caregiver of each child) |

We would like to collect two samples of blood from your child 12-59 month. The sample will be collected by using a needle to obtain blood from your veins and also through a finger prick blood specimen. Testing the blood will also help us to determine if the two readings are the same, and thus help us know if the current methods of measuring hemoglobin are accurate.

Do you agree that we can take two blood samples from your child?

RECORD WHETHER OR NOT CONSENT IS PROVIDED ON THE QUESTIONNAIRE

| Consent for non-pregnant women 15-49 years |

We would like to collect two samples of blood from you. The sample will be collected by using a needle to obtain blood from your veins and also through a finger prick blood specimen. Testing the blood will also help us to determine if the two readings are the same, and thus help us know if the current methods of measuring hemoglobin are accurate.

Do you agree that we can take two blood samples from you?
Appendix B. Sample Size Calculations

Laboratory Validation

The study design of the laboratory validation is shown in Figure B1.

Figure B1. Comparison of tests within cohorts

There are 10 different methods, distributed to be tested accordingly with figure 1. Each person of a cohort will provide:

a) Venous blood sample  
b) Capillary blood, either:  
   b.1 Pooled capillary blood (drop by drop, fill a purple cap microtube test (at least to the first line), or  
   b.2 Third drop

Among these:

- venous blood sample is considered the gold standard,  
- both venous blood sample and a capillary blood measurement are taken from the same person within each cohort

Hence, the comparison of the data must be done with statistical tools that measure concordance or agreement.

The hemoglobin measurement is a continuous numerical variable, therefore to test agreement on the results coming from the different methods, CCC will be used (L. I. Lin 1989; L. I. Lin et al. 2002; L. Lin 1992).
The CCC is calculated using pairs of observations (coming from the same person), where one of these is a gold standard (venous sample); but further analysis could be done comparing the different methods among them, which would also give us information on how these methods agree among them.

Proposed sample sizes are given based on different precision and bias, and the selected sample size should be the highest one. The selected sample size will be used for each of the four cohorts.

We will define the following terms that serve as the inputs to calculate the sample size (L. Lin 1992):

1) **Precision**: Precision refers to the "closeness of agreement between test results". Pearson correlation coefficient ($r$) evaluates how far the observation deviate from the best-fit linear line.

2) **Bias**: Bias refers to "The measure of accuracy evaluates how far the best fit line deviates from the concordance line in the scale of 1 (no deviation) to (but not including) 0 (very far away). This bias consists of a scale shift (ratio of 2 standard deviations) and a location shift (square difference in means relative to the product of 2 standard deviations)."

Scale shift and location shift allow us to estimate bias. Assume the following values from a hypothetical study:

1. Gold standard: Mean= 12 mg, standard deviation= 8 mg
2. New method: Mean= 11 mg, standard deviation= 7 mg

**Scale shift**: explains the relationship between the variability of the new method in relation to the gold standard:

\[
\text{Scale shift} = \left(1 - \frac{\text{Highest SD}}{\text{Smallest SD}}\right) \times 100 = \left(1 - \frac{8}{7}\right) \times 100 = 14.3\%
\]

**Location shift**: estimates how far the mean value of the “new” method is from mean of the gold standard, but related to the variability from both methods. In our example:

\[
\text{Location shift} = \left(\frac{(\text{Mean new} - \text{mean GS})^2}{\text{SD new} \times \text{SD GS}}\right) \times 100 = \left(\frac{(11 - 12)^2}{7 \times 8}\right) \times 100 = 1.8\%
\]

**Sample sizes for CCC** were calculated based on the assumption that the within-sample variation among methods is small (assays would be under ideal conditions). We calculated sample sizes for different levels of precision, location shift, and scale shift, as presented below;

1. 41 paired samples, based on
   a. Precision of $r^2 = 0.95$ ($r = 0.98$)
   b. 1% loss of precision
   c. Bias: location shift of 12.5% and a 10% scale shift
2. 31 paired samples, based on
   a. Precision of $r^2 = 0.95$ ($r = 0.98$)
   b. A 2% loss of precision
   c. Bias: location shift of 12.5% and a 10% scale shift
3. 17 paired samples, based on
   a. Precision of $r^2 = 0.95$ ($r = 0.98$)
   b. A 1% loss of precision
   c. Bias: location shift of 25% and a 10% scale shift
4. 16 paired samples, based on
   a. Precision of $r^2 = 0.95$ ($r = 0.98$)
   b. A 2% loss of precision
   c. Bias: location shift of 25% and a 10% scale shift
A location shift of 35% and scale shift of 10% translates to about a 0.6 g/dL in mean difference and a 0.2 g/dL difference in standard deviation of Hb measurements by the two methods. We assume those to be the variation between the results and select the third assumption in the sample size calculations. Therefore, the recommended sample size is going to be 17 for each cohort. As three persons are going to be asked to take the capillary samples, each one of them is going to work with 6 WRA and 6 children, for a total of 18 WRA and 18 children per cohort.
Appendix C. Characteristics of an Internal Quality Assessment and Control

Characteristics of an Internal Quality Assessment and Control

[Quoted from the Micronutrient Survey Toolkit (Centers for Disease Control and Prevention (CDC) 2020)]

Quality Assessment

Quality assessment (QA) ensures that the final results reported by the analyst for all the analytes measured in the laboratory are accurate and of the highest quality. To achieve high-quality results, full staff participation is required. All laboratory personnel should be aware of the necessity for quality performance to ensure that the laboratory retains its accreditation. QA ensures that all of the following items are addressed: 1) avoidance of mistakes; 2) consistency of performance; 3) data integrity; and 4) opportunities for training.

The basic components of a QA system include: 1) internal quality control (QC) through the use of bench and blind QC samples; 2) external QA via participation in proficiency testing programs; 3) equipment monitoring and maintenance; 4) documentation of policies and procedures; 5) proper staff training; and 6) laboratory audits. However, before the quality and consistency of any laboratory method can be monitored, prospect methods must be validated (for accuracy, precision, sensitivity, and ruggedness) and verified periodically (verification of calibration, verification of accuracy of pipettes, instruments, etc.).

Internal Quality Control

Bench QC: Bench QC pools are typically prepared in-house by the laboratory in quantities sufficient to last for a few years (depending on the stability of the material) and they are incorporated after characterization into each assay in an open way (analyst knows the identity and concentration expected in the sample).

Usually three levels of bench QC pools—a low, medium, and high pool—are prepared. During each assay, these three levels of bench QC pools are analyzed together with unknown subject samples by placing them at the beginning and the end of the run.

Laboratories should prepare their own bench QC pools and characterize them appropriately (throughout a 20-day period) before incorporating them into the analysis of survey samples. Separate documents provide guidance about how to prepare bench QC pools, including how to determine and apply acceptability limits for each pool. No samples should be analyzed without using bench QC samples in every run (ideally at the beginning and end of each run). If the QC pools are not within the acceptability limits, the results of the assay or the complete run should not be accepted; the analyst should determine the cause of the QC failure, and address the problem appropriately, and repeat the run.

Blind QC: Blind QC pools also typically are prepared in-house by the laboratory in quantities sufficient to last for a few years (depending on the stability of the material) and incorporated after characterization into each assay in a blinded way (analyst does not know the identity and concentration of the samples).
Two levels of blind QC samples (e.g., a low and high pool) are typically prepared by the laboratory. The blind QC samples labels are very similar to the survey subject’s labels, and only the supervisor or someone not involved with the analysis should know which samples are blind. Also, only the supervisor or someone not involved with the analysis should insert the blind QC samples into the survey samples to ensure the analyst does not know the position of the blind QC samples in the box.

Using blind QC samples helps the survey coordinator or the external party evaluating the laboratory results better judge the laboratory performance and, therefore, builds confidence in the data. Ideally, similar variability (CV) on bench and blind QC samples is expected. Using blind QC samples also helps identify potential errors that may occur when the samples are misidentified during the assay because the supervisor knows the position of the blind QC and its concentration.

Note: If it is not possible to prepare stable samples of the “Bench QC” and “Blind QC” pools that can be kept over a long span of time, please use the methods described in Appendix D on use of stabilized quality control materials and frequent external quality control testing.
Appendix D. Characteristics of an External Quality Assessment Program

Characteristics of an External Quality Assessment/ Proficiency Testing Program

[Quoted from the Micronutrient Survey Toolkit (Centers for Disease Control and Prevention (CDC) 2020)]

External Quality Assessment (EQA) is a valuable and important tool for laboratories to assess how their values compare to other methods and laboratories and, therefore, to assess the quality of their results.

The Centers for Medicare & Medicaid Services (CMS) maintains a list of official, proficiency testing (PT) programs approved by Clinical Laboratory Improvement Act (CLIA) (http://www.cms.hhs.gov/CLIA/14_Proficiency_Testing_Providers.asp). In these programs, laboratory performance is rated according to pass/fail criteria. EQA programs are not regulatory in nature, but they do provide valuable feedback to laboratories.

Some common EQA/PT programs for nutritional indicators are available at:

- College of American Pathologists (CAP) (http://www.cap.org/apps/cap.portal)
- United Kingdom Vitamin D External Quality Assessment Scheme (UK DEQAS) (http://www.deqas.org)
- United Kingdom National External Quality Assessment Service (UK NEQAS) (http://www.ukneqas-haematinics.org.uk/)
- National Institute of Standards and Technologies Micronutrients Measurement Quality Assurance Program (NIST MMQAP) (http://www.cstl.nist.gov/acd/839.02/qa.html)

These programs provide QC samples to participating laboratories several times per year (the number of samples and frequency may vary with different programs). The unknown samples are analyzed by participating laboratories and the results are reported to the organizer within a predefined reporting period for performance assessment. The organizer compiles all results and generates reports that are distributed to the participating laboratories. The EQA program sets criteria for acceptability of the participating laboratory’s results based on different criteria. Typically, laboratory results are compared to an all laboratory trimmed mean (ALTM) or a method-specific mean (if method differences exist), therefore the interpretation of results is not necessarily accuracy based. However, if a particular analyte has been standardized and/or if target values for the EQA materials have been assigned by an accuracy-based method, the EQA program can check for accuracy of results. The laboratory results are considered acceptable if they fall within the range of acceptability. Typically, 80% of laboratory results must fall within predefined acceptability limits for the laboratory to pass an EQA challenge.

Equipment Monitoring and Maintenance

Laboratory equipment should be checked regularly to ensure acceptable performance. Each analytical procedure outlines the maintenance and function tests that must be conducted on the equipment to ensure proper method performance and acceptable results. These checks must be made at the interval
specified in the procedure documentation. Maintenance and function checks should be documented in the equipment log. Failure of a function check, and remedial action taken, also should be documented in the equipment log. It is also important to regulate the temperature of instruments with temperature-dependent components or functions. Stable temperatures must be maintained in freezers in which assay materials requiring low temperatures (e.g., -20°C or -70°C) are kept. Freezer and refrigerator temperatures should be checked weekly and results should be recorded in maintenance logs. Freezers and refrigerators should be monitored regularly for excessive ice deposits and inoperative cooling fans. Eye-wash stations should be flushed weekly. Deionized water systems and fire extinguishers should be checked monthly. Problems should be reported to the laboratory chief for action.

Equipment monitoring maintenance logs: The calibration of pipettes and pipetting devices should be verified regularly (at least every 6 months) and results should be recorded in maintenance logs; pipettes should be recalibrated if they do not meet performance specifications. The calibration of balances and centrifuges should be verified at least annually and results should be recorded in maintenance logs; balances and centrifuges should be recalibrated if necessary. Other equipment such as spectrophotometers should be calibrated regularly to ensure that they provide accurate readings and results should be recorded in maintenance logs. Date, finding, and person conducting the procedure should be recorded in the maintenance log for any calibration, calibration verification, or maintenance procedure. Dates of replacement of parts, such as inline filters, columns, and guard columns, should be noted. For chromatographic methods, test chromatograms for each column should be kept. Each new column should be compared to the column it is replacing by analyzing a set of QC pools to ensure that the column gives acceptable separation before it is used for routine analyses.

**Documentation of Policies and Procedures**

Documentation is a written procedure to be followed by all the staff working on the analyses. **Safety precautions** for all methods and procedures should be well documented. The laboratory chief ensures that the primary laboratory analyst has read and is familiar with safety precautions involved in each procedure.

A written **Methods Procedure Manual** for performing all analytical methods used by the laboratory must be readily available and followed by laboratory personnel. It must be approved, signed, and dated by the laboratory director. Any changes must also be approved, signed, and dated by the laboratory director. To assure uniformity and minimize differences between analysts, all staff performing analyses must be aware of any changes so that everyone follows the same procedures.

For **analytical runs**, the run date, calibration results, QC results, and the analyst who prepared the specimens should be kept. Ideally, records of everything that might affect the results of the analyses should be kept. These records will make it simpler to determine the source of analytical problems and correct them.

**Labeling** is a key component for staff both in the laboratory and in the field:

- **Laboratory**: Reagents, solutions, and other supplies must be labeled appropriately to indicate the identity of contents, concentration, preparation and expiration date, name of preparer, recommended storage requirements, and any other pertinent information required for proper use. Chemicals should also be labeled to indicate a receipt date and open date.
- **Field**: The field and laboratory staff involved in the surveys should be well trained to properly label Vacutainers/vials used for blood or urine collection and sample storage, and slides used to prepare malaria smears. The cryovial boxes used for storing samples should be labeled appropriately and stored in freezers. All samples collected and stored should be documented using electronic lists. Bar-code labeling of tubes, vials and boxes is preferred whenever possible.
• **Shipping of samples:** The procedures for packing the Styrofoam boxes used for shipping samples on dry ice to other laboratories for analysis should follow the IATA regulations. The boxes should be labeled with a dry-ice label indicating the quantity of dry ice and other labels as applicable. An appropriate shipping list, which includes the study name, date of sample collection, number of samples, and any other relevant information, should be included with the samples. Documentation of samples shipped to various laboratories for analysis should be maintained by the sender and receiver.

**Training and Evaluation of Staff**

Qualified, well-trained, and competent personnel are essential for good laboratory performance. New staff members should be trained appropriately to perform the assay with the best possible accuracy and precision. Staff performance is evaluated by:

- Assessing intra-observer and inter-observer variability on volunteers during training.
- direct observation of test performance, specimen handling, and specimen processing and testing;
- direct observation of recording and reporting of test results;
- review of QC results, proficiency testing results, and preventive maintenance records;
- direct observation of performance of instrument maintenance and function checks;
- review of blind QC data; and
- assessment of problem-solving skills.

Evaluation is performed by the laboratory chief or their designee. If necessary, additional training must be provided to enhance the technical skills.

**Method Validation**

Any prospective method must be validated before it is used for patient testing. Validation of an analytical method is the process that establishes that the method’s performance characteristics meet the requirements for the intended analytical applications. Performance characteristics are expressed in terms of analytical parameters (accuracy, precision, sensitivity, specificity, and ruggedness).

- **Accuracy:** The closeness of test results to the “true” results.
- **Precision:** The closeness of agreement between test results.
- **Sensitivity:** Measured by the limit of detection (LOD), which is the lowest level of analyte in a sample that can be detected or the level at which the measurement has a 95% probability of being greater than zero.
- **Specificity:** The determination that the correct component is being measured. Use multiple methods, if possible, to assure specificity. Test potential interferences; test reference materials; compare results with a more definitive method (reference method).
- **Ruggedness:** The change in accuracy, precision, sensitivity and/or specificity resulting from changes in method parameters likely to occur during analyses. Method parameter changes are generally quantitative (e.g., length of incubation or reaction time, amount of enzyme used, temperature, residence time on column, concentration of buffer).

**Method Verification**

All methods must be verified regularly to ensure that they are still performing as expected.

**Calibration:** Calibration is performed using calibration material that contains a known amount of analyte. Based on the assay, various concentrations of the calibration material are used to generate a calibration curve. Generally, calibration curves are linear and every run contains a calibration curve, but that is not always the case.
**Calibration verification:** Calibration verification is the analysis of calibration materials in the same manner as patient samples to confirm that the calibration of the instrument, kit, or test system has remained stable throughout the laboratory’s reportable range for patient test results. Calibration verification is used to ensure that the accuracy of the measurement process across the reportable range is maintained over time. It is performed routinely using standard reference materials after any change in the analytical procedure that is likely to make a non-trivial difference in sample results (change of reagent lots, replacement of critical parts that may influence test performance, controls are not within the acceptable limits, etc.). If the test system calibration procedure includes three or more levels of calibration material (low, mid, and high value) and is performed at least once every six months, the requirement for calibration verification is met.

Some common standard reference materials for nutritional indicators are available at:
- National Institute for Biological Standards and Control (NIBSC) ([http://www.nibsc.ac.uk/](http://www.nibsc.ac.uk/))

**Laboratory Audits**

Internal laboratory audits verify compliance with technical and operational procedures.
Appendix E. Biological Sampling

Biological Sampling

Venous and pooled capillary blood sampling techniques follow the procedures laid out in the CDC’s Micronutrient Survey Toolkit (Centers for Disease Control and Prevention (CDC) 2020). Single-drop capillary blood collection follows the procedure laid out in the Demographic and Health Survey’s Biomarker Field Manual (ICF International 2012). We will let participant comfort dictate the decisions about whether to use the non-dominant hand for venous and capillary sampling or whether to use different sides for the venous and capillary blood collection. We suggest the non-dominant side for both venous and capillary sampling, unless indicated otherwise by the participant.

Procedure for Venous Blood Collection

(Centers for Disease Control and Prevention (CDC) 2020)

Universal Precautions - procedures to prevent exposure to HIV, hepatitis, and other infections agents are assumed during all collection and handling of biological specimens. ALL specimens should be considered potentially infectious. Practice Universal Precautions using fresh “powder-free” gloves for each patient, eye protection, and a laboratory coat.

1. Collecting a venous blood sample
   a. Obtain consent for blood collection
   b. Locate a suitable area for the blood collection procedure. Make sure participant is sitting comfortably.
   c. Lay out all blood collection supplies onto a disposable absorbent pad. Assemble needle or butterfly needle into Vacutainer holder being sure that it is firmly seated into threads. Loosely place Vacutainer tube into holder, but do not puncture top. Assemble and open supplies needed for collection.
   d. Examine both arms to find the best vein. Locate the puncture site. Apply tourniquet (not too tightly).
   e. Wipe the area with an alcohol prep in a circular motion making sure the area is thoroughly clean. Dry with gauze.
   f. If it is necessary to feel the vein again, do so. After you feel it, cleanse the area again with an alcohol prep. Dry with gauze.
   g. Fix the vein by pressing down on the vein about 1 inch below the proposed point of entry into the skin and pull the skin taut.
   h. Remove needle shield.
   i. Approach the vein in the same direction the vein is running. Hold the needle so that it is at an approximately 15-degree angle with the participant’s arm.
   j. Push the needle with bevel facing up firmly and deliberately into the vein. Activate the vacuum collection tube by pushing the tube onto the needle and puncturing the tube top. If the needle is in the vein, blood will flow freely into the tube. If no blood enters the tube, probe once or twice for the vein until entry is indicated by blood flowing into the tube.
   k. For collection, loosen the tourniquet immediately after blood flow is established and release entirely as the last tube fills, about 3 ml.
   l. If a syringe is required to obtain the blood, attach it to the appropriate size butterfly needle and withdraw 2-3 mL blood. After withdrawing the needle from the arm, quickly change the needle on the syringe and transfer blood from the syringe by puncturing the
top of the tube with the new needle and allowing the vacuum to draw the blood into the tube. Mix well with the anticoagulant to avoid clotting.
m. Withdraw the tube, then the needle. Heavy pressure as the needle is being withdrawn should be avoided.
n. If you plan to use the venous blood to measure hemoglobin on a HemoCue machine, please see instructions on that process below. Transfer a few drops of blood onto special wax paper using a small plastic transfer pipette before capping the vacuum tube.
o. When the needle is out of the arm, press gauze firmly on the puncture.
p. Have the participant raise his/her arm (not bend it) and continue to hold the gauze in place for several minutes. This will help prevent hematomas.
q. Report to the supervisor any unusual reaction experienced by the participant during the venipuncture procedure.
r. Place a bandage on the participant’s arm.
s. Label all tubes with the preprinted labels provided and use a permanent marker to add the date collected to the label (if a date or date range is not already printed). If the label contains a barcode, the barcode needs to be vertical like a ladder when placed on the tube. If the barcode is not vertical, the laboratory will not be able to read the label with the barcode reader. Place the label over the existing tube label so that it can be read from left to right starting from the cap end.
t. You may resheath the needle, but only with proper technique. With the needle top on the absorbent pad, slowly slide the needle into the needle top.
u. The needle should be discarded into a Sharps container. Place all labeled tubes in a cool box, and discard waste into appropriate biohazard container.
v. Dispose of biological waste according to the laws of the country in which the survey is taking place.

2. Using the HemoCue device for Hb measurement on a venous blood samples
   a. For venous samples, measuring needs to be initiated within 8 h after collection for HemoCue models.
   b. Transfer a few drops of blood onto special wax paper using a small plastic transfer pipette. Ensure that the specimen is mixed before using the microcuvette.
   c. Take the microcuvette out of the cuvette box, and then close the lid of the cuvette box.
   d. Apply the tip of the HemoCue microcuvette to the middle of the blood drop. The microcuvette chamber will fill itself automatically by capillary action. Filling the HemoCue microcuvette in one continuous step is important in order to avoid air bubbles. The chamber needs to be filled completely never “top off” the microcuvette. Instead, if the microcuvette is not completely filled, use a fresh microcuvette and fill it with the next blood drop that forms.
   e. Wipe any surplus blood off both sides of the microcuvette “like butter from a knife,” using the clean end of a sterile gauze pad. Ensure that no blood is sucked out of the microcuvette when wiping it – do not let the tip of the filled microcuvette touch the gauze.
   f. After filling the chamber, the microcuvette needs to be visually inspected for air bubbles. Since air bubbles may influence the hemoglobin measurement, any microcuvette containing air bubbles must be discarded. In such cases, with the permission of the parent/ responsible adult, repeat the blood drop collection using a different finger. Again, you must use new disposable supplies and follow all of the steps described previously in obtaining the new sample.
   g. Place the microcuvette in its holder and gently push the holder into the HemoCue device. Time to measurement for HemoCue 301 and HemoCue 801 needs to be within
Blood sampling methods and analytical devices in the measurement of hemoglobin

40 seconds after filling of microcuvette; for HemoCue 201, time to measurement is within 10 minutes after filled cuvette.

h. Reading the results: The microcuvette should be analyzed immediately, and no later than ten minutes after being filled. The blood hemoglobin level in grams per deciliter (g/dl) is displayed 15 to 45 seconds after the drawer is closed.

i. Record the hemoglobin level and test result:

Blood collection materials needed for venous blood sampling

1) Vacutainer Tube 3.0mL, 13 x 75mm, Plastic, K2 EDTA 5.4mg, Hemogard Closure, with Paper Label, Lavender Closure
2) Needle holder, vacuum tubes
3) Disposable needle, 22 or 23 G
4) Samco graduated transfer pipets, 3.9mL Small Bulb, Graduated to 1mL, Sterile, Individually Packed
5) Thomas Scientific Glassine Weighing Paper, 3 x 3”, for loading the HemoCue microcuvette
6) Gauze compress
7) Adhesive bandage
8) Individually wrapped alcohol pads
9) Personal protective equipment - fresh “powder-free” gloves for each patient, eye protection, and a laboratory coat
10) Materials needed for safe disposal of sharps and biological waste

Procedure for Capillary Blood Collection

A. Single-drop sampling (ICF International 2012)

1. Complete general preparation
   a. Do not remove microcuvettes or filter paper until right before pricking. These items should be taken out on an individual basis. In other words, if three individuals are being tested for anemia, only remove the microcuvettes from the canister one at a time, before pricking each person.
   b. If the respondent is a child, describe to the parent/responsible adult exactly what will be done during the collection of the blood sample and how they can assist by holding the child on their lap and holding the child’s hand during the collection of the sample.
   c. The child may be fearful or anxious about what is going to happen. Therefore, using a calm and reassuring manner with the child is important as you begin to collect the blood sample. Remember that nonverbal communication is important, so maintain eye contact with the child as you prepare to take the sample.

2. Select and prepare the fingerprick site
a. Blood collection is usually easier if you sit on the side of the respondent opposite to the hand that you will collect blood from. For example, if you want to collect the specimen from the left hand, place yourself to the right side of the respondent.

b. Use the third or fourth finger for collecting the blood. Do not use a finger with a scar, a wound or cut, an infection, swelling, a deformity, or a rash. Also, do not use a finger on which the respondent is wearing a ring, because the ring may disrupt the free flow of blood to the tip of the finger. You can ask the respondent to remove the ring.

c. Warm the skin over the puncture site by rubbing it. This will increase blood flow to the fingertip and improve the ease with which a sample can be obtained.

d. With an alcohol swab, clean the skin of the finger thoroughly. If the skin is very dirty, use a second swab. Finish cleaning the finger before preparing for the finger prick. Ensure that the fingertip is dry after disinfecting by allowing it to air dry. Do not blow on the area to dry the alcohol. Blowing may allow bacteria to contaminate the site.

e. Ensure that the correct size lancet is easily accessible. For adults, you will use adult lancets which have a needle diameter of 0.81 mm (21 G) and pierce the skin to a depth of 2.4 mm. For children, you will use children's lancets which pierce the skin to a depth of 2.25 mm.

f. Remove the blade slot cover - For the child lancet, remove the blade slot cover by first twisting it 360° and then pulling it out; for the adult lancets, push in the blade slot cover and then twist 360°. Pull it out after twisting. Do not remove the blade slot cover from the adult or child lancets other than as instructed above, as this may cause the blade not to pierce the skin.

3. Prick the Finger

a. Make sure that the finger is below the level of the respondent's heart to increase the flow of blood to the finger. Using a rolling movement of your thumb, lightly press the finger from the top knuckle toward the tip. This action will stimulate a flow of blood to the sample area.

b. For children, it may be helpful if the parent/responsible adult assists you by holding the child's hand.

c. When your thumb reaches the fingertip, maintain a gentle pressure to trap the blood in the fingertip.

d. Place the adult lancet firmly against the skin with the trigger facing upwards, so that the arrow preceding the trigger is visible; for children, place the lancet so that the wide body of the lancet faces up. Note: for both adults and children avoid placing the lancet on the very tip of the finger or the sides beyond the palmar area or you will risk piercing the underlying bone.

e. Use the lancet to prick the skin by placing the blade-slot surface against the area and pressing the trigger. The tip of the blade ejects through the blade slot, producing a micro-incision in the skin, and immediately retracts into the device. After pricking the skin, set aside the lancet and turn the finger slightly to prevent blood from running into the grooves of the fingerprints.
4. Using the HemoCue device for single-drop capillary measurement
   a. Take the microcuvette out of the cuvette box, and then close the lid of the cuvette box.
   b. Apply the tip of the HemoCue microcuvette to the middle of the blood drop. The microcuvette chamber will fill itself automatically by capillary action. Filling the HemoCue microcuvette in one continuous step is important in order to avoid air bubbles. The chamber needs to be filled completely never “top off” the microcuvette. Instead, if the microcuvette is not completely filled, use a fresh microcuvette and fill it with the next blood drop that forms.
   c. Wipe any surplus blood off both sides of the microcuvette “like butter from a knife,” using the clean end of a sterile gauze pad. Ensure that no blood is sucked out of the microcuvette when wiping it – do not let the tip of the filled microcuvette touch the gauze.
   d. After filling the chamber, the microcuvette needs to be visually inspected for air bubbles. Since air bubbles may influence the hemoglobin measurement, any microcuvette containing air bubbles must be discarded. In such cases, with the permission of the parent/responsible adult, repeat the blood drop collection using a different finger. Again, you must use new disposable supplies and follow all of the steps described previously in obtaining the new sample.
   e. Place the microcuvette in its holder and gently push the holder into the HemoCue device. Time to measurement for HemoCue 301 and HemoCue 801 needs to be within 40 seconds after filling of microcuvette; for HemoCue 201, time to measurement is within 10 minutes after filled cuvette.
   f. Reading the results: The microcuvette should be analyzed immediately, and no later than ten minutes after being filled. The blood hemoglobin level in grams per deciliter (g/dl) is displayed 15 to 45 seconds after the drawer is closed.
   g. Record the hemoglobin level and test result:

5. Take an adhesive bandage from its wrapper and apply it to the prick site. Advise the mother, especially when the child is a toddler, to watch carefully that child does not take off the bandage and put it in his/her mouth where the child may choke on it.

6. Place all biohazardous waste (lancets, microcuvettes, alcohol swabs, gauze, and gloves) into a plastic bag provided for field disposal of these items.

Blood collection materials needed for single-drop capillary blood sampling

1) Suggested Lancet: For WRA and children 24-59 months - High flow lancet - BD 366594 Microtainer Contact-Activated Lancet, High Flow, 1.5 mm x 2 m; For Children 12-23 months –1.8 mm; 23G; Unistik3 Normal yellow AT1004, Tryco, VA
2) Gauze compress - Gauze - sterile, disposable (2x2)
3) Adhesive bandage, fabric
4) Individually wrapped alcohol pads, Absorbent sheets
5) Personal protective equipment - fresh “powder-free” gloves for use with each patient [Field Gloves (latex, powder free) – LG, MED, SM, XL, XS), eye protection, and a laboratory coat
6) Materials needed for safe disposal of sharps and biological waste - Sharps container (2.2 qt, Red), Biohazard bags (12-14 gal; 25”x30”; 2-3 gal; 14”x19”;7-10 gal; 23”x24”)
7) Backpack, AA batteries for analyzers, Hemocue cleaner
A. Pooled Blood (Centers for Disease Control and Prevention (CDC) 2020)

1. Obtain informed consent for blood collection.
2. Follow Steps 1, 2, and 3, as outlined above for single-drop capillary sampling.
3. Collecting a pooled capillary sample.
   a. After puncturing the skin, apply slight pressure to start blood flow. Wipe away the first drop of blood on a gauze pad and discard pad in appropriate biohazard container.
   b. Keep the finger in a downward position and gently massage it to maintain blood flow. An effective way to do this is to apply pressure to the nail for three seconds, release for three seconds, and repeat continuously while collecting the blood.
   c. Avoid milking the puncture site
   d. Hold the Microtainer at an angle of 30 degrees below the collection site and use the scoop on the Microtainer to guide the drops into the vial. Do not scrape the skin. Fill the Microtainer to at least ~250 µL, and more if possible. If you are unable to collect ~250 µL of the sample, select another individual.
   e. Do not use hard pressure to squeeze the finger because the blood may be diluted with interstitial fluid or hemolysis may occur.
   f. It is important to collect the sample within two minutes.
4. The Phlebotomist will have ONLY two opportunities to collect blood from participants. After two unsuccessful attempts, no blood collection should be done and indicated on the participant’s questionnaire.
5. Cap the Purple top Microtainer and gently invert it 10 times to prevent clot formation.
6. After blood collection, place a gauze pad over the puncture site until bleeding has stopped and apply a sterile bandage on finger.
7. Label the Microtainer with the “Microtainer” label. The barcode needs to be vertical like a ladder when placed on the vial. Place the label from left to right starting from the cap end and leave the graduated numbers on the tube visible.

8. After mixing the pooled sample, place a few drops of blood onto special wax paper using a small plastic pipette. The pooled sample should not stand on the surface of the wax paper for more than 30 seconds. Follow the same instructions in using the HemoCue device, as with single drop capillary samples. Time to measurement for HemoCue Hb 301 and HemoCue Hb 801 needs to be within 40 seconds after filling of microcuvette. Filling the HemoCue microcuvette in one continuous step is important in order to avoid air bubbles.

9. After testing, discard the Microtainer if it is no longer needed.

10. Dispose of the lancet in the appropriate sharps container and remaining items in the biohazard bag.

11. Thank them for their participation.

Blood collection materials needed for pooled capillary blood sampling

1) Suggested Lancet: For WRA and children 24-59 months - High flow lancet - BD 366594 Microtainer Contact-Activated Lancet, High Flow, 1.5 mm x 2 m; For Children 12-23 months –1.8 mm; 23G; Unistik3 Normal yellow AT1004, Tryco, VA

2) BD 365974 Microtainer Tube with BD Microgard Lavender Closure, K2EDTA Additive, 250-500 uL

3) Samco graduated transfer pipets, 3.9mL Small Bulb, Graduated to 1mL, Sterile, Individually Packed

4) Thomas Scientific Glassine Weighing Paper, 3 x 3", for loading the HemoCue microcuvette

5) Gauze compress

6) Adhesive bandage

7) Individually wrapped alcohol pads

8) Personal protective equipment - fresh “powder-free” gloves for each patient, eye protection, and a laboratory coat.

9) Materials needed for safe disposal of sharps and biological waste

Other materials needed for the study

1) Hematology autoanalyzer – devices that is used in the reference laboratory

2) HemoCue devices - Hb 201+, Hb 301, and Hb 801

3) Microcuvettes for HemoCue 201

4) Microcuvettes for HemoCue 301

5) Microcuvettes for HemoCue 801