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Best practices for haemoglobin measurement in population-level anaemia surveys

Technical brief

This brief describes the current best practices for haemoglobin measurement. It provides guidance for people who are planning or implementing field surveys that will assess anaemia prevalence at a population level.

Inside

Background

Haemoglobin concentration measured in a blood specimen is most commonly used to screen for and diagnose anaemia *(1)*. Accurate and precise measurements of haemoglobin concentration are essential to reliably estimate the prevalence of anaemia at a population level. In large-scale surveys, haemoglobin is most commonly measured using single-drop capillary blood specimens in point-of-care devices. In some instances, venous blood specimens are used.

However, emerging evidence suggests that the use of single-drop capillary blood can introduce a substantial amount of random and/or systematic error. These errors may lead to inaccurate estimates. Based on this, there are growing concerns about the use of capillary blood specimens in population-based anaemia prevalence surveys.

Ongoing efforts to examine relevant evidence

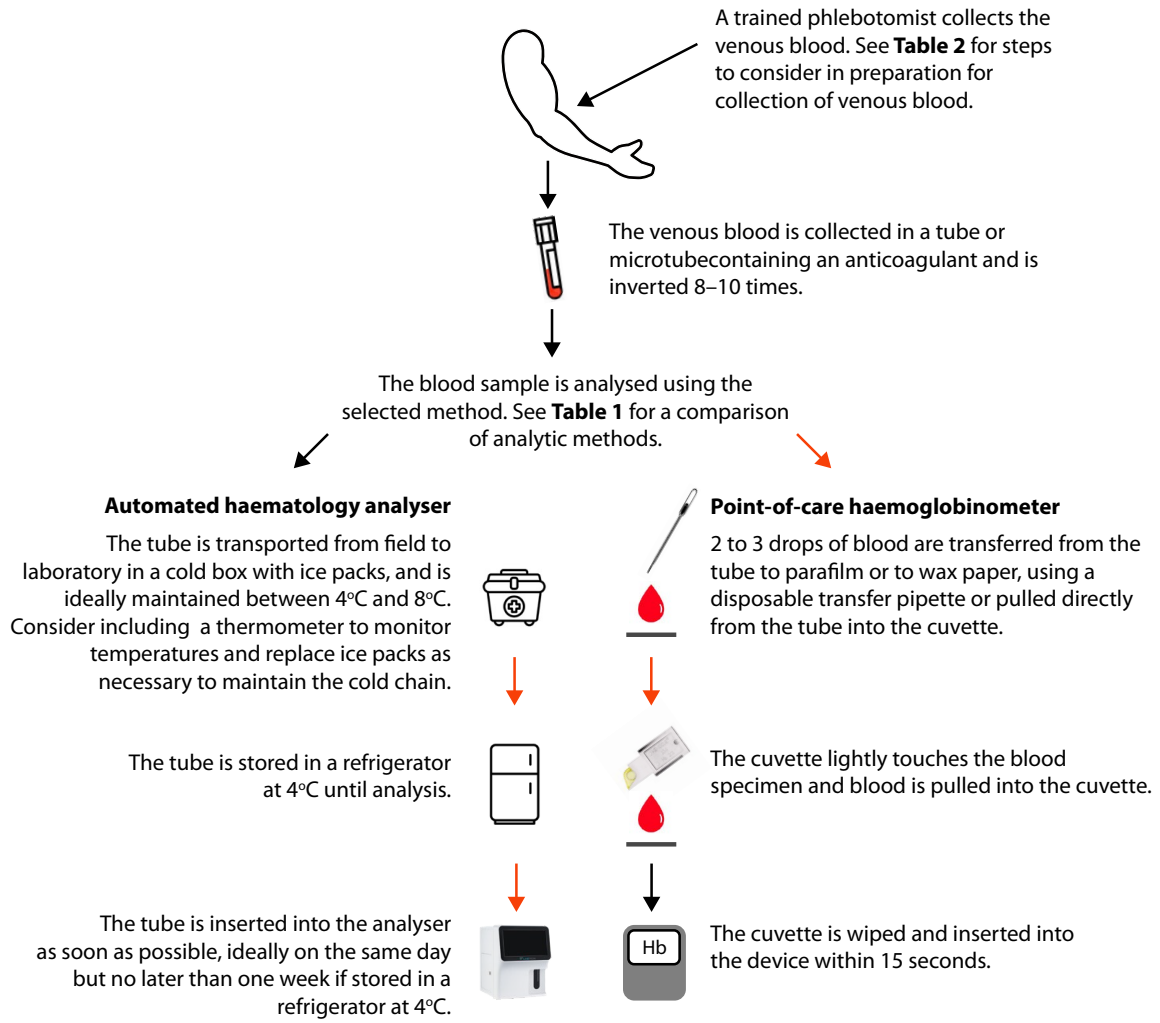
Details of the emerging evidence and critical issues are being examined by members of the WHO-UNICEF Technical Expert Advisory group on nutrition Monitoring (TEAM) and this brief describes best practices discussed within the TEAM working group for Anaemia. This working group is aiming to publish emerging evidence on the types of errors (random and systematic) in haemoglobin measurement that can influence the accuracy and precision of estimates. They will summarize selected examples of evidence from epidemiological research and validation studies that use different analytical methods to measure haemoglobin, and will highlight the gaps where further research is needed.

Significant efforts are under way to rigorously appraise and inform global policy on haemoglobin measurement in population-level anaemia surveys. WHO is commissioning a systematic review of the literature to assess the accuracy and precision of methods for collecting and analysing data for haemoglobin assessment in populations. WHO will also release global recommendations for updating haemoglobin thresholds for diagnosing anaemia, and adjustments for factors known to affect haemoglobin concentrations. The HEmoglobin MEasurment (HEME) Working Group, convened by the United States Agency for International Development (USAID), is undertaking research in six countries[1] with the aim of identifying optimal procedures for measuring haemoglobin, including the potential use of pooled (multiple drops) capillary blood.

Current best practices

At the time of this writing, it is recommended to use venous blood for measuring haemoglobin with an automated haematology analyser, following standard operating procedures *(2–6)* and generally accepted quality control assurance measures. These measures include daily quality control (QC) checks, regular instrument maintenance and verification, and/or participation in proficiency testing schemes. If the use of an automated analyser is not possible, the use of a point-of-care haemoglobinometer by trained phlebotomists or specimen collectors could be considered. Typically, haemoglobinometers are calibrated against international reference standards by the manufacturer before release and require no further calibration. The current best practices for collecting venous blood for use with either option is shown in Figure 1.

Figure 1: Best practices for collecting venous blood for use with an automated haematology analyser or a point-of-care haemoglobinometer



[1] The Kingdom of Cambodia, the Federal Democratic Republic of Ethiopia, the Republic of Guatemala, the Lebanese Republic, the Federal Republic of Nigeria, the United Republic of Tanzania.

Factors to consider when planning a survey to assess population-level anaemia prevalence

The ***Micronutrient Survey Manual*** & ***Toolkit*** *(2, 3)* provides guidance on the steps needed to plan and conduct a population-based representative anaemia survey. The document includes comprehensive modules on survey design, sample size, and the selection of clusters, households and participants. Other resources that describe survey planning and design are also available *(7)*.

Survey planning should consider the measurement of additional analytes (for example, ferritin concentration), selection of the analytical device, and financial and logistical implications (Table 1). Note that several steps are required to plan and carry out venous blood collection (Table 2).

If it is not feasible to measure haemoglobin in the full study population of interest (for example, in the case of a national survey), it may be reasonable to consider selecting a subsample of participants and extrapolating the results to the wider population. In this case, planning will need to take into account sample size calculations, sampling frame design (such as geographical clusters), eligibility criteria and recruitment of participants *(2, 3)*.

Table 1: Comparison of analytic methods

Considerations	Automated haematology analyser	Point-of-care haemoglobinometer
Desired haematological analytes to be measured	Can provide a complete blood count (CBC), which typically includes haemoglobin, haematocrit, mean corpuscular volume (MCV), and other indices which may help differentiate the type of anaemia (for example, microcytic anaemia).	Measurement is limited to haemoglobin, no other analytes.
Logistics	Not portable, and must be housed relatively close to the collection site. Requires a reliable and consistent source of electricity. If possible, specimens should be analysed on the same day; otherwise they can be stored for a maximum of one week, if refrigerated at 4°C.	Portable and battery-operated, and thus can be taken to any location site. However, functionality may be affected by extreme weather conditions such as humidity and temperature.
Technical resources and support required	Requires a high level of technical expertise to maintain and operate; some types may require specialized laboratory technicians.	Easy to use with basic, yet essential, training to operate correctly. Requires little maintenance beyond cleaning.
Financial resources and support required	Cost varies widely depending on the number of analytes to be measured and on throughput. Purchase price can range from US$ 1000 to US$ 25 000. Reagents incur a small additional cost per specimen.	Cost varies by model: devices can range from US$ 300 to US$ 800, and cuvettes can range from US$ 0.50 to US$ 1.50 per piece. Cuvettes are for single-use only.
Reagents	Requires a regular supply of reagents that are specific to each analyser. An adequate supply is crucial to ensure that analysis is reliable and consistent during the survey.	Uses disposable cuvettes (specific characteristics vary by device). Some require protection from humidity because of reagent degradation, and/or may need protection from damage such as scratching.
Required materials for quality control (QC) checks	QC materials/reagents are specific to each type of analyser and can be procured from the supplier.	Manufacturers may recommend QC solutions specific to each model or type.
QC assurance	Laboratory should be accredited and participating in a QC assurance programme for haemoglobin measurement (such as the College of American Pathologists Proficiency Testing). Most have built-in QC calibration, which should be regularly assessed and monitored, along with daily QC checks. A qualified technician should service and/or calibrate the analyser to ensure its proper functioning before it can be used.	Factory-calibrated against a reference method and need no further calibration. If a haemoglobinometer does not comply with manufacturer QC standards (for example if it reads outside of the range of acceptable measurement), it should be cleaned and rechecked. If the problem is confirmed with a second failed reading, it should be returned to the company, and a new device should be tested and used.

Table 2: Steps and considerations in preparation for collecting venous blood specimens

Step	Relevant activities, materials and/or details to consider
1. Procure supplies and materials	Supplies, survey tools and materials for training, pre-testing and survey data collection should be procured well in advance of the survey to ensure an adequate stock and supply chain, while ensuring that no supplies expire before or during data collection (see *Step 3*). Blood collection and safety supplies, reagents, cuvettes, devices, labels and QC materials are required. Conduct QC checks on the analytical devices, and reserve the scheduled use of the analyser if needed.
2. Recruit and train phlebotomists	Certified phlebotomists should be recruited and trained as per the specific survey protocol. They may need to have specific experience for defined populations, such as young children.
3. Perform training and practise exercises	Standardized training exercises should be conducted to allow phlebotomists to practise blood collection multiple times under close supervision. This ensures that the protocol is being followed, and allows errors or deviations to be corrected before the survey begins. In addition to the phlebotomists, the exercises should include all other survey staff, and should cover all survey procedures including data collection and the and labeling, handling and transport of specimens. During practice, the survey staff must be able to demonstrate that they produce consistent results within certain pre-defined parameters, for example within a margin of 3% across five replicates of measurement.
4. Prepare field site(s) for blood collection	When possible, the collection site should be in a quiet, clean, and well-lit area. Participants should be seated in a comfortable chair, with easily-accessible blood collection supplies and safety materials.
5. Prepare each participant for blood collection	The phlebotomist must check that the participant's identification and labelled collection tube are correctly matched, that the participant has been informed of the procedure and has provided consent, and that all required participant information (such as their fasting state) is recorded.
6. Ensure safety considerations and the proper disposal of biohazardous material	Blood specimens should be handled according to universal safety precautions, as the specimens may be infectious. Protective gloves should be worn at all times, and gloves must be changed between participants. Biohazardous material such as needles should be disposed of in specialized safety containers, according to established safety protocols. A system for reporting adverse events should be established to record accurate details of and prevent future incidents.

Assessing and reporting anaemia survey data

Example templates for the reporting of haemoglobin results can be found online in the ***Micronutrient Survey Manual & Toolkit*** *(2, 3)*. Calculating and reporting mean haemoglobin concentrations with standard deviations or confidence intervals among subgroups of the study population can be helpful and sometimes more informative than reporting anaemia prevalence rates alone. Visual assessment of frequency distributions of haemoglobin concentrations among subgroups of the study population may also be useful *(2, 3, 8)*.

Once haemoglobin measurements have been obtained, data should be cleaned and assessed for quality. This includes checking for potentially erroneous values (for example, when the value is so extremely low or high that it would be considered biologically implausible). Values that appear erroneous should be carefully reviewed, but only after adjustment for external influencing factors with established adjustment values (including the altitude of residence, and smoking habits) *(9)*. Haemoglobin values of <40 and >180 g/L for non-pregnant women and children, and of <40 and >200 g/L for men have been suggested as implausible values in previous literature *(10)*. Table 3 describes additional specific information that is important to report, in terms of assessing validity and allowing reproducibility across surveys.

Table 3: Information to include in a report of population-based anaemia surveys

Methodology section
• Type of survey (for example cross-sectional, sentinel, administrative/routine data, longitudinal)
• Population groups assessed (age, sex, pregnancy status, gestational age) and other characteristics such as the elevation of residence, smoking status
• Sampling methodology including design and procedure, sample size determination, and sampling frame (for example by defining the strata, primary sampling unit and cluster)
• The type and serial numbers of analytical devices (automated analyser or type/model of haemoglobinometer)
• Blood collection materials such as tubes, lancets and needles
• Blood source (venous, single-drop capillary, or pooled capillary specimen) and blood volume collected. If single or pooled drops were collected, then describe which finger was used for blood collection, the procedures to wipe blood away, the number of drops collected, and whether they were collected from parafilm or directly from tubes
• Fasting state (non-fasting or number of hours fasting)
• Position of the participant (for example, seated)
• Quality control procedures for equipment
• Training of field staff in data collection and use of equipment
• Monitoring of data collection
• Data entry procedures, data cleaning (for example, for biologically implausible values) and data analysis plan
• Assessment of and adjustments for elevation and smoking applied to haemoglobin concentrations

Results section

- Total number of primary sampling units sampled vs primary sampling units completed
- Total number of sample households/clinics
- Total number of individuals in the sample who were eligible, as well as those who completed and those who refused
- Total number of haemoglobin measurements, and if applicable, the number of haemoglobin measurements that were excluded due to biologically implausible values
- Mean or median haemoglobin concentration (and standard errors or 95% confidence intervals), and anaemia prevalence. This should be based on WHO anaemia thresholds and anaemia classification on severity thresholds (mild, moderate or severe), for each applicable subgroup of the study population (subgroups may include non-pregnant women, pregnant women, children 6–23 and 24–59 months) The description should also include the region, sub-region and demographic characteristics of the study population, depending on the survey design
- Whether haemoglobin measurements were adjusted for elevation and smoking
- If applicable, weighted and unweighted prevalence estimates
- Quality control results may include data tracking on how and when QC testing was conducted for all analytical devices, and what materials were used, for example QC solutions or QC lot number

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References

1. Nutritional anaemias: tools for effective prevention and control. Geneva: World Health Organization; 2017 (https://iris.who.int/handle/10665/259425, accessed 7 November 2023). License: CC BY-NC-SA 3.0 IGO
2. Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), Nutrition International (NI), UNICEF. Micronutrient survey manual & toolkit [website]. Ottawa: Nutrition International; 2022 (https://mnsurvey.nutritionintl.org/, accessed 7 November 2023).
3. Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), Nutrition International (NI), UNICEF. Micronutrient survey manual. Geneva: World Health Organization; 2020. (https://iris.who.int/handle/10665/336010, accessed 7 November 2023) License: CC BY-NC-SA 3.0 IGO.
4. Biomarker manual: Demographic and Health Survey. Training program for measuring and testing biomarkers. Rockville: ICF; 2021. (https://dhsprogram.com/publications/publication-dhsm7-dhs-questionnaires-and-manuals.cfm, accessed 7 November 2023).
5. Burger S, Pierre-Louis J. A procedure to estimate the accuracy and reliability of HemoCueTM measurements of survey workers. Washington: International Life Sciences Institute; 2003.
6. WHO guidelines on drawing blood: best practices in phlebotomy. Geneva: World Health Organization; 2010 (https://iris.who.int/handle/10665/44294, accessed 7 November 2023).
7. WHO, UNICEF. Recommendations for data collection, analysis and reporting on anthropometric indicators in children under 5 years old. Geneva: World Health Organization; 2019 (https://apps.who.int/iris/handle/10665/324791, accessed 7 November 2023). License: CC BY-NC-SA 3.0 IGO
8. Pasricha S, Drakesmith H. Iron deficiency anemia: problems in diagnosis and prevention at the population level. Hematol Oncol Clin N Am. 2016;30:309–25.
9. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva: World Health Organization; 2011 (https://iris.who.int/handle/10665/85839, accessed 7 November 2023).
10. Sullivan KM, Mei Z, Grummer-Strawn L, Parvanta I. Haemoglobin adjustments to define anaemia. Trop Med Int Heal. 2008;13:1267–71.

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