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Hemoglobin determination: how good is good enough?

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Anemia is a well-known global public health problem mainly affecting women and children. To assess the prevalence in populations, hemoglobin (Hb) measurements are typically conducted in resource limited field settings, generally relying on portable analyzers such as the HemoCue® device, and often sampling capillary blood collected through finger pricking (1). Due to growing concern that methodological factors associated with Hb measurements can influence the accuracy and precision of results and thus affect the estimated prevalence of anemia by up to 20 percentage points in children and up to 28 percentage points in non-pregnant women (2), efforts are underway to shed light on pre-analytical (e.g., type of blood collected) and analytical (e.g., device used for measurement) issues. The WHO is developing a *Comprehensive framework for integrated action on the prevention, diagnosis and management of anemia* (1) and has released a technical brief on *Best practices for haemoglobin measurement in population-level anaemia surveys* (3). The US Agency for International Development (USAID) Advancing Nutrition program is sponsoring an intercountry study to determine the most appropriate procedures to improve Hb determination using field photometers (4).

The paper by Hackl et al. in the current issue of *The Journal of Nutrition* is a comprehensive report from the USAID study presenting data from 6 evaluation sites using 3 HemoCue® models (201+, 301, and 801) and 3 blood types (venous [VB], capillary pooled [CPB], and capillary single-drop [CDB]) and comparing results to those obtained with VB measured by an automated hematology analyzer (AHA) (5). The authors found large variations across study sites regardless of blood type or instrument used, suggesting differences in personnel skills. The common findings across study sites were a positive bias for the HemoCue® 301 vs. AHA and decreasing measurement precision from VB to CPB to CDB.

Despite earnest attempts over the last few years to disentangle effects of the instrument/analytical method, biological variation, and/or other pre-analytical factors (2, 5–11), important questions remain. Let's first consider pre-analytical factors. Results from recent studies comparing VB and capillary blood using the same instrument were mixed

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(community, not acute care setting): VB produced either lower (5), similar (8 [for women of reproductive age], 9, 10 [for CPB], 11), or higher Hb results (6, 8 [for pre-school children]); however most studies showed similar Hb concentrations within ≤±3 g/L and all studies achieved Hb concentrations within <±10 g/L. Data on measurement precision across blood sample types (HemoCue® vs. AHA) were consistent across studies, in that VB showed the smallest and CDB showed the largest imprecision, but the magnitude varied. De la Cruz-Gongora et al. (10) reported tolerance limits (2*SD) of ±6.6 g/L for VB, ±7.2 g/L for CPB, and ±16.2 g/L for CDB. Namaste et al. (9) reported tolerance limits (children and women, controlled and survey setting) ranging from ±5.8 to 10 g/L for VB, from ±10.2 to 11.6 g/L for CPB, and from ±11.4 to 17.4 g/L for CDB. Finally, Hackl et al. (5) reported the widest ranges of tolerance limits (across 6 sites) from ±5 to 16 g/L for VB, from ±9 to 28 g/L for CPB, and from ±9 to 37 g/L for CDB.

Next, let's consider analytical factors. A few recent HemoCue® model comparisons of 301 vs. 201+ indicated higher Hb results by the newer model (5, 8, 11). Investigations on the comparison of the HemoCue® 201+ to the AHA were mostly consistent showing no or a small (≤3 g/L) bias (2, 6, 9, 10). Lastly, 2 important reports arrived independently to the conclusion that the instrument/method variability is minimal, unlikely to influence the diagnosis of anemia: Neufeld et al. (6) reviewed studies where split VB samples were tested by the HemoCue® device vs. the AHA reference method; Larson et al. (2), using 2010–2019 data from a large international quality assurance program, found a small (<4 g/L) mean Hb difference from the sample mean (which excluded the instrument under investigation) for 16 different instrument groups comprising mostly of AHAs but also including the HemoCue® 201+.

Given convincing evidence that the HemoCue® 201+ model can measure Hb accurately, one may wonder why 2 recent reports attempted to harmonize HemoCue® results to results obtained for VB by AHA. De la Cruz-Gongora et al. (10) demonstrated that after adjusting for a 3.1 g/L bias between the HemoCue® 201+ and the AHA, CPB showed no bias compared to VB, while CDB still showed a positive bias of 4.25 g/L. Hackl et al. (5) showed that adjustment to the AHA reference point "harmonized mean errors for all devices across study-sites to <1 g/L using venous blood". This begs the question of how good is good enough and how to reconcile the desire for the highest quality data with the realities of field work, resource constraints, and participant reluctance to provide certain biological samples, to name just a few concerns.

Several questions come to mind: (a) what are appropriate criteria during the training and standardization exercise of personnel; (b) what is an acceptable device bias and how is the bias determined; (c) what approach is taken when an excessive device bias is found; and (d) when is data adjustment appropriate. To minimize the chance of anemia misclassification, Hackl et al. propose a tolerance limit (i.e., 95% LOA) of <±10 g/L around the target mean as achievable with training and experience, based on data from their 3 better performing sites. They suggest that study implementers use this criterion on each training sample with 5 replicates per survey personnel (question a). They suggest a device bias ≤±3 g/L as acceptable (part of question b). Experts need to consider questions that address the design (e.g., how many samples, how much replication), frequency (e.g., annually, prior to a large

survey), and location (e.g., laboratory, training site) of a comparison study to determine the device bias (remainder of question b), as well as the interpretation and application of findings from the comparison study (e.g., regression approach, mean difference approach, rotate devices across study sites to generate small, balanced mean bias per site) (question c). Careful consideration needs to be given to the possibility of the AHA being slightly biased in one direction and the HemoCue® in the other direction, making the adjusted HemoCue® result less accurate than if no action was taken (question d).

One would assume that a tolerance limit of ±10 g/L should be wide enough to accommodate a small device bias (as typically seen with the HemoCue® 201+) and the soon-to-come, tighter CLIA target of ±4% for acceptable analytical performance for single blood samples (not for mean method bias). The challenge is to assess with simple means whether a particular device performs within that tolerance. Unfortunately, commutable whole blood-based reference materials are not commercially available. While the concerted efforts over the last few years have generated important insights into the pre-analytical and analytical factors influencing Hb measurements, we still have a long way to go.

Abbreviations:

AHA	automated hematology analyzer
CPB	capillary pooled blood
CDB	capillary single-drop blood
Hb	hemoglobin
USAID	US Agency for International Development
VB	venous blood

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