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Evaluation of automatic mixing versus manual mixing for point of care hemoglobin measurement

Ghaith Altawallbeh [a], Pedro Castaneda [b], Gitte Wennecke [c], Amy B. Karger [a,*]

[a] Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA
[b] M Health Fairview, Minneapolis, MN, USA
[c] Radiometer Medical Inc., Copenhagen, Denmark

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ABSTRACT

Objective: We compared hemoglobin results from manually mixed blood gas (BG) Portex syringes (Smiths Medical) and automatically mixed *safe*PICO BG syringes (Radiometer Medical) measured on the ABL90 FLEX blood gas analyzer at the Point of Care with a laboratory hematology analyzer (XN-series, Sysmex) to determine whether automatic mixing improved the accuracy and precision of hemoglobin measurement relative to the standardized method.
Methods: Ninety-nine lithium heparin tubes and EDTA tubes were collected simultaneously from selected patients at five participating institutions. The lithium heparin tubes were then split between the Portex ABG syringe and the *safe*PICO aspirator. The Portex syringe was mixed manually according to routine procedures, while the *safe*PICO syringe was mixed automatically on the ABL90 FLEX using the automatic mixing ball. The two syringes were mixed and run on the ABL90 FLEX analyzer by the same lab personnel. Total hemoglobin results obtained from the EDTA tubes on the Sysmex XN hematology analyzer were used as the reference values.
Results: Manual mixing demonstrated a larger scatter of hemoglobin values ($R^2 = 0.515$ and Mean diff.: -0.9 g/dL) while automatic mixing at the point of care yielded a significantly better correlation ($R^2 = 0.986$ and Mean diff.: -0.2 g/dL) when compared to a reference hematology analyzer.
Conclusion: Using the *safe*PICO syringe with automatic mixing at the point of care results in a significantly better correlation of hemoglobin with the standardized method.

1. Introduction

Preanalytical errors, which have a profound impact on the accuracy of laboratory tests, can lead to incorrect diagnostic and therapeutic decisions [1,2]. In order to avoid the risk of potential errors, all clinical laboratory procedures must be simplified, standardized, and automated if feasible. Implementation of such actions in central laboratory testing has significantly improved the quality outcome of laboratory results [3].

Blood gas measurements, widely used at the point of care for prompt lab results, are particularly prone to preanalytical errors [4]. The quality and integrity of whole blood specimens used for blood gas and hemoglobin analysis can be altered by numerous factors. Specifically, lack of homogeneity due to inadequate mixing of whole blood specimens can lead to inaccurate blood gas and hemoglobin analyses. Inadequate mixing is a particular concern when non-laboratory professionals perform testing on waived instruments, since they are less likely to be adherent to pre-analytical processing procedures than laboratory professionals. While a prior study has shown

* Corresponding author. University of Minnesota, 420 Delaware St SE MMC 609, Minneapolis, MN, 55455, USA.
E-mail address: karge026@umn.edu (A.B. Karger).

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that automatic mixing in *safe*PICO syringes decreases variability of results [5], no studies have compared results of both manual mixing and automatic mixing to a standardized method for hemoglobin measurement.

The aim of this study was to evaluate hemoglobin measurements obtained from samples mixed manually alongside samples mixed using the automatic method on the ABL90 FLEX in comparison to the automated hematology analyzer.

2. Methods

Five different institutions {(AMITA Health Adventist Medical Centers in La Grange, Hinsdale, Bolingbrook, and GlenOaks, Illinois) and the University of Minnesota (UOM) in Minneapolis, Minnesota} followed the protocol developed by the UOM, described below. Each site collected two venous samples simultaneously from 20 selected patients (n = 100 patients). Samples from one subject were excluded from analysis due to a very large bias between the two syringes indicating an error in collection. Blood was drawn into one green top tube containing lithium heparin as anticoagulant and a purple top tube containing EDTA anticoagulant. The heparinized tube was split into two ABG syringes: A 1 mL Portex syringe with no automatic mixing capacity, and a 1.5 mL *safe*PICO syringe containing a mixing ball for automatic mixing.

The EDTA tube was considered the reference sample and hemoglobin was measured on the laboratory hematology analyzer (XE-series, Sysmex). The two syringes were initially mixed and stored under equivalent conditions and handled by one laboratory personnel only. The Portex syringe was mixed manually according to routine procedures based on the International Federation of Clinical Chemistry (IFCC) recommendations [6], by inverting the syringe five times and rolling it between the palms for 5 s, whereas the *safe*PICO syringe was mixed using the automatic method on the ABL90 FLEX. Hemoglobin was determined in both syringes on the ABL90 FLEX.

The ABL90 FLEX is an automated blood gas analyzer that utilizes sensor cassettes, an oximetry module, and solution packs to measure blood gases, electrolytes, metabolites, and hemoglobin status in whole blood samples [7]. The ABL90 FLEX measures total hemoglobin (tHb) by spectrophotometry. The central hematology analyzer (Sysmex®) measures hemoglobin concentration based on a gold standard method recommended by the International Committee for Standardization in Hematology (ICSH) [8]. Statistical analyses were performed using JMP®, Version 14 (SAS Institute Inc.). The linear fit was determined using bivariate analysis with least squares regression.

3. Results

The manual mixing method (Fig. 1A) resulted in a large scatter of hemoglobin results when compared to the gold standard hematology analyzer ($R^2 = 0.515$). The Bland-Altman plot revealed a negative systematic bias using the Portex syringes, with a mean difference of −0.9 g/dL (Fig. 2A). The range of differences between the manual mixing method and Sysmex results was wide and consistent with random error (−7.5 to 5.0 g/dL).

In contrast, the data obtained from the automatic magnetic mixing on the ABL90 FLEX with the *safe*PICO syringes (Fig. 1B) were in a good agreement with the reference method ($R^2 = 0.986$). The Bland-Altman plot revealed a mean difference of −0.2 g/dL independent of hemoglobin concentration, with a small range of variability (−0.4 to 0.8 g/dL) (Fig. 2B).

4. Discussion

Use of automatic mixing ensures a reproducibly homogenous sample and accurate hemoglobin results when compared to a standardized hematology analyzer, whereas manual mixing shows a large amount of scatter and a relatively poor correlation when

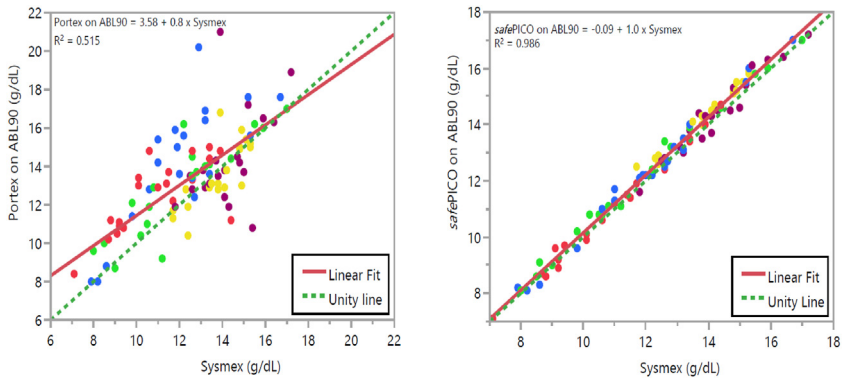

Fig. 1. Linear regression analysis. (A) Hb results obtained by manual mixing on ABL90 FLEX compared to Sysmex. (B) Hb results obtained by automatic mixing on ABL90 FLEX compared to Sysmex. Hospital color coding: Adventist Bolingbrook, (Green); Adventist GlenOaks (Blue); Adventist Hinsdale (Bordeaux); Adventist LaGrange (Red); University of Minnesota (Yellow). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

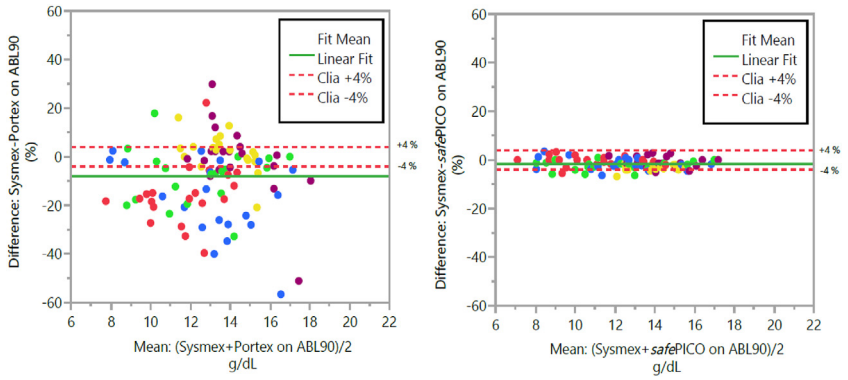

Fig. 2. Bland-Altman plots. Bland-Altman plots of agreement in the measurement of hemoglobin given by the ABL-90 FLEX for manual (A) versus automatic mixing (B) in reference to Sysmex. Hospital color coding: Adventist Bolingbrook, (Green); Adventist GlenOaks (Blue); Adventist Hinsdale (Bordeaux); Adventist LaGrange (Red); University of Minnesota (Yellow). The mean difference (solid line) and the CLIA allowable total error acceptability limits for hemoglobin of ±4% (dotted lines) are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

compared to the standardized method. Variability in mixing techniques can alter the integrity of the sample and thus affect the quality of results. In an earlier study, manually mixed samples by nurses demonstrated a poor correlation with the automatic laboratory instrument when compared to a uniform mixing approach by laboratory staff using a mechanical mixer [9]. The lack of proper mixing will result in both positive and negative bias values dependent on which portion of the syringe content is aspirated into the analyzer.

Our finding is consistent with other studies where they have shown that automatic mixing in *safe*PICO syringes decreases variability of results [5,10]. Grenache and Parker reported that manually mixed specimens produced more variation in paired hemoglobin measurements on the ABL700 compared to automatic mixing on the ABL800 FLEX [5]. Andersen et al. also found statistically significant differences between manual and automatic approaches for hemoglobin measurement on the ABL800 FLEX, however they concluded that the differences were clinically insignificant based on a cutoff of a mean difference of 10% or less [11]. However, both of these prior studies did not compare their results to a reference or standardized method. Benoit and Paul also showed a good concordance between automatically mixed samples on the ABL 825 FLEX with the LH 750 hematology analyzer (Beckman Coulter Inc.) for hemoglobin concentration [10]; however their study did not simultaneously assess manual mixing relative to the standardized hematology analyzer.

One limitation of our study includes the difference in sample volume used. Our study focused on comparing automatic mixing using the 1.5 mL *safe*PICO syringes to the current syringe type (1 mL Portex) which is mixed manually. The sample volume may influence the mixing capability, but since the samples were handled per routine procedure in accordance with manufacturer's instructions, our results reflect the process that would be used by laboratories. Another drawback is the use of double heparinized samples, drawing already heparinized blood into the two different heparin-containing syringes. This could explain the small observed systematic differences, however since this effect would impact both syringes it should not change the overall conclusions of the study. In addition, it is important to recognize that our data and conclusions are relevant to the specific manual mixing procedure used, which was based on IFCC guideline recommendations. Therefore, correlation studies may look different when different manual mixing procedures are used.

Results obtained with automatic mixing fulfill the previous criteria recommended by CLIA for acceptable performance for hemoglobin (target value ± 7%). Newly proposed CLIA rules, published on February 4, 2019, tightened the acceptance limits for hemoglobin to ±4%. In our study, only six results out of 99 (6%) measured with automatic mixing were slightly below −4%. However, 59% of results measured with manual mixing fell outside the limits of ±4%, and more than 40% were outside the prior CLIA limits of ±7% allowable total error. These findings support the conclusion that the degree of error seen with manual mixing is clinically significant.

In comparing the two syringe types, the *safe*PICO syringe with its automatic mixing capability provides features to minimize preanalytical errors. For example, the ability to mix properly at bedside with the magnetic ball facilitates heparin distribution and more readily prevents clot formation. It also addresses a safety issue to the user, as the *safe*PICO tip cap stays on for testing on the ABL90 FLEX while the Portex tip needs to be removed and blood expelled in gauze to check for clots and cancel the test if clots are present.

In conclusion, using *safe*PICO syringes with automatic mixing at the point of care results in a significantly better correlation of hemoglobin with the laboratory analyzer. Automatic mixing ensures a homogenous sample and accurate hemoglobin results, which could potentially prevent inappropriate blood transfusions and unnecessary testing as well as timely blood management when necessary. Accurate hemoglobin results are essential when the focus is on patient blood management, i.e. collecting the lowest possible blood volume with the highest possible sample quality to minimize patient blood loss.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Radiometer America Inc. provided financial and statistical support for this study. G. Wennecke is employed by Radiometer Medical.

CRediT authorship contribution statement

Ghaith Altawallbeh: Data curation, Writing - original draft, Visualization. **Pedro Castaneda:** Investigation, Writing - review & editing. **Gitte Wennecke:** Formal analysis, Resources, Data curation, Writing - review & editing, Visualization, Funding acquisition. **Amy B. Karger:** Resources, Writing - review & editing, Visualization, Supervision, Project administration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2020.e00163.

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