Comparison of the safePICO Automatic Mixer to Manual Mixing of Specimens for Blood Gas Analysis on the ABL80 FLEX

Christopher R. McCudden[1], Christopher Parker[2], and David G. Grenache[1]

[1]Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC, 27599, United States
[2]McLendon Clinical Laboratories, University of North Carolina Hospitals, Chapel Hill, NC, 27514, United States

Objective:
The objective of this study was to compare the efficacy of the stand-alone *safe*PICO mixing device and syringe (Radiometer America, Inc., Westlake, OH) to manual mixing of heparinized whole blood collected into standard blood gas syringes.

Background:
Blood gas analysis is most often performed urgently where rapid turnaround time is critical for patient care. An increasing demand for decreased turnaround times has led to the development of point-of-care blood gas instruments, such as the ABL80 FLEX (Radiometer America, Inc.), which performs quantitative measurements of pH, PCO2, PO2, Na^+, K^+, Cl^-, Ca^{2+} and hematocrit on whole blood collected into standard or *safe*PICO blood gas syringes. The *safe*PICO syringe contains a magnetic ball that mixes the whole blood specimen when placed on the *safe*PICO portable automated mixing device. A homogeneous whole blood specimen is required to ensure accurate blood gas results, particularly when determining hemoglobin or hematocrit. While this is frequently accomplished by manual mixing of the specimen, the absence of any dead space in a typical blood gas syringe can result in inadequate mixing. Guidelines for handling blood gas specimens call for mixing blood gas specimens by gentle rotation for 2 minutes immediately prior to analysis[1], yet this practice is inconsistently utilized. It has been reported that true practice manual mixing procedures fail to produce a homogeneous specimen compared to automatic mixing[2]. This study was designed to compare the efficacy of automatic mixing using the *safe*PICO syringe in conjunction with the *safe*PICO Mixer to both standardized ($manual_{std}$) and true practice ($manual_{tp}$) manual mixing of a blood gas syringe in common use today.

Methods:
This study compared two different manual mixing methods to automatic mixing using the *safe*PICO Mixers. The manual mixing protocols consisted of either a 2 minute standardized manual mixing protocol or a non-standardized protocol based on prior observations of true laboratory practice where samples were mixed randomly from 2-15 seconds. In each experiment, heparinized whole blood was obtained from seven healthy volunteers to generate a baseline and 15 sets of seven specimens by combining packed red cells and plasma in various ratios to achieve hematocrit levels ranging from 25-75%. Each set of seven specimens consisted of three *safe*PICO and standard syringes with one standard syringe for use as a baseline. Syringes were filled with 1.0 mL of whole blood without any dead space. Immediately after sample preparation, a baseline hematocrit and K^+ for each set was analyzed on the ABL80 FLEX. The remaining six syringes were stored horizontally and undisturbed at room temperature for 10, 20, or 30 minutes. In the standard mixing protocol, specimens in standard syringes were manually mixed after the

appropriate storage time by inversion for one minute followed by horizontal palm rolling for one minute. For the non-standardized protocol, samples in standard syringes were manually mixed for 2-15 seconds by hand rolling and/or inversion. For both experiments the *safe*PICO syringes were automatically mixed for seven seconds using the automated mixing device. Immediately after mixing, specimens were analyzed by the ABL80 FLEX. Hematocrit was used as an indicator of specimen homogeneity and K^+ was used as a marker of red blood cell lysis. Deming regression was used to determine the linear relationships for hematocrit and K^+ between mixing methods. A paired, non-parametric ANOVA (Friedman) test was used to analyze the variances of the differences between the hematocrit and K^+ results between mixing methods and over the time course in comparison to baseline values.

Results:

Deming regression analysis results comparing the hematocrit and K^+ data for the automatic versus standardized 2 minute manual mixing were well correlated and revealed clinically insignificant differences. Analysis by Bland-Altman plots revealed neither clinically significant bias nor scatter indicating that both mixing protocols performed similarly. Analysis of true practice vs. automatic mixing by Deming regression revealed larger standard errors of the estimates (Sy.x) as compared to standardized mixing (Table 1). Using medical decision points of 35% for hematocrit, systematic error was 1.6-fold higher at 10 min, 5.1-fold higher at 20 min and 5.9-fold higher after 30 minutes of storage relative to standardized mixing. Similar differences between methods were observed at decision point of 50% hematocrit. Differences in true practice mixing are also evident from Bland-Altman plots shown in Figure 1. Bland-Altman plots revealed inaccuracy exceeding the recommended total allowable error of 6% for hematocrit[3] after each of the storage times. An additional measure of mixing efficacy is to statistically analyze the variances of the differences between baseline and timed specimens for hematocrit. Similarly, the variances of the K^+ concentrations between baseline and timed specimens can be used as an indicator of red blood cell hemolysis. Paired, non-parametric ANOVA (Friedman test) analysis of the differences between the mixing protocols did not reveal any statistically significant differences between any of the mixing protocols (standard manual vs. automatic; true practice manual vs. automatic) or over any of the storage periods for either hematocrit or K^+ ($P>0.05$). Furthermore, K^+ results from each set of experiments did not reveal any clinically significant differences between the mixing methods (Table 1). Therefore, although there were no statistically significant differences for either analyte between mixing methods over the various incubation times, there were clinically relevant differences observed for hematocrit using the true practice manual mixing protocol relative to automatic mixing.

Conclusions:

While both automatic and standardized mixing methods produce a homogeneous whole blood specimen without damaging red blood cells, true practice manual mixing is not effective and may lead to erroneous hematocrit results. The *safe*PICO Mixer and syringe are a rapid alternative to manual mixing for blood gas analysis.

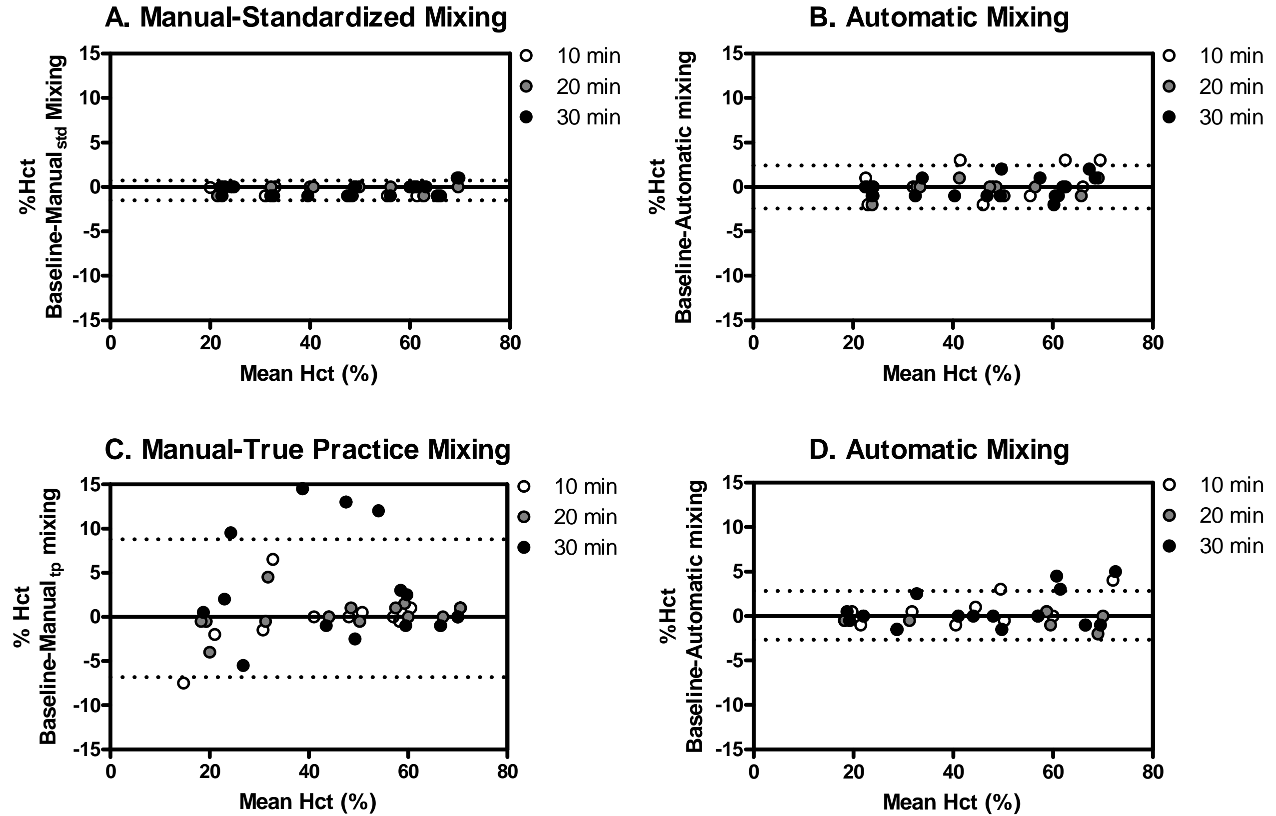
.

Table 1. Deming regression statistics comparing true practice manual mixing to automatic mixing for hematocrit and potassium reveal clinically important differences in hematocrit results after 10 minutes of storage.

Analyte*	Storage Time* (min)	Slope	Y-Intercept	Sy.x	R
Hematocrit	10	0.980	1.41	3.33	0.969
	20	0.965	0.05	3.73	0.961
	30	1.087	-6.66	6.59	0.891
K^+	10	1.059	-0.11	0.11	0.935
	20	1.106	-0.34	0.11	0.938
	30	1.068	-0.17	0.09	0.960

*n=15 pairs for each time point for both analytes.

Figure 1. Bland-Altman plots comparing standardized (A; $manual_{std}$) to automatic mixing (B) were not significantly different. However, a comparison of true practice (C; $manual_{tp}$) mixing to automatic mixing (D) revealed clinically significant bias and scatter for hematocrit. Therefore, whereas the *safe*PICO device and standardized mixing procedures are equivalent and effective, true practice mixing can result in unacceptably high error rates based on recommended guidelines[3]. Dashed lines represent 95% confidence intervals.



References:

1. Clinical Laboratory Standards Institute. Blood gas and pH analysis and related measurements; Approved guideline. CLSI document C-46A. 2001. 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, 19087-1898.

2. Grenache DG, Parker C. Integrated and automatic mixing of whole blood: an evaluation of novel blood gas analyzer. *Clin Chim Acta* 2007;375:153-7.

3. Wennecke G. Hematocrit – a review of different analytical methods. www.bloodgas.org. Sept. 2004