Original Article

Blood sample quality using Greiner Bio-One HOLDEX® Single-Use Holder and VACUETTE® SAFELINK holder with male luer lock: a comparative study

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Contributions: (I) Conception and design: G Lippi; (II) Administrative support: None; (III) Provision of study materials or patients: GL Salvagno, M Pucci; (IV); Collection and assembly of data: GL Salvagno, M Pucci; (V) Data analysis and interpretation: G Lippi; (VI) Manuscript writing: G Lippi; (VII) Final approval of manuscript: All authors.

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Background: Hemolysis is the most frequent and potentially one of the most harmful causes of poor sample quality. Many strategies have been attempted for minimizing the risk of generating spurious hemolysis during blood collection, such as using the HOLDEX® single-use holder, which features a chamber that decelerates blood flow pressure before entering evacuated blood tubes. We have hence designed a study to establish whether the new VACUETTE® SAFELINK, which shares different structure but similar strategy affecting blood flow pressure, may ensure a comparable sample quality as using HOLDEX® single-use holder.

Methods: A total number of 24 clinical chemistry, hematologic and hemostasis parameters were measured in paired blood samples collected from 30 healthy volunteers using either HOLDEX® single-use holder or VACUETTE® SAFELINK. Test results were compared with paired Student's t-test, Pearson's correlation and Bland-Altman plots.

Results: All test results were non-significantly different in blood samples collected with HOLDEX® single-use holder or VACUETTE® SAFELINK except potassium, whose values were marginally higher in plasma collected with VACUETTE® SAFELINK. All Pearson's correlation coefficients were excellent except potassium, chloride and H-index. In this latter case, the correlation did not reach statistical significance. The percent bias of different parameters in samples collected with HOLDEX® single-use holder or VACUETTE® SAFELINK was minimal, comprised between –4.5% and +1.6%. In all cases the percent bias was comprised within the quality specifications tests. The rate of plasma samples with H-index >3 was eventually lower when blood was collected using SAFELINK than with HOLDEX® single use holder (odds ratio, 0.52; 95% confidence interval, 0.17–1.60).

Conclusions: The results of this comparative study suggest that sample quality is thoughtfully comparable using HOLDEX® single-use holder and VACUETTE® SAFELINK, thus translating into the concept that VACUETTE® SAFELINK may also be an effective means for reducing spurious hemolysis, especially when drawing blood from catheters.

Keywords: Blood sampling; venipuncture; phlebotomy; quality; errors; preanalytical variability

Received: 29 April 2019; Accepted: 26 August 2019; Published: 02 September 2019.

doi: 10.21037/jlpm.2019.08.03

View this article at: http://dx.doi.org/10.21037/jlpm.2019.08.03

Introduction

Laboratory diagnostics has become a virtually unavoidable part of the clinical decision making in recent years (1). This essential medical discipline can be reasonably defined as the science of providing valuable clinical information by analyzing the concentration or composition of many analytes in different body fluids (2). This clear-cut definition has been inherently associated with a straightforward partitioning of the total testing process into—at least—three different parts, which entail the preanalytical, analytical and postanalytical phases (3). Several lines of evidence now attest that many manually-intensive preanalytical activities are highly vulnerable to errors and uncertainties (4), so that major efforts shall be made to enhance both quality and safety of this essential part of the total testing process (5).

Until *in vivo* diagnostic testing will replace laboratory diagnostics, but there is no reliable evidence that this may happen soon, blood samples collection will remain an unavoidable part of the total testing process for long. The collection of quality samples would hence necessitate that the entire blood drawing procedure is accurately standardized and appropriately performed, by thoughtfully applying currently available recommendations and guidelines (6). This also encompasses that the different materials used for drawing blood will need to fulfill strict quality criteria. Notably, the recent recommendations of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) also clearly endorse that an analytical and clinical validation is necessary before new blood collection devices are introduced into clinical practice (7).

Previous evidence has been published that the HOLDEX® single-use holder (Greiner Bio-One GmbH, Kremsmuenster, Austria) may be effective to lower the risk of generating spurious hemolysis during blood drawing (8). This promising evidence has been attributed to the specific conformation of the device, characterized by the presence of an eccentric luer on the top which may effectively reduce the differential of pressure between the evacuated blood tube and the vein, especially when blood is drawn through catheters. More recently, Greiner Bio-One has manufactured a new type of holder (VACUETTE® SAFELINK, Greiner Bio-One GmbH, Kremsmuenster, Austria), which is supposed to allow efficient and practical handling, along with eased and more rapid blood drawing when used in combination with blood sampling devices encompassing a standardized female luer lock adapter. Unlike HOLDEX® single-use holder, which is characterized by the integrated luer needle with off-centre luer slip connector, the luer lock mechanism in VACUETTE® SAFELINK is centered at the top of the holder, but the device still contains a small chamber which allows seeing the flash of vein penetration and also has the effect of decelerating blood flow prior to entering the tube, thus potentially reducing the risk of spurious hemolysis during blood drawing and ultimately enhancing sample quality. In accordance with the recent EFLM recommendations (7), we have hence designed a comparative study to establish whether VACUETTE® SAFELINK may ensure comparable sample quality as using HOLDEX® single-use holder.

Methods

The study population consisted of 30 healthy volunteers recruited from the local laboratory staff (15 women and 15 men; mean age, 38±14 years), regularly undergoing laboratory testing and health assessment visits for establishing the fitness for job. The protocol of the study was based on two sequential venipunctures. The first standard venipuncture was performed by an expert phlebotomist on one arm, using a 19 gauge (G) straight luer needle attached to a Greiner HOLDEX® single-use holder, whilst the second venipuncture was then sequentially performed on the other arm, also using a 19 G straight luer needle, but attached to the new Greiner VACUETTE® SAFELINK holder. Three sequential blood tubes were drawn in each venipuncture, containing lithium-heparin, K_2EDTA (dipotassium ethylenediaminetetraacetic acid) and 3.2% buffer sodium citrate (all from Greiner Bio-One GmbH, Kremsmuenster, Austria). The new disposable VACUETTE® SAFELINK holder with male luer lock is manufactured with unbreakable plastic and does not contain natural rubber latex. The specific luer lock mechanism grants secure connection and has the advantages of being compatible with the vast majority of female luer lock adapters and can sustain blood pressures as high as 185 mmHg.

Clinical chemistry and immunochemistry testing was performed in lithium-heparin plasma, using Roche Cobas 8000 and proprietary reagents (Roche Diagnostics, Basel, Switzerland). Specifically, aspartate aminotransferase (AST) was measured using the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) assay with pyridoxal phosphate activation, lactate dehydrogenase (LDH) with the reference UV assay, whilst sodium, potassium and chloride were tested with an indirect method using ion-selective electrodes. The serum

Table 1 Comparison of laboratory test results in samples collected with Greiner Bio-One HOLDEX® single-use and VACUETTE® SAFELINK holders

Parameter	HOLDEX®	SAFELINK	P
Complete blood cell count			
Red blood cells (×10^{12}/L)	4.70±0.50	4.67±0.47	0.099
Hemoglobin (g/L)	137.6±16.9	136.9±16.5	0.070
Hematocrit	0.42±0.05	0.42±0.04	0.134
Mean corpuscular volume (fL)	89.0±6.2	89.2±6.1	0.081
RDW (%)	13.0±1.2	13.0±1.2	0.339
White blood cells (×10^{9}/L)	6.27±1.80	6.16±1.81	0.054
Platelets (×10^{9}/L)	275±64	272±65	0.261
Mean platelet volume (fL)	10.7±1.1	10.7±1.1	0.354
Clinical chemistry			
Potassium (mmol/L)	4.38±0.24	4.49±0.23	0.011
Chloride (mmol/L)	100.2±1.4	100.7±1.9	0.114
Sodium (mmol/L)	140.4±2.0	140.1±1.8	0.326
Aspartate aminotransferase (U/L)	20.5±6.9	20.7±7.2	0.508
Lactate dehydrogenase (U/L)	160.1±26.3	160.9±27.3	0.273
Hemostasis			
Prothrombin time (sec)	11.46±0.77	11.45±0.73	0.792
Activated partial thromboplastin time (sec)	30.23±2.04	30.46±2.46	0.362
Fibrinogen (g/L)	2.55±0.42	2.56±0.47	0.740
D-dimer (ng/L)	69.5±43.2	69.8±47.5	0.920
TG-lag time (sec)	2.88±0.64	2.90±0.65	0.532
TG-peak height (nM)	197±88	201±97	0.556
TG-time to peak (sec)	6.12±1.17	6.10±1.18	0.731
TG-endogenous thrombin potential (nM)	1,281±348	1,280±369	0.965
Sample quality			
H-index	4.9±2.5	4.5±1.8	0.459
I-Index	22.7±8.9	22.6±8.6	0.639
L-index	9.4±4.5	9.3±5.2	0.823

RDW, red blood cell distribution width; TG, thrombin generation.

indices, thus including the hemolysis-index (H-index), icteric-index (I-I) and lipaemic-index (L-I) were also assayed using Roche Cobas 8000, with spectrophotometric techniques, as comprehensively described elsewhere (9). The complete blood cell count was performed in whole blood anticoagulated with K_2EDTA, using Sysmex XN (Sysmex Corporation, Kobe, Japan), whose basic characteristics have been previously described elsewhere (10,11). Routine hemostasis testing, thus including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and D-dimer, was assayed in citrate plasma, using Instrumentation Laboratory ACL TOP 700 (Instrumentation Laboratory, Bedford, MA, USA) and proprietary reagents (RecombiPlasTin, SynthASil, Fibrinogen-CXL and HemosIL HS D-dimer). The characteristics of this analyzer and reagents have been earlier summarized elsewhere (12). Finally, thrombin generation (TG) was assayed in citrate plasma, using the fully-automated analyzer ST Genesia (STG-BleedScreen; Diagnostica Stago, Asnières sur Seine, Cedex, France), as previously summarized (13). These measurements included the assessment of lag phase (or lag time), time to reach the peak (or time to peak), peak height and endogenous thrombin potential (ETP).

The test results in samples collected with the two different holders followed a substantially normal distribution, as assessed with Kolmogorov-Smirnov test, and were hence expressed as mean and standard deviation (SD) and compared with paired Student's *t*-test, Pearson's correlation and Bland-Altman plots, using Analyse-it (Analyse-it Software Ltd, Leeds, UK). The mean percent bias of values in samples collected with the two different holders was compared with the quality specifications calculated from desirable biological variation (14). All subjects participating to this study provided a written informed consent and the study was cleared by the local Ethics Committee (970CESC; July 20, 2016).

Results

The main results of this study are shown in *Table 1*. All test results of hematological, clinical chemistry and hemostasis testing were non-significantly different in blood samples collected with HOLDEX® single-use holder or VACUETTE® SAFELINK except for potassium, whose values were marginally higher in plasma collected with VACUETTE® SAFELINK. The Pearson's correlation of the different parameters in blood samples collected with HOLDEX® single-use holder or VACUETTE® SAFELINK is shown in *Table 2*. All correlation coefficients were excellent except for potassium, chloride and,

Table 2 Comparison of laboratory test results in samples collected with Greiner Bio-One HOLDEX® single-use and VACUETTE® SAFELINK holders

Parameter	Correlation	Quality specifications (%)	Bias (%)
Complete blood cell count			
Red blood cells ($\times10^{12}$/L)	0.982 (P<0.001)	1.7	–0.6 (–1.3 to 0.2)
Hemoglobin (g/L)	0.991 (P<0.001)	1.8	–0.5 (–1.2 to 0.1)
Hematocrit	0.976 (P<0.001)	1.7	–0.6 (–1.7 to 0.4)
Mean corpuscular volume (fL)	0.997 (P<0.001)	1.3	0 (–0.2 to 0.2)
RDW (%)	0.995 (P<0.001)	1.7	0.2 (–0.2 to 0.5)
White blood cells ($\times10^{9}$/L)	0.986 (P<0.001)	6.1	–1.9 (–3.9 to 0.2)
Platelets ($\times10^{9}$/L)	0.983 (P<0.001)	5.9	–1.0 (–2.7 to 0.7)
Mean platelet volume (fL)	0.993 (P<0.001)	2.3	0.2 (–0.3 to 0.7)
Clinical chemistry			
Potassium (mmol/L)	0.532 (P=0.002)	1.8	1.6 (0.7 to 2.5)
Chloride (mmol/L)	0.572 (P=0.001)	0.5	0.5 (–0.1 to 1.0)
Sodium (mmol/L)	0.770 (P<0.001)	0.2	–0.2 (–0.5 to 0.2)
Aspartate aminotransferase (U/L)	0.953 (P<0.001)	6.5	0.9 (–3.5 to 5.4)
Lactate dehydrogenase (U/L)	0.974 (P<0.001)	4.3	1.0 (–6.6 to 8.6)
Hemostasis			
Prothrombin time (sec)	0.963 (P<0.001)	2.0	–0.1 (–0.7 to 0.6)
Activated partial thromboplastin time (sec)	0.828 (P<0.001)	2.3	0.7 (–1.0 to 2.3)
Fibrinogen (g/L)	0.933 (P<0.001)	4.8	0.1 (–2.3 to 2.5)
D-dimer (ng/L)	0.953 (P<0.001)	8.8	–3.3 (–14.9 to 8.4)
TG-lag time (sec)	0.958 (P<0.001)	N/A	0.8 (–1.5 to 3.1)
TG-peak height (nM)	0.931 (P<0.001)	N/A	0.8 (–6.6 to 8.2)
TG-time to peak (sec)	0.976 (P<0.001)	N/A	–0.3 (–2.0 to 1.4)
TG-endogenous thrombin potential (nM)	0.860 (P<0.001)	N/A	–0.5 (–6.7 to 5.7)
Sample quality			
H-index	0.122 (P=0.521)	N/A	–4.5 (–23.8 to 14.8)
I-Index	0.922 (P<0.001)	N/A	–0.1 (–1.9 to 1.7)
L-index	0.885 (P<0.001)	N/A	–0.3 (–7.8 to 7.5)

N/A, not available; RDW, red blood cell distribution width; TG, thrombin generation.

especially, H-index. In this latter case, the correlation did not even reach statistical significance. The percent bias of the different parameters in blood samples collected with HOLDEX® single-use holder or VACUETTE® SAFELINK was always minimal, ranging between –4.5% for the H-index and +1.6% for potassium (*Table 2*). In all cases the percent bias was comprised within the quality specifications. Importantly, the rate of samples with H-index >3 was marginally lower when blood was collected using VACUETTE® SAFELINK than with HOLDEX® single-use holder (i.e., 19/30 versus 23/30; odds ratio, 0.52; 95% CI, 95 0.17–1.60; P=0.263).

Discussion

Blood sample quality is essential to guarantee reliable results of downstream laboratory testing (15). Among the various sources of preanalytical mistakes, hemolysis is indeed the most frequent and one of the most harmful when overlooked or left unmanaged (16). Several strategies have been attempted to minimize the risk of producing spurious hemolysis during blood collection, such as using discard or low vacuum tubes (17), decreasing the pressure of blood aspiration within the tube (18), use of new venous catheter blood draw devices (19), as well as the usage of holders with features that effectively decelerate blood flow pressure (8). VACUETTE® SAFELINK is one of these last devices, which has been recently commercialized by Greiner Bio-One GmbH, with the aim of lowering the risk of generating spurious hemolysis during blood drawing, and thereby improving clinical effectiveness by reducing the number of hemolyzed tests suppressed and avoiding the need to recollect blood samples. This innovative device is equipped with a small flash chamber which has two effects: allowing to visualize the blood and decelerating blood flow pressure before entering the tube. Altogether these two characteristics would enable the phlebotomist to enhance the rate of successful venipunctures and contextually lowering the risk of hemolyzing samples. To validate this second theoretical advantage, we planned a comparative study, where 24 clinical chemistry, hematology and hemostasis test results were compared among samples collected using the HOLDEX® single-use holder or the new VACUETTE® SAFELINK holder.

Taken together, the results of our study clearly suggest that the performance of these two devices is fully comparable. More specifically, the values of all analytes tested (except potassium) were non-significantly different in blood samples collected with either holder and, even more importantly, in no case the percent bias exceeded the currently quality specifications. The correlation between measures was also optimal except for potassium, chloride and, especially, for the H-index. Albeit the correlation of potassium and chloride plasma concentrations was still significant and thereby acceptable, H-index values obtained with HOLDEX® single-use holder and VACUETTE® SAFELINK were definitely incomparable (r=0.122; P=0.521), with VACUETTE® SAFELINK exhibiting a trend towards lower risk of generating spurious hemolysis (i.e., –48%). This is not really unexpected, and may be either due to the small sample size or to the different structure of the two devices, with HOLDEX® single-use holder presenting an eccentric position of the luer slip connection, compared to the luer centered at the top of the holder in VACUETTE® SAFELINK. This would inevitably generate a different path of blood flow within the two devices, thus explaining the different predisposition towards *in vitro* erythrocyte injury depending on flow perturbation and erythrocyte mechanical fragility (20). After that said, however, the data obtained in our experimental study clearly show that the quality of laboratory testing is virtually identical between HOLDEX® single-use holder and VACUETTE® SAFELINK (*Table 2*), whilst the risk of injuring blood cells appears eventually lower with VACUETTE® SAFELINK. Of particular note are also the results of the TG assay, which would enable us to reliably conclude that hemostasis may be equally safeguarded with both devices.

Conclusions

In conclusion, the results of this comparative study suggest that sample quality is thoughtfully comparable using HOLDEX® single-use holder and VACUETTE® SAFELINK, thus translating into the concept that VACUETTE® SAFELINK may also be an effective means for reducing spurious hemolysis, especially when drawing blood from catheters.

Acknowledgments

The manufacturer provided in-kind support in the form of equipment and consumables to enable study performance. The manufacturer had no role in directing the study or influencing the interpretation of the study results.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/jlpm.2019.08.03
Cite this article as: Salvagno GL, Pucci M, Lippi G. Blood sample quality using Greiner Bio-One HOLDEX® Single-Use Holder and VACUETTE® SAFELINK holder with male luer lock: a comparative study. J Lab Precis Med 2019;4:27.

