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Preanalytical variability in laboratory testing: influence of the blood drawing technique

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Abstract

The predominant technique used to draw blood for laboratory testing is a conventional straight needle attached to an evacuated tube system. However, alternative tools might be advantageous in exceptional circumstances. The use of butterfly devices has been traditionally discouraged for reasons of costs and due to the high risk of obtaining unsuitable samples, but there is no convincing evidence to support the latter indication. The purpose of this study was to compare results of hematological and clinical chemistry testing, after drawing blood into evacuated tubes, employing either a traditional 21-gauge straight needle or a 21-gauge butterfly device with 300-mm-grade polyvinyl chloride tubing. Blood samples and complete sets of data were successfully obtained for 30 consecutive outpatients. Of the 43 hematological and clinical chemistry parameters measured, means for paired samples collected by the two alternative drawing techniques did not differ significantly, except for serum sodium, white blood cells and platelets counts. Bland-Altman plots and limits-of-agreement analysis showed mean bias of between −7.2% and 1.7% and relative coefficients of variation ranging from 0.2% to 21.2%. The 95% agreement interval in the set of differences was acceptable and was mostly within the current analytical quality specifications for desirable bias. The rate of hemolysis in plasma was not statistically different between the two collection techniques. Taken together, the results of the present investigation suggest that, when a proper technique is used and within certain limitations, the butterfly device may be a reliable alternative to the conventional straight needle to draw blood for laboratory testing.

Keywords: blood collection; butterfly device; laboratory testing; preanalytical variability; standardization.

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Introduction

Most recipients of laboratory testing ignore the possibility of other factors contributing to the test values, especially those that are abnormal. It is essential for everyone who either performs tests or uses their results for patient care to have a clear understanding of all the factors that can generate erroneous and misleading laboratory results (1). Any improvement in laboratory-related patient outcomes ultimately includes accurate standardization and monitoring of all pre-, intra-, and postanalytical phases. A considerable body of clinical and laboratory evidence suggests that most laboratory errors occur in the preanalytical phase, highlighting the need for the acquisition or implementation of more rigorous methodologies to detect and classify potential pitfalls in this crucial step of laboratory work-up (2). Most problems in the preanalytical phase concern factors immediately associated with collection of the specimen. Therefore, the choice of device for blood collection becomes a pivotal aspect in optimizing the preanalytical phase and achieving reliable results. Venepuncture has traditionally been carried out using ordinary straight needles and syringes; the blood was then injected into sample tubes by piercing the rubber bung using the same needle. The introduction of disposable needles attached to evacuated tube collection systems in the early 1980s represented substantial progress in blood collection techniques, almost completely replacing the classical syringes (3). These evacuated tube collection systems generally consist of a double-pointed needle, a plastic holder or adapter, and a series of vacuum tubes with rubber stoppers. It has been proven that blood collection using these devices produces the best blood samples for laboratory testing and concomitantly ensure greater safeness for the operator, as the patient's blood flows directly into appropriate test tubes. In addition, the sheath makes it possible to draw several tubes of blood while preventing blood leakage as tubes are changed. There are several sizes of needle available for these disposable devices, with the size depending on the length and gauge (G) of the needle that goes into the vein.

Among the major determinants of preanalytical variability, the blood drawing technique exerts considerable influence on the reliability of results of laboratory testing. The butterfly device, a small needle attached to flexible plastic wings and connected with extension flexible tubing, might be regarded as a reliable alternative to the classic straight needle for collecting blood in selected categories of patients. In fact, an adapter can easily be added, so that it fits into a vacuum needle holder and a vacuum system. Many

patients receiving invasive medical treatment or diagnostic investigations undergo subcutaneous venous cannulation by permanent or butterfly devices. The venous cannula may be in place for a few minutes or hours, as for patients undergoing general anesthesia, sedation before surgery, noxious clinical procedures, or diagnostic radiological investigations. In other clinical settings, long-term cannulation may be required (hemodialyzed, critically ill or cancer patients). Through these devices, blood products, fluids, electrolytes, antimicrobial drugs, long-term infusions for pain relief or chemotherapy and other essential therapeutic agents are delivered. In these circumstances, it is integral to the care of patients to maintain catheter patency and a second venepuncture to draw blood for laboratory testing might be inopportune or inconvenient, as the same venous access can be used. This approach is cheaper, as no other devices for blood collection might be required, and much safer for both patient and phlebotomist. Blood collection by butterfly systems might be necessary or easier and less painful in newborns, children, small animals and patients with small, difficult and atypical venous access, where it might be very difficult to rest the supporting hand (venepuncture in the hand, leg, heel or cranium). Finally, the use of a butterfly device, which is less intimidating due to the reduced dimension of the needle, might be advisable in some other circumstances, especially when approaching nervous or anxious patients. The simplicity of use for unskilled or nervous operators is an additional advantage of this system, because the needle does not need to be held once it is in the vein. Practical disadvantages are represented by the major cost, the increased chance of needlestick injury and the possibility that a small amount of blood will be spilled when the needle is withdrawn from the skin. In laboratory practice, the use of butterfly needles and intravenous lines for specimen collection has been traditionally discouraged, unless more conventional routes have failed, for reasons of costs and for the high risk of obtaining unsuitable samples (incomplete filling of the vacuum tube, hemolysis, activated or clotted samples) (1). Little scientific research exists on optimal methods for obtaining blood samples from catheters, and clinicians use a variety of unproven techniques (4). In particular, there are few reliable studies investigating the influence of the device employed for specimen collection on the imprecision of routine laboratory testing. Therefore, we planned to compare the accuracy of routine clinical chemistry and hematologic testing on specimens collected into evacuated tubes from two sequential venepunctures by a butterfly device and a conventional straight needle.

Materials and methods

The primary purpose of this study was to investigate the influence of a butterfly device on routine laboratory testing. Blood was collected into siliconized vacuum tubes, either by a sterile infusion set with a 21-gauge (G), 0.80×19-mm butterfly needle and 300-mm grade polyvinyl chloride (PVC) tubing with a Luer adapter (Artsana, Casnate, Italy) or a classic 21G, 0.80×19-mm Venoject® multi-sample straight needle (Terumo Europe NV, Leuven, Belgium). The study was performed according to the following protocol: two independent blood samples were successively drawn from each patient; samples labeled as "A" were collected after discarding a first vacuum tube by venepuncture using a classic 21G straight needle on the median cubital or basilic vein of the left arm. Samples labeled as "B" were collected after discarding a first vacuum tube by a second venepuncture using a 21G needle butterfly device on the median cubital or basilic vein of the right arm. Alternate arms were preferred for repeated venepuncture to avoid potential contamination with endogenous material (thromboplastin, fibrin, cellular and subendothelial components, etc.) likely released during the first venepuncture. In each phlebotomy, the first tube was discarded to avoid incomplete filling of subsequent tubes. Venepuncture was carried out in the morning of the same day on fasted volunteers by a single experienced phlebotomist. All phases of the sample collection were accurately standardized, including identical resting time for the subjects (>5 min), tourniquet placement (<30 s), and the use of vacuum tubes from the same lot. The tourniquet was released when blood began flowing into the first tube. After collection into siliconized vacuum tubes containing either K_2 EDTA (for hematological measurements) (Becton-Dickinson, Oxford, UK) or Gel+45 USP U Lithium Heparin (Terumo Europe, Haasrode, Belgium) (for clinical chemistry testing) as anticoagulants, samples were gently mixed by inverting the tubes four–six times. The lithium-heparin tubes were centrifuged at 1200×*g* for 10 min at room temperature within 30 min of collection; plasma was separated and immediately analyzed. No specimen was discarded for non-fulfillment of the criteria formerly established for suitability (unsatisfactory attempts, difficulty in locating easy venous accesses, vein missing, manifest hemolysis or lipemia), and the final study population consisted of the originally intended 30 consecutive outpatients (18 women, 12 men; mean age, 47 years).

Clinical chemistry measurements were carried out using enzymatic procedures on a Roche/Hitachi modular system (Roche Diagnostics GmbH, Mannheim, Germany), according to manufacturer specifications and employing proprietary reagents. Sodium, chloride and potassium were measured on a Roche/Hitachi modular system using ion-selective electrodes. Total imprecision, as expressed by the coefficient of variation (CV), was less than 2.2% for all analytes tested. Hematological measurements and differential blood counts were carried out on an ADVIA 120™ analyzer (Bayer Diagnostics, Newbury, UK). Analytical imprecision, expressed in terms of mean inter-assay CV, was quoted by the manufacturer as being between 2 and 10%. Sample hemolysis was assayed by measuring the concentration of free hemoglobin in plasma using a reference cyanmethemoglobin method (5) on a UV-1700 spectrophotometer (Shimadzu Italia Slr, Milan, Italy). All measurements were carried out in duplicate within a single analytical session and final results were finally averaged. Significance of differences between samples was assessed by a paired Student's t-test; the level of statistical significance was set at $p<0.05$. Bland-Altman plots and limits-of-agreement analysis was used to compare the results of the independent measurements on samples A and B; plot differences were finally reported as a percentage of the averages.

Results

Results of the present evaluation are shown in Tables 1 and 2. Statistically significant differences according

Table 1 Statistical analysis of clinical chemistry testing in specimens collected into evacuated tubes employing a 21G butterfly device and 300-mm PVC tubing (Sample B) vs. a 21G conventional straight needle (Sample A).

	Sample A	Sample B	p	Passing-Bablock regression (r)	Desirable bias, %	Mean bias (%)	CV, %	95% CI, %
Alanine amino transferase, U/L	31±16	31±16	0.475	$y=1.00x$ (r=0.997)	±12.0	−0.17 (−0.5%)	2.53	−2.1 to 1.0
Albumin, g/L	42.4±2.5	42.8±2.7	0.276	$y=1.06x-2.42$ (r=0.753)	±1.3	0.38 (0.9%)	1.75	−0.8 to 2.4
Alkaline phosphatase, U/L	77±40	77±40	0.495	$y=1.00x$ (r=0.999)	±6.4	−0.20 (−0.3%)	1.01	−1.0 to 0.5
Amylase, U/L	73±29	73±28	0.133	$y=1.00x$ (r=0.999)	±7.8	−0.37 (−0.5%)	0.71	−1.4 to 0.1
α-Amylase pancreatic, U/L	27±13	26±13	0.118	$y=1.00x$ (r=0.998)	±8.0	−0.27 (−0.1%)	1.87	−2.3 to 0.3
Aspartate amino transferase, U/L	30±8	29±9	0.125	$y=0.97x+1.00$ (r=0.991)	±5.4	−0.37 (−1.2%)	2.44	−2.8 to 0.4
Bilirubin total μmol/L	12.7±6.2	12.6±6.1	0.055	$y=1.00x$ (r=0.999)	±10.0	−0.11 (−0.9%)	1.20	−1.7 to 0.1
Bilirubin conjugated, μmol/L	3.1±1.7	3.0±1.7	0.076	$y=1.00x$ (r=0.993)	±14.2	0.07 (−2.3%)	4.55	−4.5 to 0.3
Calcium, mmol/L	2.44±0.13	2.42±0.11	0.335	$y=0.99x+0.01$ (r=0.828)	±0.8	−0.02 (−0.7%)	1.01	−2.0 to 0.7
C-Reactive protein, mg/L	5.80±2.55	5.73±2.67	0.573	$y=1.00x$ (r=0.972)	±24.9	−0.07 (−1.2%)	4.71	−2.9 to 5.3
Chloride, mmol/L	105.7±2.7	105.6±2.3	0.601	$y=1.00x$ (r=0.860)	±0.5	−0.13 (−0.10%)	0.67	−0.4 to 0.6
Cholesterol HDL, mmol/L	1.49±0.38	1.48±0.39	0.326	$y=1.00x$ (r=0.997)	±5.2	−0.01 (−0.4%)	1.23	−1.3 to 0.7
Cholesterol total, mmol/L	5.31±0.99	5.29±1.01	0.291	$y=0.98x-0.12$ (r=0.995)	±4.0	−0.02 (−0.4%)	1.16	−1.1 to 0.4
Creatine kinase, U/L	101±39	101±39	0.933	$y=1.00x$ (r=0.999)	±11.5	0.03 (0.03%)	1.33	−0.8 to 0.8
Creatinine, μmol/L	91.0±33.9	90.4±34.5	0.175	$y=0.99x-1.48$ (r=0.998)	±3.4	−0.62 (−0.7%)	1.70	−1.7 to 0.3
γ-Glutamyl transferase, U/L	53±70	53±69	1.000	$y=1.00x$ (r=0.999)	±10.8	0 (0.00%)	3.10	−2.3 to 2.3
Glucose, mmol/L	5.66±1.51	5.65±1.50	0.880	$y=0.98x+0.1$ (r=0.992)	±2.2	−0.01 (−0.1%)	1.79	−1.4 to 1.2
Iron, μmol/L	17.1±7.0	17.1±6.9	0.741	$y=1.00x$ (r=0.998)	±8.8	−0.03 (−0.2%)	1.26	−1.2 to 0.9
Lactate dehydrogenase, U/L	382±120	379±98	0.748	$y=0.99x+0.51$ (r=0.922)	±4.3	−2.90 (−0.8%)	3.46	−5.5 to 3.9
Lipase, U/L	31±14	31±14	0.550	$y=1.00x+1.00$ (r=0.980)	±10.1	−0.30 (−1.0%)	5.67	−4.3 to 2.3
Magnesium mmol/L	0.96±0.06	0.96±0.07	0.917	$y=1.00x+0.01$ (r=0.948)	±1.8	0 (0.00%)	1.34	−1.0 to 1.0
Inorganic phosphorus, mmol/L	3.02±0.51	3.05±0.50	0.080	$y=1.01x-0.01$ (r=0.990)	±3.2	0.02 (0.8%)	1.61	−0.1 to 1.7
Potassium, mmol/L	4.13±0.42	4.20±0.44	0.179	$y=0.93x+0.21$ (r=0.801)	±1.8	0.07 (1.7%)	3.67	−0.7 to 4.1
Protein total, g/L	74.9±3.4	74.6±3.3	0.325	$y=0.95x+4.1$ (r=0.846)	±1.2	−0.35 (−0.5%)	1.28	−1.4 to 0.5
Sodium, mmol/L	141.0±2.5	140.4±2.1	0.067	$y=1.00x$ (r=0.763)	±0.3	−0.57 (−0.4%)	0.62	−0.8 to 0.03
Triglycerides mol/L	1.72±1.47	1.73±1.50	0.521	$y=1.00x$ (r=1.000)	±10.7	0.01 (0.4%)	1.38	−0.8 to 1.7
Urea nitrogen, mmol/L	13.7±4.0	13.6±3.9	0.221	$y=1.00x+0.07$ (r=0.997)	±5.5	−0.07 (−0.5%)	1.37	−1.4 to 0.4
Uric acid, μmol/L	343±92	344±93	0.109	$y=1.00x$ (r=0.999)	±4.8	1.39 (0.4%)	0.76	−0.1 to 0.9

Values are expressed as mean±standard deviation. Differences between samples A and B were analyzed by a paired Student's t-test (p), Passing-Bablock regression analysis and relative coefficient of correlation (r). Mean differences between samples A and B are shown as absolute and percentage bias, coefficient of variation (CV) and relative Altman-Bland 95% coefficient of interval (CI) limits of agreement. Values are finally compared to the current analytical quality specifications for desirable bias derived from biological variation, as indicated by Ricos et al. (6).

to the paired Student t-test could be observed between samples A and B in only two out of 43 parameters tested (platelet count, $p<0.01$ and white blood cell count, $p=0.041$). The substantial agreement between specimens collected by the two alternative techniques, as evaluated by Passing-Bablock regression analysis and calculation of the relative correlation coefficients, is shown in Tables 1 and 2. Although

Table 2 Statistical analysis of hematological testing of specimens collected into evacuated tubes employing a 21G butterfly device and 300-mm PVC tubing (Sample B) vs. a 21G conventional straight needle (Sample A).

	Sample A	Sample B	p	Passing-Bablock regression (r)	Desirable bias, %	Mean bias (%)	CV, %	95% CI, %
Leukocyte count, 10^3 μL	6.20±1.31	6.10±1.27	0.041	$y=0.98x-0.07$ (r=0.976)	±5.6	−0.105 (−1.7%)	2.5	−3.3 to −0.1
Erythrocyte count, 10^6 μL	4.97±0.50	4.98±0.51	0.671	$y=1.00x-0.01$ (r=0.993)	±1.7	0.01 (0.1%)	0.7	−0.4 to 0.6
Hematocrit	0.44±0.03	0.44±0.03	0.955	$y=1.00x-0.1$ (r=0.978)	±1.7	0.01 (0.0%)	0.8	−0.5 to 0.6
Hemoglobin mmol/L	9.01±0.66	8.98±0.67	0.085	$y=1.00x$ (r=0.993)	±1.8	−0.03 (−0.3%)	0.5	−0.7 to 0.1
Hemoglobin free, mmol/L	0.09±0.03	0.09±0.03	0.811	$y=0.99x$ (r=0.981)	±1.8	−0.0003 (−0.5%)	4.1	−3.3 to 3.3
Mean corpuscular hemoglobin, pg	29.4±2.8	29.3±2.8	0.061	$y=1.00x-0.01$ (r=0.995)	±1.4	−0.11 (−0.3%)	0.6	−0.7 to 0.0
Mean corpuscular volume, fL	89.8±6.9	89.7±6.9	0.335	$y=1.00x$ (r=0.999)	±2.3	−0.06 (−0.1%)	0.2	−0.2 to 0.1
Platelet count, 10^3 μL	246±92	239±90	<0.01	$y=1.00x-5$ (r=0.996)	±5.9	−7.2 (−4.1%)	2.4	−4.3 to −1.6
Mean platelet volume, fL	7.9±0.7	7.8±0.8	0.219	$y=1.00x$ (r=0.977)	±2.3	−0.05 (−0.6%)	1.4	−1.6 to 0.4
Leukocyte differential								
Neutrophil count, 10^3 μL	3.45±1.02	3.42±0.99	0.136	$y=1.04x-0.11$ (r=0.990)	±9.1	−0.04 (−1.8%)	2.6	−2.6 to 0.3
Lymphocyte count, 10^3 μL	2.01±0.59	1.98±0.51	0.065	$y=1.01x-0.01$ (r=0.985)	±7.4	−0.04 (−2.2%)	2.8	−3.5 to 0.1
Monocyte count, 10^3 μL	0.34±0.09	0.34±0.09	0.536	$y=1.00x$ (r=0.938)	±13.2	0.004 (1.2%)	6.4	−2.6 to 5.0
Eosinophil count, 10^3 μL	0.21±0.14	0.20±0.14	0.275	$y=1.00x$ (r=0.979)	±19.8	−0.01 (−1.0%)	7.6	−6.7 to 1.9
Basophil count, 10^3 μL	0.05±0.02	0.05±0.02	0.182	$y=1.00x-0.01$ (r=0.721)	±15.4	0.004 (−7.2%)	21.2	−4.0 to 2.2
Large unstained cells, 10^3 μL	0.14±0.05	0.14±0.05	0.310	$y=1.00x$ (r=0.920)	Not available	−0.005 (−2.8%)	9.3	−10.0 to 3.6

Values are expressed as mean±standard deviation. Differences between samples A and B were analyzed by a paired Student's t-test (p), Passing-Bablock regression analysis and relative coefficient of correlation (r). Mean differences between samples A and B are shown as absolute and percentage bias, coefficient of variation (CV) and relative Altman-Bland 95% coefficient of interval (CI) limits of agreement. Values are finally compared to the current analytical quality specifications for desirable bias derived from biologic variation, as indicated by Ricos et al. (6).

the statistical analysis was satisfactory for most analytes tested, the clinical acceptability was somehow lower for platelet counts. However, with the exception of sodium, the precision of repeated measurements ascertained by Bland-Altman plots and the 95% agreement interval in the set of differences between samples was always within the current analytical quality specifications for desirable bias derived from biological variation, as proposed by Ricos et al. (6). The imprecision, expressed by the relative CVs of measured analytes between individual samples A and B, was modest overall and clinically acceptable, with few exceptions. When comparing results of specimens obtained from straight-needle venepuncture versus those from the butterfly device, 8 out of 43 calculated CVs exceeded the current analytical quality specifications for desirable bias (serum albumin, calcium, chloride, potassium, total proteins, sodium, free hemoglobin, basophil count), but were still within the relative critical difference specifications, as estimated in accordance with the reported biological variability (6) and the total imprecision quoted by the manufacturer. The substantial agreement by paired Student's t-test and the limited bias observed between the measurement of free hemoglobin, potassium, LDH and AST, indicate that the extent of clinical erythrocytolysis and the leakage of intracellular constituents in samples drawn by the butterfly device were not greater than those typical of straight-needle venepuncture.

Discussion

Spurious variations in hematological parameters and clinical chemistry analytes that cannot be clinically explained and do not correspond to the status of the patient might be harmful and misleading, consuming valuable healthcare resources and leading to potential errors or delays in patient care (7). The standardization of each aspect of laboratory testing, including the preanalytical phase, is essential in achieving reliable results. Unsuitable samples (hemolyzed, insufficient, activated or clotted), due to problems in drawing blood from the patient, might account for over 80% of the errors occurring in the preanalytical

phase (2). From this perspective, identification and understanding of the mechanisms by which some preanalytical variables of specimen acquisition and processing can affect laboratory test results are crucial steps, together with the definition of corrective actions to overcome such problems.

There is general consensus on problem with the use of intravenous catheters and butterfly devices, as these expensive phlebotomy systems are often overutilized, especially to draw blood for routine laboratory testing. Although a high risk of obtaining unsuitable samples has frequently been associated with the material used for venepuncture, especially short catheters (8), there are few reliable studies investigating the influence of butterfly devices on hematological and clinical chemistry testing to the best of our knowledge. Most laboratory measures are not apparently influenced by the site of sample withdrawal, the presence of a tourniquet, or the time elapsed between blood sampling and analysis (9, 10). However, earlier investigations emphasized that blood drawn through intravenous catheters, butterfly needles and other similar devices often shows significantly more hemolysis and results in higher test cancellation than that drawn with a conventional straight needle (8, 11–13). This has influenced the development of pertinent guidelines and suggestions for specimen collection from indwelling catheters or cannulae by the International Federation of Clinical Chemistry (IFCC) (4, 14). Exposure of blood cells to sufficiently large shear stress and mechanical strain, such as occurs using PVC, polyurethane and Teflon catheters, might affect membrane function and integrity, causing shape modifications, cell activation, damage, and efflux of intracellular constituents into the serum (12). The possible alteration of cell integrity during blood collection and hemolysis, in particular, might lead to inaccurate assay results, requiring repeated blood draws, especially for hematological, electrolytic and enzymatic determinations (8). Evidence from previous investigations is fragmentary, inconclusive and occasionally controversial. Due to shear-induced hemolysis, it was previously reported that the prevalence of hemolyzed specimens might range from 15 to 25% for catheter diameters from 22G to 20G, compared to 3.8% in samples collected by a 21G conventional straight needle (11). Other investigations concluded that obtaining blood samples from peripheral catheters might be clinically acceptable for hemoglobin and most clinical chemistry analytes (15–19), but not for potassium (20), bicarbonate and glucose (19), albumin and total protein (21) measurements. Although PVC and other plastic materials are extensively used for disposable medical devices, it has been suggested that they might produce adverse reactions when in contact with body tissues and fluids. As the surface hydrophobicity of several artificial surfaces and the impact of the blood cells against the tubing walls are potential causes of hemostatic activation in vitro, it is conceivable that the transit of whole blood within butterfly devices might introduce variations in comparison to blood collection directly into vacuum tubes by traditional straight needles. Due to the increased risk of platelet activation and hemolysis within the long plastic tubes of the butterfly systems, platelet counts and potassium measurements are thought to be more susceptible to variations introduced by the blood collection technique. At variance with earlier studies, the degree of hemolysis, as assessed by measurement of free plasma hemoglobin, potassium, LDH and AST concentrations, in blood specimens collected via a 21G needle, 300-mm PVC tubing device was not greater than that of blood collected with a traditional 21G straight needle. This result is consistent with the study of Sonntag (22), who showed that the concentrations of plasma potassium and LDH are significantly affected by hemoglobin concentrations >0.2 g/L. For hematological parameters, an earlier investigation observed no major differences between samples obtained using saline locks and those drawn from direct venepuncture (23). Our results are consistent with those of Sliwa, except for platelet count (-4.1%, $p<0.001$) and white blood cell count (-1.7%, $p=0.041$), which were consistently lower in samples collected using the butterfly device (Figure 1). However, it should be pointed out that the overall discrepancy between the two collection devices, although reaching statistical significance, was limited and mostly within the relative critical differences and current analytical quality specifications for desirable bias (6). In addition, the hematological analyzer unveiled no major signs of cells aggregation in any sample, as testified by the absence of the corresponding instrumental flags. Taken together, these data suggest that, although statistically significant, the differences for both counts did not reach clinical relevance in either case.

The underestimation observed in platelet and leukocyte counts in samples collected by the butterfly system is not really surprising and there is some reliable evidence that might explain this finding. Optimal blood compatibility depends on a combination of biomaterial wettability and the shear stress prevailing in the device (24). Platelet and leukocyte activation and aggregation are well-recognized consequences of contact with several biopolymer surfaces, including PVC (25). Moreover, the alteration in fluid dynamics, leading to a mechanical load on blood corpuscle membranes due to the shear stress that seems to occur within the narrow butterfly tubing, might further promote and trigger platelet aggregation, an additional mechanism that can explain the drop in absolute platelet count (26). Consistent with our findings, it was shown that incubation of blood with PVC slides results in the rapid binding of leukocytes and platelets (27). Likewise, it is well known that in high-shear applications, in combination with blood activation, platelet deposition appears to be a major concern (24), as the in vitro flow of blood within uncoated tubes causes immediate platelet aggregation and loss (28).

Among all measurements, the mean bias of repeated measurements for serum sodium slightly exceeded the current analytical quality specification for

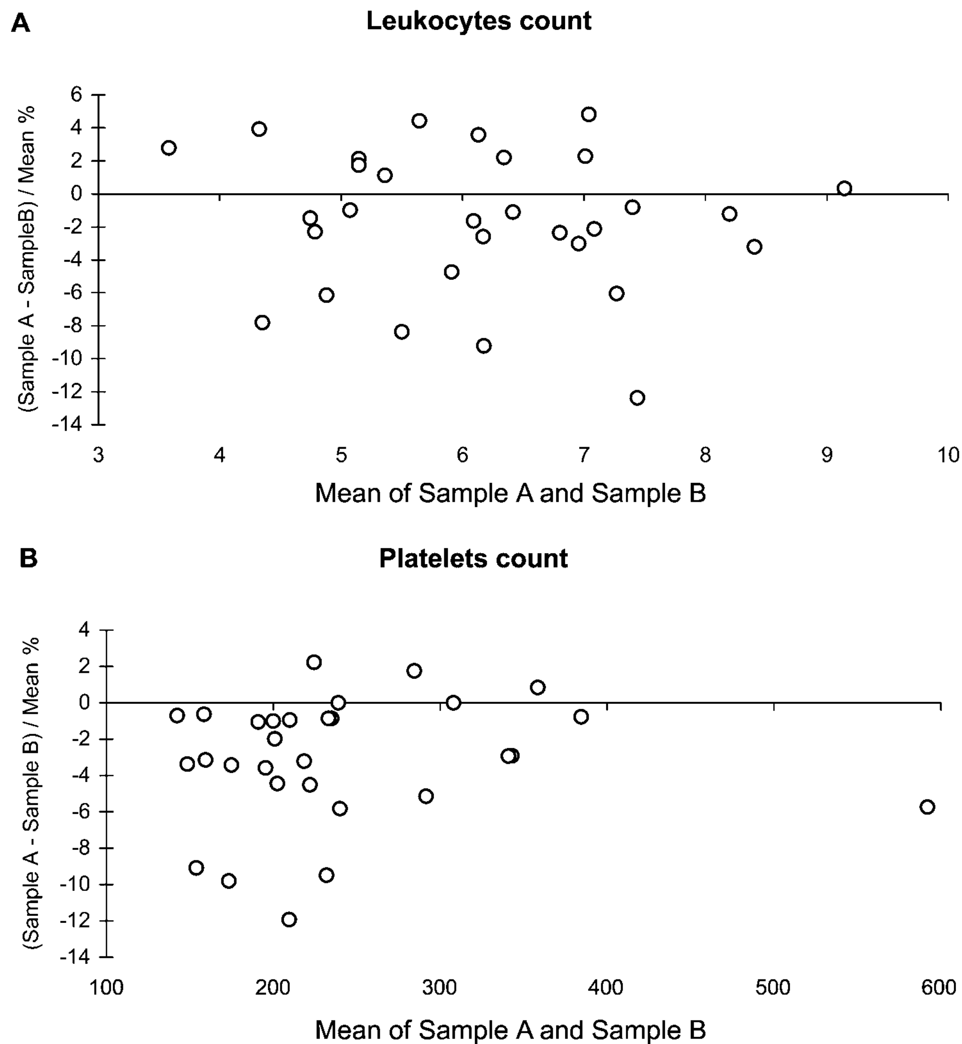

Figure 1 Bland-Altman plots for (A) leukocyte and (B) platelet counts in samples A and B. Plot differences are expressed as a percentage of the means.

desirable bias (0.4% vs. 0.3%). This is an unexpected observation, although the CVs calculated for most inorganic ions (calcium, sodium, chloride and potassium) are above the desirable bias values, despite substantial agreement of measurements (Table 1). However, it must be considered that CVs and bias values observed between samples A and B for inorganic ions were still largely within the relative critical differences, as estimated in accordance with the reported biological variability (6) and the total imprecision quoted by the manufacturer (6.7% for calcium, 2.9% for sodium, 4.2% for chloride and 13.5% for potassium).

In conclusion, besides serum sodium, platelet and leukocyte counts, we did not observe substantial differences in routine hematological and clinical chemistry testing for samples collected with the two different drawing techniques. The innovative value of this investigation is represented by the use of a butterfly device assigned exclusively for blood drawing. In most cases, specimens have been obtained from patients with permanent or semi-permanent peripheral intravenous catheters, who were receiving infusion therapy or saline solution. Specimens were collected after aspiration and discarding of a small volume of withdrawn blood directly from the saline solution lock, and results of laboratory testing were compared with concomitant venepuncture on the other upper extremity. In addition, each phase of blood collection was accurately standardized according to our study design, as indicated and suggested by the current guidelines (29). As there might be an inverse correlation between intravenous catheter diameter and the risk of obtaining unsuitable samples for laboratory testing (11), we standardized the needle diameter to 21G for both conventional and butterfly needles. Although the order of drawing when taking blood using vacuum-based blood collection systems seems negligible for the purposes of laboratory testing (30), the influence of additional hypothetical variables was ruled out by collecting and discarding a first vacuum tube immediately after venepuncture by both devices, collecting the lithium-heparin and K_2 EDTA tubes as the second and third specimens, respectively.

Presumably, phlebotomy equipment will continue to evolve; the choice of the most suitable blood-collection system depends mainly on considerations of

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cost, reliability, safety, and convenience (4). Along with these considerations, the bias observed for the analytes tested between the use of one device and the other was always lower than both the relative biological variability and the critical difference. Therefore, the use of a butterfly needle device (or winged infusion device) had little or no clinical repercussions and might be a reliable alternative to the ordinary needle system, when indicated and within certain limitations, for collecting specimens for routine laboratory testing.

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