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The effect of anticoagulant, storage temperature and dilution on cord blood hematology parameters over time

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SUMMARY

The objective of the study was to determine whether selected hematologic parameters measured on umbilical cord blood samples using an automated hematology analyzer (Sysmex XE-2100) were affected by (i) anticoagulant (the specimens were collected in EDTA *vs.* sodium heparin), (ii) temperature (the specimens were maintained at 4° C *vs.* room temperature for up to 72 h) and (iii) 1 : 5 dilution *vs.* undiluted using the manufacturer's diluting solution. Use of heparin, instead of EDTA, had little effect on the hematologic results ($n = 8$) except for lower platelet and progenitor cell counts. Results were remarkably stable for 72 h at either room temperature or 4° C except for modest red blood cell swelling at 24 h. Specimens of blood diluted at 1 : 5 had an immediate small, but significant change on white cell count (+13.3%), reticulocyte count (−11.2%) and reticulocyte hemoglobin content (−19.6%). Diluted samples did not change further over 4 h at room temperature. With a 1 : 5 dilution, analysis of 40 μ l of cord blood stored for 3 days at room temperature may provide useful hematologic information with little phlebotomy loss.

Keywords

Neonate; reticulocytes; red blood cell parameters; automated hematology analyzer; heparin; EDTA

INTRODUCTION

Phlebotomy blood loss for clinical laboratory testing in the neonatal intensive care unit (NICU) is a major contributor to anemia and the resultant red blood cell (RBC) transfusions administered to critically ill, very low birth weight (VLBW) infants (Lin *et al.*, 2000; Madan *et al.*, 2005; Widness *et al.*, 2005). Indeed, reduction in the volume of blood drawn for laboratory testing has been shown to reduce the number of red blood cell (RBC) transfusions (Madan *et al.*, 2005; Widness *et al.*, 2005). Therefore, the development of laboratory methodologies that require smaller blood volumes for clinical and research testing is an important objective for improving neonatal care by preventing anemia and thereby avoiding the risks and cost associated with RBC transfusion.

Hematology analyzers utilizing flow cytometry are routinely used to provide rapid, reliable and accurate determination of hematology parameters in the clinical setting. Generally, blood samples are collected in tubes containing the recommended anticoagulant,

ethylenediaminetetraacetic acid (EDTA), and sent to the laboratory for immediate analysis. In the setting of the neonatal intensive care unit (NICU), 250–500 µl samples of whole blood are typically required for hematologic testing. In the NICU setting, blood collected for clinical laboratory testing (i) is often drawn in heparin or anticoagulants other than EDTA and (ii) is often drawn in volumes that are in excess of that needed. If properly saved and labeled, the limited volumes of leftover anticoagulated whole blood ordered for clinically indicated purposes could be retrieved, diluted and analyzed to yield valuable clinical and/or research data without the need for drawing additional blood. Available samples are commonly collected in sodium heparin containing tubes for analysis of blood gases, electrolytes and other blood chemistries.

Previous studies in adults have demonstrated the stability of many hematologic parameters over time (Paxton & Bendele, 1993; Peng *et al.*, 2001; deBaca *et al.*, 2006; Hedberg & Lehto, 2008) and have documented which parameters are affected when sodium heparin is used in place of EDTA (Paxton & Bendele, 1993; Peng *et al.*, 2001). None of the studies have investigated these effects on fetal or neonatal blood, nor have they investigated the effect of sample dilution on hematologic parameters. Furthermore, these effects have not been evaluated for the most recent hematologic variables analyzed with the newer, highly sophisticated hematology analyzers. Examples of these newer measurements include the hematopoietic progenitor cell (HPC) count, nucleated red blood cell (NRBC) count, immature reticulocyte fraction (IRF) and reticulocyte hemoglobin content (Ret He).

Therefore, using fetal umbilical cord blood as a surrogate for neonatal blood, the objective of the current study was to determine the effect of EDTA vs. sodium heparin on selected hematologic parameters kept at different storage temperatures for up to 72 h. We also sought to examine the effect of a 1 : 5 dilution of cord blood on hematologic parameters using the manufacturer's cellular diluting solution while maintaining samples at room temperature for 4 h. The findings of these studies have important clinical and research implications for critically ill, VLBW infants relative to reducing the volume of blood required for laboratory testing and the resultant number of RBC transfusions.

MATERIALS AND METHODS

Eight study subjects born after uncomplicated pregnancy, labor and delivery were enrolled. This included six term and two preterm (<37 weeks postmenstrual age) infants. All procedures were approved by the University of Iowa Human Subject Internal Review Board Committee and parental written consent was obtained for all study subjects.

Immediately after delivery of the placenta, fetal cord blood samples were collected in EDTA and sodium heparin containing Vacutainer® tubes (BD Vacutainer® sodium heparin, 143 USP Units, Reference #367874, and K₂ EDTA, 10.8 mg, Reference #367899, Franklin Lakes, NJ, USA). Only tubes that filled completely were included. Blood samples from individual collection tubes were split into twelve 0.5 ml aliquots and stored at room temperature (~20° C) or 4° C (six aliquots per storage temperature). Selected hematologic parameters were determined serially on individual aliquots over 72 h, the period of time over which stored, retrieved blood remaining from clinical analyses might commonly be available. Separate aliquots from each anticoagulant tube at the two temperatures were measured in manual mode at 0, 4, 8, 24, 48 and 72 h using the Sysmex XE-2100 automatic hematology analyzer (Sysmex Corporation, Kobe, Japan). The analyzer was calibrated and quality controlled according to the manufacturer's recommendations. Immediately after collection, aliquots from both the EDTA and heparin samples were diluted 1 : 5 with the cellular suspension medium CELLPACK (Sysmex Corporation). The diluted EDTA and heparin blood samples were aliquoted in five 0.2 ml volumes and stored at room temperature for up to 4 h, the estimated

period of time before diluted blood samples might commonly be analyzed. Aliquots of the 1 : 5 diluted blood samples were analyzed in capillary mode after 0, 0.5, 1, 2 and 4 h using the same hematology analyzer.

The effects of anticoagulant and dilution at time 0 were analyzed for the hematologic parameters using a linear mixed effects model, with subject as a random effect and anticoagulant, dilution and their interaction as fixed effects. The effects of anticoagulant and storage temperature over time for undiluted cord blood hematologic results were analyzed using a linear mixed effects repeated measures model, with subject as a random effect and anticoagulant, temperature, time and their interactions as fixed effects. The effect of anticoagulant over time on diluted cord blood was analyzed similarly using subject as a random effect, and using anticoagulant, time and their interactions as fixed effects.

The correlation between repeated measurements on the same subject for both diluted and undiluted blood was modeled as a Toeplitz (diagonal constant) variance–covariance matrix structure, based on the Akaike's Information Criterion (Akaike, 1974). The null hypothesis that $|\mu_1 - \mu_0| \leq 5\% \mu_0$ was tested against the alternative hypothesis that $|\mu_1 - \mu_0| > 5\% \mu_0$ using Student's *t*-test, where μ_1 is the mean value of the treatment combination of interest and μ_0 is the mean baseline value of the control treatment. A difference of larger than 5% from μ_0 was selected for the alternative hypothesis as smaller differences are not generally of clinical and/or biological relevance. For the effect of anticoagulant and dilution at time 0 analysis, the baseline control treatment was the mean value of the EDTA/undiluted/time 0 treatment for both the 4° C and room temperature samples. Similarly, for the effect of anticoagulant and storage temperature over time for undiluted blood analysis and for the effect of anticoagulant over time on diluted blood analysis, the baseline control treatments were the 'EDTA/4° C/undiluted/time 0 treatment' and 'EDTA/diluted/time 0 treatment', respectively. Statistically significant differences were determined at $\alpha = 0.05$ probability of type I error rate. Dunnett's *post hoc* adjustment was performed for multiple comparisons. All statistical analyses were conducted using SAS[®] for Windows (Version 9.1.3, Service Pack 4; SAS[®] Institute Inc., Cary, NC, USA).

RESULTS

Collection of cord blood with sodium heparin as the anticoagulant did not result in any statistically significant effects on any of hematologic parameters that were greater than 5% of the EDTA values of undiluted blood samples measured immediately after collection except for platelets, immature platelet fraction (IPF) and HPC (Figure 1). For undiluted samples, the platelet count was lower and the IPF was higher when heparin was used as the anticoagulant rather than EDTA. Similarly, dilution of fresh cord blood with CELLPACK did not affect any of the hematologic parameters more than 5% of the EDTA–undiluted blood parameters values except for reticulocyte count and Ret He (Figure 1). With dilution, reticulocyte count dropped from 214 ± 10.8 (mean $\pm$ standard error of the mean) to $190 \pm 8.88 \times 10^3$ cells/ μ l (–11.2%) and Ret He dropped from 32.6 ± 0.415 to 26.3 ± 0.510 (–19.6%), irrespective of the anticoagulant. Additionally, there was a statistically significant effect of dilution greater than 5% of the control treatment on white blood cell (WBC) counts when a statistical test was conducted across both diluted EDTA and heparin treatments simultaneously. However, the individual EDTA/diluted and heparin/diluted WBC count effects were not statistically greater than 5% of the control treatment. Unlike reticulocytes and Ret He, however, dilution with CELLPACK increased the WBC count across anticoagulants by 13.3%.

In general, the undiluted cord blood hematologic parameters changed little over the 72 h postcollection period irrespective of the storage temperature or the anticoagulant (Table 1). Exceptions to this were the WBC count, immature granulocyte (IG) count, HPC count, IPF,

mean corpuscular volume (MCV) (Figure 2c), platelets (Figure 2d) and Ret He. Other than platelets, IG count, IPF and MCV, none of these changes were statistically greater than 5% of the EDTA/4° C/undiluted/time 0 h value until after 24 h after blood collection. While the 5% difference limit seems large relative to quality control variances for hematologic parameters, in the NICU even greater variances are observed for capillary blood sampling relative to venous or arterial sampling (Linderkamp *et al.*, 1977; Rivera, 1982). For all hematologic parameters evaluated other than HPC count, whole blood collected into EDTA tubes and stored at 4° C did not change more than 5% of the time 0 h value through 72 h of storage. When blood was collected into heparin containing tubes, storage at room temperature was superior to storage at 4° C after 48 to 72 h for WBC count, IG count, IPF and Ret He. Conversely, for MCV storage at 4° C was superior to storage at room temperature over time irrespective of anticoagulant. The RBC and reticulocyte count did not change statistically over time, irrespective of the anticoagulant or storage temperature (Figure 2a,b, respectively). In contrast, MCV slowly increased over time with either EDTA or heparin as anticoagulants when stored at room temperature.

Following 1 : 5 dilution in CELLPACK, there were no statistically significant changes in the hematologic parameters studied which were greater than 5% of mean EDTA/diluted/time 0 h value over the 4 h study period when stored at room temperature (Table 2). As noted above, the only statistically significant effect was that of heparin as the anticoagulant on platelets.

DISCUSSION

In this study, we demonstrate that the Sysmex XE-2100 hematology analyzer is capable of reducing physician-ordered laboratory blood loss by nearly an order of magnitude (from 250–500 µl commonly requested in by hospital laboratories to 40 µl while providing hematologic results for the same expansive group of analytes. In using umbilical cord blood at delivery as a surrogate for neonatal blood, we also demonstrated that with few exceptions that anticoagulated whole blood results are unaffected by (i) storage for up to 72 h at 4° C, (ii) storage for up to 24 h room temperature, after which cell swelling occurs as indicated by greater MCV and (iii) the use heparin over EDTA, the recommended anticoagulant for hematologic parameters, except for lower platelet and HPC counts and increases in IPF with heparin. The findings of this study have potentially important clinical and research implications for reducing iatrogenic phlebotomy loss in the critical care setting without sacrificing either the accuracy or the scope of laboratory test results.

As technology advances, the capability of chemistry and hematology analyzers used in clinical settings continues to expand while the sample volumes required by these instruments continue to decrease. In the intensive care setting, an important consequence of these technologic advances is less iatrogenic blood loss. As a result, patients are less prone to develop anemia and require fewer RBC transfusions. Despite these technologic advances, even greater progress is needed, particularly in the NICU setting where iatrogenic blood loss and resultant anemia among VLBW infants remains as the primary reason for RBC transfusions (Lin *et al.*, 2000; Madan *et al.*, 2005; Widness *et al.*, 2005).

For measurement of all hematologic parameters, EDTA is the recommended anticoagulant (Haematology, 1993) Nonetheless, we found that analysis of cord blood immediately after collection using heparin as the anticoagulant had no effect on the primary hematologic parameters other than lower platelet counts (Figure 1). Similar findings have been reported for hematologic parameters in adults (Reinhart *et al.*, 1990; Kawamoto *et al.*, 2000). The effect of heparin on lowering progenitor cell counts and increasing IPF has not been previously reported in adults perhaps because both are relatively recent reported additions of some hematologic analyzers.

While dilution of fresh cord blood with CELLPACK initially affected only reticulocyte count, Ret He and WBC count (Figure 1), hematology parameters did not vary further over 4 h at room temperature following dilution (Table 2). This indicates that anticoagulated whole blood samples do not need to be analyzed immediately following dilution to obtain accurate results. Despite the effects of dilution on these hematology parameters, the ability to analyze hematologic parameters with as little as 40 μ l of whole blood when using a 1 : 5 dilution and analysis in capillary mode on the Sysmex XE-2100 instrument offers the advantages of markedly decreasing phlebotomy loss and of allowing capillary blood draws as a reliable alternative to venous sampling (2005). If similar laboratory findings are demonstrated in adults, these features may also be of importance for the elderly in nursing homes and for critically ill, VLBW premature infants, who by virtue of their extremely small size and need for frequent laboratory monitoring, quickly become clinically anemic to the extent that they require frequent RBC transfusions in the early weeks of life (Lin *et al.*, 2000; Madan *et al.*, 2005; Widness *et al.*, 2005). Recent studies by our group in VLBW infants have demonstrated that reducing the volume of blood drawn for laboratory testing may reduce the number of RBC transfusions (Madan *et al.*, 2005; Widness *et al.*, 2005).

In the NICU setting, blood collected for clinical laboratory testing is (i) often drawn in heparin or anticoagulants other than EDTA and (ii) often drawn in volumes in excess of that needed. Hence, the reduced blood volume needed when using dilutions and capillary mode allows for substantially reduced blood volumes to be collected and combined with the stability of the hematologic values over several days (Tables 1 and 2) permits 'leftover' anticoagulated blood from other routine clinical tests to be retrieved, diluted and analyzed for clinical and/or research purposes. As in the critical care setting, a majority of samples are collected in heparin, this provides a rich source of data without incurring additional blood loss.

Though most hematologic parameters were stable over 72 h, the MCV increase indicated cell swelling over time when undiluted blood was stored at room temperature; however, it did not change when stored at 4° C (Figure 2c). The temperature-dependent swelling in RBC for both EDTA and heparin is consistent with previous reports in humans (Brittin *et al.*, 1969; Paxton & Bendele, 1993; deBaca *et al.*, 2006; Hedberg & Lehto, 2008) and dogs at room temperature (Medaille, Briend-Marchal & Braun, 2006). Both the undiluted RBC and reticulocyte counts did not substantially change over 72 h regardless of anticoagulant and storage temperature (Table 1 and Figure 2a,b, respectively). The stability of reticulocytes is consistent with other reports using Sysmex instrumentation demonstrating that reticulocytes drawn in EDTA from adults are stable for 48 h when stored at either room temperature or 4° C (Peng *et al.*, 2001; deBaca *et al.*, 2006). Other reports have demonstrated reticulocyte stability between 1 and 3 days when stored at 4° C or 24° C (Perry *et al.*, 1996). Of note, in our study, key clinical hematologic parameters including hemoglobin concentration, mean corpuscular hemoglobin (MCH), RBC distribution width (RDW), mean platelet volume (MPV), IRF and NRBC were also not affected by anticoagulant, storage temperature or storage time. Our results of RDW, MPV, IRF and NRBC are in variance from those of deBaca *et al.* (2006), who have reported significant changes/variability in these parameters upon storage of EDTA-anticoagulated blood at room temperature for over 24 h. The observed variances may, however, be attributed to the differences in sample size (8 vs. 40) and specimen type (normal vs. normal and abnormal) utilized in the two studies. Recently, Hedberg & Lehto (2008) reported changes in RDW at 48 and 72 h of EDTA samples stored at room temperature (but not in IRF).

Not surprisingly, some of the newer hematologic parameters that have inherently low cell numbers were more variable than the more 'classical' parameters. In particular, the IG, HPC and NRBC count were substantially more variable (Figure 1). Accordingly, detecting changes in the mean value using heparin over time required larger percent changes (Tables 1 and 2).

Given the high variability of these parameters, caution is required when interpreting change over time, particularly when heparin is used as the anticoagulant.

In conclusion, we have demonstrated that with its capability of utilizing 40 µl anticoagulated whole blood samples diluted 1 : 5 with CELLPACK, the Sysmex XE-2100 hematology analyzer is capable of reducing iatrogenic laboratory blood loss by nearly an order of magnitude (from 250–500 µl to 40 µl). The results provided include nearly the same expansive panel hematologic determinations as determined on undiluted whole blood samples. Using umbilical cord blood at delivery as a surrogate for neonatal blood, we also demonstrated that the use of sodium heparin instead of EDTA as anticoagulant has little effect on the measured hematologic parameters of cord blood other than lowering platelet and HPC counts and increasing IPF. In general, the primary hematologic parameters were stable over 72 h at both room temperature and 4° C storage temperatures, except for swelling of RBCs after 24 h of room temperature storage. However, dilution of cord blood 1 : 5 in CELL-PACK had a significant initial effect on WBC count, reticulocyte count and reticulocyte hemoglobin amount values. Of note, after dilution with CELL-PACK, no further changes in any measured hematology values were observed through 4 h of storage at room temperature. The stability of the hematologic parameters over time and the ability to reduce the blood volume required for hematologic testing has important clinical and research implications for improving patient care by reducing anemia and the need for and the risks associated with RBC transfusions. This is particularly true of VLBW premature infants for whom iatrogenic blood loss remains as the primary cause of symptomatic anemia.

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Abbreviations

EDTA	ethylenediaminetetraacetic acid
Hgb	hemoglobin
HPC	hematopoietic progenitor cells
IG	immature granulocytes
IPF	immature platelet fraction
IRF	immature reticulocyte fraction
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MPV	mean platelet volume
NICU	neonatal intensive care unit
NRBC	nucleated red blood cell
RBC	red blood cell
RDW	red blood cell distribution width
Ret He	reticulocyte mean corpuscular hemoglobin
VLBW	very low birth weight

WBC white blood cell

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Figure 1.

Mean hematologic values immediately after collection normalized by the mean EDTA/undiluted/time 0 h sample value. Horizontal line indicates the normalized value of 1.0 and bars represent the normalized standard error ($n = 8$). Hematologic parameters missing for the diluted samples are not reported by the Sysmex XE-2100 automatic hematology analyzer when operated in capillary (i.e. diluted) mode.

Figure 2. Mean RBC (a), reticulocytes (b), MCV (c) and platelets (d) vs. time for EDTA - 4° C (—●—), EDTA - room temperature (—○—), heparin - 4° C (—▲—) and heparin - room temperature (—△—) - undiluted treatments. Bars represent the standard error (n = 8).

Table 1

Mean undiluted cord blood hematologic parameter results by anticoagulant, storage temperature and duration of storage ($n = 8$)

Variable	Anticoagulant	Temperature	Time (h)						
			0	4	8	24	48	72	
WBC (/ μ l)	EDTA	4° C	9418	9464	9604	9894	9943	9725	
		RT	9619	9533	9480	9544	9751	10084	
	Heparin	4° C	9110	9219	8904	8645	7991	7511*	
IG (/ μ l)	EDTA	RT	9003	9036	9166	9131	8970	9238	
		4° C	178	188	203	175	156	135	
	Heparin	RT	205	198	180	179	188	129	
HPC (/ μ l)	EDTA	4° C	179	213	296	370*	416*	431*	
		RT	183	168	164	164	176	199	
	Heparin	4° C	9.14	15.1	12.6	14.0	16.0	20.3*	
Platelets (10^3 / μ l)	EDTA	RT	5.43	10.7	10.0	11.7	12.0	12.0	
		4° C	2.14	2.57	1.00	2.00	4.29	4.14	
	Heparin	RT	2.43	1.00	0.71	0.43	0.43	0.43	
MPV (fl)	EDTA	4° C	271	244	243	240	250	255	
		RT	275	282	282	279	278	277	
	Heparin	4° C	198*	49.8*	60.9*	53.4*	72.0*	85.9*	
IPF (%)	EDTA	RT	191*	171*	180*	161*	204*	199*	
		4° C	10.2	10.4	10.5	10.4	10.7	10.6	
	Heparin	RT	10.2	10.5	10.4	10.6	10.8	10.9	
Hgb (g/dl)	EDTA	4° C	10.0	10.4	10.4	10.5	10.8	11.2	
		RT	9.7	10.3	10.3	10.4	9.9	10.1	
	Heparin	4° C	3.00	3.74	3.65	4.19	4.06	4.30	
IPF (%)	EDTA	RT	3.20	3.26	3.74	3.74	4.45	4.99	
		4° C	4.63	16.1*	13.8*	14.8*	16.1*	18.0*	
	Heparin	RT	4.51	7.75	7.56	8.39	5.60	6.64	
Hgb (g/dl)	EDTA	4° C	15.9	15.9	15.8	16.0	15.9	15.9	
		RT	15.8	15.8	15.7	16.0	15.9	16.0	
	Heparin	4° C	15.4	15.4	15.3	15.5	15.6	15.6	

Variable	Anticoagulant	Temperature	Time (h)					
			0	4	8	24	48	72
RBC ($10^6/\mu\text{l}$)	EDTA	RT	15.4	15.4	15.4	15.4	15.5	15.0
		4° C	4.32	4.32	4.32	4.34	4.35	4.33
	Heparin	RT	4.31	4.27	4.28	4.28	4.29	4.28
		4° C	4.19	4.21	4.20	4.24	4.26	4.26
MCV (fl)	EDTA	RT	4.23	4.22	4.19	4.23	4.21	4.20
		4° C	108	108	108	108	109	110
	Heparin	RT	108	110	111	116*	121*	123*
		4° C	106	106	106	107	108	108
RDW (%)	EDTA	RT	106	107	108	114	119*	121*
		4° C	16.8	16.7	16.6	16.6	16.4	16.3
	Heparin	RT	16.8	16.8	16.9	17.3	17.4	17.4
		4° C	16.6	16.6	16.5	16.3	16.2	16.1
MCH (pg)	EDTA	RT	16.6	16.6	16.6	17.1	17.1	17.0
		4° C	36.9	36.7	36.7	36.7	36.6	36.7
	Heparin	RT	36.7	36.9	36.7	37.3	37.1	37.3
		4° C	36.8	36.6	36.4	36.7	36.5	36.6
Ret. He (pg)	EDTA	RT	36.4	36.6	36.6	36.5	36.9	35.6
		4° C	32.3	31.6	31.6	32.6	34.4	35.1
	Heparin	RT	32.4	33.1	33.2	33.7	34.3	34.7
		4° C	32.8	32.5	32.6	33.7	35.3*	35.7*
Ret. ($10^3/\mu\text{l}$)	EDTA	RT	33.1	33.7	33.9	34.4	34.4	34.1
		4° C	216	214	214	217	217	210
	Heparin	RT	215	215	217	219	220	226
		4° C	213	215	216	223	233	237
IRF (%)	EDTA	RT	212	214	212	217	216	241
		4° C	37.9	37.9	37.9	38.3	39.5	39.0
	Heparin	RT	40.1	39.7	41.4	39.0	40.1	39.6
		4° C	37.2	37.4	38.4	38.1	36.9	36.6
NRBC (μl)	EDTA	RT	38.0	37.3	38.6	38.0	36.6	34.5
		4° C	960	956	995	1011	1078	1048

Variable	Anticoagulant	Temperature	Time (h)				
			0	4	8	24	48
		RT	973	953	998	901	895
	Heparin	4° C	868	926	961	943	901
		RT	878	886	855	856	864
							851

RT, room temperature.

* Absolute value of the difference from the mean EDTA/4°C/undiluted/time 0 h treatment significantly greater than 5% ($P < 0.05$).

Table 2Mean diluted cord blood hematologic parameter results by anticoagulant and duration of storage ($n = 8$)

Variable	Anticoagulant	Storage period (h)				
		0	0.5	1	2	4
WBC (/ μ l)	EDTA	10846	10896	10908	10898	11020
	Heparin	10216	10196	10335	10310	10334
Platelets (10^3 / μ l)	EDTA	304	306	306	300	311
	Heparin	229*	222*	226*	228*	229*
IPF (%)	EDTA	4.48	5.04	4.46	4.39	4.73
	Heparin	5.40	5.09	4.74	5.05	4.95
Hgb (g/dl)	EDTA	15.8	15.8	15.8	15.7	15.8
	Heparin	15.6	15.5	15.4	15.5	15.5
RBC (10^6 / μ l)	EDTA	4.33	4.35	4.35	4.32	4.34
	Heparin	4.25	4.24	4.21	4.27	4.24
MCV (fl)	EDTA	110	110	110	110	110
	Heparin	109	109	108	108	109
MCH (pg)	EDTA	36.6	36.4	36.3	36.4	36.3
	Heparin	36.7	36.6	36.6	36.4	36.6
Ret He (pg)	EDTA	26.1	26.5	26.6	27.7	28.2
	Heparin	26.2	26.5	26.9	27.3	28.1
Reticulocytes (10^3 / μ l)	EDTA	188	196	196	191	201
	Heparin	190	188	192	199	196
IFR (%)	EDTA	40.8	44.1	41.1	42.3	40.4
	Heparin	41.8	42.4	42.6	40.8	44.0

* Absolute value of the difference from the mean EDTA/diluted/time 0 h treatment significantly greater than 5% ($P < 0.05$).