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Effects of Storage Conditions on Complete Blood Cell Count Parameters

[Saklama Şartlarının Tam Kan Sayım Parametrelerine Etkisi]

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ABSTRACT

Purpose: Delineating the changes occuring in various parameters of automated complete blood cell count of normal and abnormal specimens due to prolonged storage at different conditions.

Methods: The study was conducted in Ankara Numune Education and Research Hospital Emergency Biochemistry Laboratory. 117 randomly selected K_2EDTA anticoagulated blood specimens were processed through the Coulter Gen S hematology analyzer, before and after 24 and 48 h storage at room temperature and +4°C. Among the 117 specimens; 48 were normal, 14 were leukopenic, 27 had leukocytosis, 19 were thrombocytopenic and 9 had thrombocytosis.

Results: Room temperature storage caused MCV and MPV increase in all normal and pathalogical specimens. At 48 hours, Htc and RDW were increased in all specimens except the ones with leukocytosis. MCHC was decreased in all but leukopenic ones. Refrigerated storage caused a decline in WBC and platelet counts of normal specimens. MPV was increased in the specimens with leukocytosis whereas MCHC was decreased in leukopenic ones. Specimens with thrombocytosis and thrombocytopenia were stable up to 48 hours at + 4°C.

Conclusion: In CBC measurements, it is preferred to analyze the specimens in a short time after venipuncture. The only stable parameters seem to be RBCs and hemoglobin if the measurements are carried out following a delay.

Key words: Hematology, blood cell count, refrigeration

ÖZET

Amaç: Ankara Numune Eğitim ve Araştırma Hastanesi Acil Biyokimya Laboratuarı'nda analiz edilen normal ve patolojik hemogram kanlarının, farklı saklama koşullarının tam kan sayım parametreleri üzerindeki değişimlerini saptamak.

Gereç ve Yöntemler: Çalışma Ankara Numune Eğitim ve Araştırma Hastanesi Acil Biyokimya Laboratuarı'nda yapılmıştır. Rastgele seçilmiş 117 K_2EDTA'lı kan örneği, oda sıcaklığında ve +4°C'de buzdolabında 2 gün saklanarak, gelir gelmez, 24 saat ve 48 saat sonra Coulter Gen S hemogram cihazında çalışılmıştır. 117 kan örneğinden 48'i normal, 14'ü lökopenik, 27'si lökositoz, 19'u trombositopenik, 9'u trombositoz'lu idi.

Bulgular: Oda sıcaklığında bekletilen normal ve patolojik tüm kan örneklerinde MCV ve MPV artmıştır. Ayrıca ikinci gün, lökositozlu örnekler haricinde tüm örneklerde Htc ve RDW artmış, lökopenik örnekler haricinde ise MCHC azalmıştır. Buzdolabında bekletme ile normal kan örneklerinde WBC ve trombosit sayısı azalmış olup patolojik kan örneklerinden lökositozlu olanlarda MPV artışı, lökopenik kan örneklerinde ise MCHC azalması görülmüştür. Trombositozlu ve trombositopenik kan örnekleri buzdolabında bekletildiğinde iki gün stabilitesini korumuştur.

Sonuç: Tam kan sayım analizinde tercih edilen kanın alımından kısa bir süre sonra ölçülmesidir. Eğer ölçümlerde bir gecikme olursa sadece eritrosit ve hemoglobin stabil parametreler olarak görülmektedir.

Anahtar kelimeler: Hematoloji, tam kan sayımı, soğutma

Introduction

Laboratory practices carried out by centralized laboratories make it critical to know the specimen storage conditions well. Transport of collected blood specimens to centralized laboratories can lead to a delay in testing for several hours. Cellular elements are known to have a limited stability in blood containing ethylenediamine tetraacetate (EDTA) [1]. Excessive delays in processing, however, might compromise the reliability of results. Limited number of studies have been conducted with all the currently used hematology analyzers for the effects of storage on analysis [2-4]. Delays in analysis may have caused different results in both normal and pathological specimens [2].

Manufacturers of automated analyzers and published literature often state that blood specimens, kept at either room temperature or at +4°C (refrigerated) for up to 24 hours, generally reveal reliable results for Complete Blood Cell Count (CBC) [2-4]. However, these studies may not be satisfactory as the high variety of analyzers used are considered. Besides, no consensus was reached about which paremeters can still be reliable in delays over 24 hours when the analyzers were out of order due to different reasons [2-4,6].

In order to decide whether to accept or reject the aged specimen, laboratory staff needs to be familiar with the changes known to occur in blood specimens during storage [3].

In the present study, Beckman Coulter Gen–S (COULTER Corp, Miami, USA) hematology analyzer was used in order to compare the stability of normal and pathological blood specimens collected in K_2EDTA tubes and stored at +4°C and +25°C up to 48 hours and evaluated if there is an advantage of storing at +4°C.

Materials and Methods

A total of 117 randomly selected K_2EDTA anticoagulated blood specimens were processed through the Coulter Gen S hematology analyzer at Ankara Numune Education and Research Hospital Emergency Biochemistry Laboratories. Two specimens were taken from each patient into two separate tubes. Among the 117 specimens; 48 of them were normal, 14 of them were leukopenic (white cell count, $<4.0 \times 10^3/\mu L$), 27 of them had leukocytosis (white cell count, $>11.0 \times 10^3/\mu L$), 19 of them were thrombocytopenic (platelet count, $<140 \times 10^3/\mu L$) and 9 of them had thrombocytosis (platelet count, $>400 \times 10^3/\mu L$). Blood specimens were kept at either room temperature or in refrigerator prior to analysis up to 48 hours.

Each sample was drawn into 4 mL capacity K_2EDTA BD Vacutainer tubes. Each sample was analyzed at time point of <20 min (baseline measurement). After this initial measurement, each sample was divided into two separate tubes; and stored at room temperature or at +4°C throughout the study for stability evaluations. All specimens were re-analyzed at 24 and 48 hours. The specimens stored at +4°C were allowed to equilibrate at room temperature for 30 minutes prior to analysis. Room temperatures were measured with a standard indoor thermometer and temperature follow-up charts were daily filled as a part of quality control standards. The range of temperature during the study was 21 ± 3°C.

According to the recommendations of the International Committee of Haematology Standardization; the maximized storage intervals for CBC and differential count were 6 hours at 18-22°C and 24 hours at 2-6°C [5]. With the guidance of these information, it is known that no change occurs in the first 6 hours. Actually, we carried out the study up to 72 and 96 hours. But we neither splitted the specimens to aliquotes in different tubes nor made a specimen pool. So, the device could not make proper readings due to some inadequate specimen volumes. The number of specimens with no measured value at these hours were high enough to affect our statistical evaluations. For that reason we excluded these time intervals from the study.

The Coulter Gen-S uses impedance technology so as to generate a 12-part CBC and volume (impedance), conductivity, and light scatter measurements, commonly referred to as VCS technology, to generate a 5-part differential. In addition; it gives the percent and absolute number of reticulocytes making a total of 24 parameters. However, we did not carry out reticulocyte count in this study. Because this device could not make a reticulocyte count. The Software version used in the study was 4A5. Calibration and quality control of the analyzer were performed using S-cal and 3 levels of 5C control (both from Beckman Coulter), respectively according to the manufacturer's instructions. External Quality Control was carried out with Bio-Rad Laboratories EQAS system. (External Quality Assurance Services). External quality control reports are among the goals of performance.

Table 1 illustrates the reference values and within-run precision values for the CBC parameters used in this study. Within-subject and between-subject CV values of analytes and desirable analytical quality specifications for total error are shown (Table 2) [7].

Statistical analysis

The groups were compared for each parameter and percent changes were calculated. The values of the initial measurements were taken into account for comparisons. The percent change value obtained for each parameter was compared with the pre-determined imprecision value calculated for individual biological variation. The allowable total error (TE_a) was accepted as the target value for imprecision. Between groups differences were evaluated with paired Student's T test. Values of p less than 0.05 were considered to be significant.

Table 1. Range of initial automated CBC and differential of specimens included in the study

Parameter	Range	Mean	%*
WBC, x10^9/L	4.4-11.3	9	2.13
NE, %	45.5-73.1	59.8	1.81
LY, %	18.3-44.2	22.6	5.13
MO, %	2.6-13	9.05	3.36
EO, %	0.00-7.00	8.5	6.14
BA, %	0.00-1.2	0.2	22.36
NE, x10^9/L	1.31-6.71	5.4	3.56
LY, x10^9/L	0.9-3.22	2	4.47
MO, x10^9/L	0.12-1.13	0.8	5.59
EO, x10^9/L	0.00-0.700	0.75	7.30
BA, x10^9/L	0.00-1.2	0.05	4.25
RBC, x10^{12}/L	4.1-5.1	5.35	1.21
Hemoglobin, g/dL	12.3-15.3	16.05	0.71
Hematocrit, %	36-45	47.15	0.45
MCV, fL	80-97	88.05	1.53
MCH, pg	27.5-33.2	30.05	1 .22
MCHC , g/dL	32.0-36.0	34.1	0.74
RDW-CV, %	11.5-14.5	14.9	2.79
Platelet, x10^9/L	150-450	210.5	2.75
MPV, fL	7.0-12.0	10.5	1.78

* within-run precision values from analyses of medium samples

Table 2. Within-subject and between-subject CV values of analytes and desirable analytical quality specifications for total error

Parameter	CV_i	CV_g	TE_a
WBC, x10^9/L	10.9	19.6	14.6
NE, x10^9/L	16.1	32.8	22.4
LY, x10^9/L	10.4	27.8	16
MO, x10^9/L	17.8	49.8	27.9
EO, x10^9/L	21	76.4	37.1
BA, x10^9/L	28	54.8	38.5
RBC, x10^{12}/L	3.2	6.1	4.4
Hemoglobin, g/dL	2.8	6.6	4.1
Hematocrit, %	2.8	6.4	4.1
MCV, fL	1.3	4.8	2.3
MCH, pg	1.6	5.2	2.7
MCHC, g/dL	1.7	2.8	2.2
RDW-CV, %	3.5	5.7	4.6
Platelet, x10^9/L	9.1	21.9	13.4
MPV, fL	4.3	8.1	5.8

CV_i: : within-subject biological variation
CV_g : between-subject biological variation
TE_a: allowable total error

Results

Among the normal specimens stored at room temperature; WBC, RBC, hemoglobin and platelets were relatively stable; the MCV, RDW and MPV each increased over time at day 1 and day 2; Htc increased at day 2 while MCHC descended over time; each change was statistically significant ($p<0.05$). When the specimens stored at room temperature were compared with regard to target value dependent on biological variation; obtained percent change values were higher than the target values determined for imprecision for only RDW in day 1, and Htc, MCV, RDW and MPV in day 2 (Table 3). Among the WBC differentials; the number of monocytes tended to decrease over time both statistically and with regard to biological variation. (especially at day 2). The 95% CIs for the monocyte number ranged between 0.0373 to 0.2585 throughout the 2 –day period. The ranges for other WBC differential counts were from -0.2996 to 0.0954 for the number of neutrophils, from – 0.1283 to 0.1366 for the number of lymphocytes, from – 0.0215 to 0.0173 for the number of eosinophils, and from – 0.0140 to 0.0265 for the number of basophils.

The same specimens stored at +4^{o}C exhibited lower mean WBC and platelet count at day 1 and day 2. MCV was stable for the first day, but an increase was observed at day 2. MPV and MCH were increased ($p<0.05$). When compared with biological variation values, only the changes in WBC and MPV in day 2 were higher than the predetermined imprecision values. From the results of the differential count; a downward trend was observed for lymphocytes and neutrophiles at day 2. (Table 4). The 95% CIs during the 2 –day period ranged from 1.264 to 2.372 for the number of neutrophils. The ranges for other WBC differentials were from - 0.1965 to 0.3507 for the number of monocytes, from – 0.0685 to 0.2481 for the number of lymphocytes, from – 0.0029 to 0.0279 for the number of eosinophils, from – 0.0011 to 0.0364 for the number of basophils. Higher values for neutrophiles at day 1 and for basophiles at day 1 and day 2 were detected according to biological variation.

Among the pathological specimens; MCV and MPV increased at day 1 and 2 when bloods having leukocytosis were kept at room temperature. MCHC was decreased. An increase was detected in Htc and MPV at day 1 and day 2; and in MCV, MCHC and RDW at day 2 in reference to biological variation values. Since the refrigerator stored counterpart of the same specimens, only MPV increased at day 1 and 2 ($p<0.05$). Among the samples with leukocytosis kept at refrigerator Htc, MCV, MCHC, RDW ve MPV were elevated when evaluated due to biological variation values (Table 5). In leukopenic specimens kept at room temperature, MCV, RDW, Htc and MPV increased at day 2, MPV at day 1 and RDW at day 1 and day 2 increased according to biological variation but in leukopenic specimes kept at +4^{o}C ; only MCHC was decreased at day 2 ($p<0.05$), RBC, HGB, Htc, MCHC and Plt were increased at day 2, MPV was increased at day 1 with regard to biological variation (Table 6).

Table 3. Mean percent changes induced by storage of normal specimens at room temperature and refrigerated

	Refrigerated									Room Temperature							
Normal (n= 48)	Fresh	24 h	Change (%)	CI(95%)		48 h	Change (%)	CI(95%)		24h	Change (%)	CI(95%)		48h	Change (%)	CI(95%)	
				Lower	Upper			Lower	Upper			Lower	Upper			Lower	Upper
WBC (x 10^9/L)	7.7	7.6*	1.30	0.005	0.219	5.70*	25.97^	1.549	2.575	7.7	0	-0.052	0.069	7.7	0	-0.042	0.126
RBC (10^{12}/L)	4.73	4.71	0.42	-0.006	0.033	4.72	0.21	0.012	-0.033	4.68	1.06	-0.034	0.129	4.71	0.42	0.003	0.030
HGB (g/dL)	13.6	13.6	0.00	-0.123	-0.018	13.70	-0.74	-0.123	-0.031	13.5	0.74	-0.198	0.264	13.6	0.00	-0.071	0.000
HCT (%)	40.6	40.7	-0.25	-0.277	0.181	40.80	-0.49	-0.464	0.048	41.1	-1.23	-1.304	0.300	42.3	-4.19^	-1.909	-1.432
MCV (fL)	85.9	85.6	0.35	-0.884	1.405	86.50*	-0.70	-0.940	-0.221	87.8*	-2.21	-2.337	-1.516	89.8*	-4.54^	-4.316	-3.513
MCH (pg)	28.8	29*	-0.69	-0.309	-0.111	29*	-0.69	-0.310	-0.106	29	-0.69	-0.307	-5.131	28.9	-0.35	-0.285	-0.064
MCHC (g/dL)	33.4	33.5	-0.30	-0.323	0.040	33.40	0.00	-0.209	0.172	32.9*	1.50	0.276	0.677	32.1*	3.89^	1.057	1.463
RDW (%)	13.9	13.8	0.72	-0.024	0.145	13.70	1.44	0.013	0.228	14.8*	-6.47^	-1.073	-0.697	15.5*	-11.51^	-1.824	-1.426
PLT (x 10^9/L)	257	247*	3.89	4.841	15.82	225*	12.45	22.601	42.066	258	-0.39	-3.676	2.218	258	-0.39	-3.777	2.694
MPV (fL)	8.8	9.3*	-5.68	-0.670	-0.383	9.60*	-9.09^	-1.114	-0.569	9.3*	-5.68	-0.394	-7.230	10*	-13.64^	-1.424	-1.100

* p<0.05 significance level

^ the ones with the percent change values higher than the target imprecision values when compared with the (TE_a) for biological variation

Table 4. Mean percent changes in WBC automated differential count with storage

NORMAL (n= 48)	Refrigerated					Room Temperature			
	Fresh	24 h	CHANGE (%)	48 h	CHANGE (%)	24 h	CHANGE (%)	48 h	CHANGE (%)
NE (x 10^9/L)	5.03	4.96	-1.39	3.215*	-36.18^	5.05	0.39	5.13	1.98
LY (x 10^9/L)	2.07	2.10	1.44	1.921*	-7.24	2.07	-0.28	2.07	-0.19
MO (x 10^9/L)	0.5	0.427	-16	0.423	-16	0.46	-8	0.352*	-30^
EO (x 10^9/L)	0.14	0.158	7.14	0.127	-14.28	0.148	5.71	0.142	1.42
BA (x 10^9/L)	0.029	0.013	-55.17^	0.01	-65.51^	0.029	0	0.023	-20.6

* p<0.05 significance level

^ the ones with the percent change values higher than the target imprecision values when compared with the (TE_a) for biological variation

• Counts are given as absolute count: the change is the percentage of change

Table 5. Mean percent changes induced by storage of specimens having leukocytosis at room temperature and +4°C

	Refrigerated								Room Temperature							
L .cytosis (n=27)	Fresh	24 h	Change (%)	CI(95%)		48 h	Change (%)	CI(95%)		24h	Change (%)	CI(95%)		48h	Change (%)	CI(95%)	
				Lower	Upper			Lower	Upper			Lower	Upper			Lower	Upper
WBC (x 109/L)	16.6	16.32	1.69	-0.157	1.572	14.67	11.63	-1.198	5.1838	16.20	2.41	-0.283	1.195	16.70	-0.60	-1.114	0.744
RBC (1012/L)	4.51	4.57	-1.33	-0.228	0.373	4.41	2.22	-0.232	0.4350	4.56	-1.11	-0.202	0.078	4.57	-1.33	-0.195	0.087
HGB (g/dL)	12.69	13.08	-3.07	-0.552	0.623	12.64	0.39	-0.740	1.2368	13.10	-3.23	-0.561	0.139	13.07	-2.99	-0.490	0.246
HCT (%)	38.13	41.21	-8.08^	-3.835	0.299	40.19	-5.40^	-4.832	1.6177	39.80	-4.38^	-2.440	-0.507	40.88*	-7.21^	-3.435	-1.460
MCV (fL)	85.37	90.1*	-5.54^	-5.942	-3.485	90.97*	-6.56^	-7.042	-4.1725	87.16*	-2.10	-2.815	-1.480	89.52*	-4.86^	-5.185	-3.688
MCH (pg)	28.55	28.63	-0.28	-0.211	0.204	29.09	-1.89	-1.307	0.2334	28.70	-0.53	-0.243	0.043	28.63	-0.28	-0.149	0.216
MCHC (g/dL)	33.36	31.72	4.92^	1.282	2.282	31.79	4.71^	0.639	2.5010	32.86	1.50	0.449	0.935	31.90*	4.38^	1.372	2.004
RDW (%)	15.07	16.15	-7.17^	-1.793	-0.363	16.06	-6.57^	-1.753	-0.2241	15.8	0.00	-1.647	-0.071	16.50	-9.49^	-2.219	-0.662
PLT (x 109/L)	292	283	3.08	-2.918	37.918	252	13.70^	-17.11	96.965	283	3.08	-1.929	19.114	289	1.03	-5.431	7.283
MPV (fL)	8.23	9.37	-13.85^	-1.436	-0.841	9.33	-13.37^	-1.383	-0.8685	8.94*	-8.63^	-0.951	-0.552	9.68*	-17.62^	-1.904	-1.236

* $p<0.05$ significance level

^ the ones with the percent change values higher than the target imprecision values when compared with the (TE_a) for biological variation

Table 6. Mean percent changes induced by storage of specimens having leukopenic at room temperature and +4°C

	Refrigerated									Room Temperature							
L.copenic (n=14)	Fresh	24 h	Change (%)	CI(95%)		48 h	Change (%)	CI(95%)		24h	Change (%)	CI(95%)		48h	Change (%)	**CI(95%)**	
				Lower	Upper			Lower	Upper			Lower	Upper			Lower	Upper
WBC (x 10^9/L)	2.02	1.91	5.45	0.031	0.195	1.80	10.89	-0.255	0.712	1.84	8.91	-0.038	0.409	2.02	0.00	-0.090	-0.090
RBC (10^{12}/L)	2.9	2.91	-0.34	-0.022	0.009	2.59	10.69^	-0.150	0.777	2.90	0.00	-0.010	0.011	2.90	0.00	-0.025	0.029
HGB (g/dL)	8.6	8.64	-0.47	-0.109	0.024	7.64	11.16^	-0.570	2.484	8.60	0.00	-0.064	0.050	8.67	-0.81	-0.163	0.006
HCT (%)	25.16	25.55	-1.55	-0.889	0.104	22.60	10.17^	-2.410	7.539	25.36	-0.79	-0.467	0.067	25.88*	-2.86	-1.154	-0.288
MCV (fL)	88.12	88.71	-0.67	-2.044	0.858	88.92	-0.91	-2.980	1.380	88.55	-0.49	-1.025	0.168	90.25*	-2.42	-3.161	-1.096
MCH (pg)	30.47	30.45	0.07	-0.274	0.316	30.85	-1.25	-1.310	0.553	30.38	0.30	-0.089	0.274	31.38	-2.99	-2.487	0.672
MCHC (g/dL)	34.47	34.22	0.73	0.201	0.687	30.85*	10.50^	-1.416	0.945	34.20	0.78	-0.023	0.566	33.88	1.71	0.200	0.970
RDW (%)	16.13	16.4	-1.67	-0.812	0.269	15.82	1.92	-0.772	1.401	16.47	-2.11	-0.630	-0.055	17.32*	-7.38^	-1.695	-5.128
PLT (x 10^9/L)	96	96	0.00	-3.468	2.896	83.00	13.54^	-16.21	41.786	99.00	-3.13	-7.032	0.603	98.00	-2.08	-5.432	2.003
MPV (fL)	8.12	8.68	-6.90^	-0.942	-0.186	8.52	-4.93	-1.304	0.489	8.71	-7.27^	-1.005	-0.179	9.35*	-15.15^	-1.588	-0.868

* $p<0.05$ significance level

^ the ones with the percent change values higher than the target imprecision values when compared with the (TE_a) for biological variation

In specimens with thrombocytosis, storage at room temperature caused an increase in Htc, MCV, RDW, MPV and a decrease in MCHC ($p<0.05$). MCV, MCHC, RDW were increased at day 2 and MPV was increased at day 1 and day 2 according to biological variation. No significant changes were observed when same specimens were refrigerated. When the specimens were kept at refrigerator; WBC, RBC, HGB, Hct, MCH, MCHC, Plt and MPV were increased at day 2 (Table 7). In thrombocytopenic specimens room temperature storage caused an increase in Htc, MCV, RDW ve MPV at day 2 ($p <0.05$), MCHC was decreased. At days 1 and 2 in RDW, and at day 2 MCHC and MPV were increased due to biological variation, but refrigerated storage showed correlation with the initial measurements. In these specimens only MPV at day 1 was increased biologically (Table 8).

Among the pathological specimens; results of the differential count were not significant changes. So their results were not shown in the tables.

Discussion

According to the recommendations of the Committee of International Haematology Standardization; Hematology analyzer manufacturers often quote that blood specimens kept at either at room temperature or at +4 ^{o}C up to 24 hours generally reveal reliable results for complete blood count and automated differential count [5]. The recent trend towards large centralized laboratories have brought some obligations about the transfer and storage times of the blood specimens properly. Laboratories now test specimens that have been dispatched over a long distance; and the testing is often delayed by several hours or in some cases for days [1,3].

Refrigerated storage of anticoagulated blood has been noted to improve the stability of CBC. However, the number of systematic reviews including all the commonly used hematology analyzers and patological specimens along with normal ones is limited [4,8]. The studies of Wood et al. with Cell-Dyne 3500, Hedberg et al. with Cell-Dyne Saphire and Warner et al. with Coulter STKS have all claimed that refrigerated storage improves stability with these analyzers [3,8,9]. However; the results of the study of Gulati et al. with Coulter Gen S analyzer have shown that stability of normal and pathological specimens at room temperature can be acceptable with some limitations, but the same can not be claimed for the differential count [2]. In this study, we also used Coulter Gen S analyzer and investigated if there were any advantages of refrigerated storage over room temperature storage.

The evaluation of the study results was not only compared statistically but according to the biological variations of defined analytical goals as well. Each of the analytes within subject CV and between subject CV results and desirable analytical quality spesifications for total error values are given in Table 2.

The parameters measured directly like WBC, RBC, hemoglobin and platelet remained stable for 2 days at room temperature as stated earlier in the study by Gulati et al. The degree of change observed in WBC count of both normal and pathological specimens throughout the study could be considered acceptable for clinical applications. However, a significant decrease in WBC and platelet count was observed when the same specimens were kept at + 4^{o}C . This downward trend was prevented by using impedance technology instead of optical measurement in the study of Wood et al [8]. We did not have the chance to compare optical and impedance measurement techniques as the Beckman Coulter Gen S analyzer uses only impedance technology. However; we used the impedance method of Coulter Gen S to analyze the specimens stored at +4^{o}C; and concluded that the decrease can not be prevented both for biological variation and statistically.

Unexpectedly we obtained negative percentage change in HTC and positive percentage change in MCV in normal specimens. But the situation which was found at the first 24 hours period measurement was not significant for both biological variation and statistically (Table 3). This unsignificant change is considered as the calculations of Htc and MCV are in within run presicion values.

The increase in MCV in the specimens stored at room temperature reflects the swelling of RBCs. As also indicated by Wood et al. this increase is largely prevented by refrigerated storage [8]. In our study; we also concluded that the increase in MCV at room temperature was significant both biologically and statistically; whereas in refrigerated storage the increase in MCV was significant statistically while the target imprecision values for biological variation could not be passed. An increase in MPV is also observed besides MCV. But the increase in MPV was over the biological variation limits in specimens kept at refrigerator.

The decrease in MCHC is caused by hematocrit increase when the hemoglobin level is still stable. As it is commonly known, MCHC is a parameter calculated by the ratio of hemoglobin to hematocrit multiplied by 100. Changes in the components of this equation will also lead to a change in MCHC value. But the changes detected in MCHC were not higher than the biological variation target values.

In our study, it is obviously seen that especially the storage of pathological specimens at +4^{o}C improves the stability when compared to normal specimens. However, we argue that this result is due to the higher number of studies with normal specimens than the pathological ones. As a result, we decided to increase the number of pathological specimens so as to make the changes more meaningful.

In CBC measurements, it is preferred that the specimens are analyzed in a short time after venipuncture. The only stable parameters seem to be RBCs and hemoglobin if

Table 7. Mean percent changes induced by storage of specimens having thrombocytosis at room temperature and +4°C

	Refrigerated									Room Temperature							
T.cytosis (n=19)	Fresh	24 h	Change (%)	CI(95%)		48 h	Change (%)	CI(95%)		24h	Change (%)	CI(95%)		48h	Change (%)	CI(95%)	
				Lower	Upper			Lower	Upper			Lower	Upper			Lower	Upper
WBC (x 10^9/L)	11.53	11.28	2.17	-0.016	0.505	6.26	45.71^	-5.287	15.821	11.64	-0.95	-0.289	0.066	11.33	1.73	-0.219	0.619
RBC (10^{12}/L)	4.01	4.05	-1.00	-0.076	0.003	3.58	10.72^	-0.579	1.439	4.01	0.00	-0.023	0.023	4.00	0.25	-0.016	0.034
HGB (g/dL)	10.77	10.91	-1.30	-0.199	-0.066	9.53	11.51^	-1.828	4.317	10.81	-0.37	-0.099	0.033	10.80	-0.28	-0.073	0.029
HCT (%)	32.88	33.6	-2.19	-1.955	0.532	29.38	10.64^	-6.163	13.163	33.08	-0.61	-0.457	0.057	34.11*	-3.74	-1.686	-0.758
MCV (fL)	83.66	84.88	-1.46	-4.486	2.042	84.80	-1.36	-5.026	2.759	84.17	-0.61	-1.253	0.231	87.00*	-3.99^	-4.601	-2.065
MCH (pg)	27.58	27.6	-0.07	-0.233	0.211	28.93	-4.89^	-4.175	1.486	27.63	-0.18	-0.316	0.227	27.70	-0.44	-0.321	0.076
MCHC (g/dL)	32.78	32.46	0.98	-0.981	1.625	33.58	-2.44^	-3.845	2.245	32.66	0.37	-0.157	0.401	31.70	3.29^	0.591	1.586
RDW (%)	16.92	17.24	-1.89	-1.140	0.496	16.91	0.06	-1.200	1.222	17.14	-1.30	-0.602	0.157	18.23*	-7.74^	-1.732	-0.889
PLT (x 10^9/L)	541	522	3.51	-0.167	37.94	452	16.45^	-103.99	283.10	540	0.18	-10.415	13.526	544	-0.55	-16.20	9.765
MPV (fL)	7.22	8.15	-12.88^	-1.890	0.023	8.42	-16.62^	-2.615	0.215	7.86	-8.86^	-0.753	-0.535	8.55*	-18.42^	-1.756	-0.910

* $p<0.05$ significance level

^ the ones with the percent change values higher than the target imprecision values when compared with the (TE_a) for biological variation

Table 8. Mean percent changes induced by storage of specimens having thrombocytopenic at room temperature and +4°C

	Refrigerated									Room Temperature							
T.cytopeni (n=19)	Fresh	24 h	Change (%)	CI(95%)		48 h	Change (%)	CI(95%)		24h	Change (%)	CI(95%)		48h	Change (%)	CI(95%)	
				Lower	Upper			Lower	Upper			Lower	Upper			Lower	Upper
WBC (x 109/L)	6.25	5.99	4.16	0.093	0.432	6.25	0.00	-0.304	0.304	5.76	7.84	-0.196	1.186	6.14	1.76	-0.041	0.273
RBC (1012/L)	3.52	3.5	0.57	-0.006	0.038	3.41	3.13	-0.087	0.300	3.51	0.28	-0.006	0.019	3.51	0.28	-0.001	0.027
HGB (g/dL)	10.58	10.61	-0.28	-0.082	0.040	10.34	2.27	-0.416	0.911	10.60	-0.19	-0.059	0.027	10.62	-0.38	-0.077	0.014
HCT (%)	31.32	32.31	-3.16	-1.614	-0.375	31.75	-1.37	-2.616	1.753	31.74	-1.34	-0.763	-0.078	32.33*	-3.22	-1.478	-0.552
MCV (fL)	89.21	89.76	-0.62	-4.372	-0.732	90.19	-1.10	-5.234	-0.723	90.24	-1.15	-1.802	-0.250	92.03*	-3.16	-3.876	-1.765
MCH (pg)	30.54	30.63	-0.29	-0.281	0.102	31.21	-2.19	-1.588	0.251	30.52	0.07	0.119	0.151	30.63	-0.29	-0.293	0.103
MCHC (g/dL)	34.18	33.43	2.19	0.258	1.257	33.68	1.46	-0.501	1.511	33.81	1.08	0.112	0.634	33.31*	2.55^	0.493	1.252
RDW (%)	15.67	16.61	-6.00	-1.542	-0.349	16.06	-2.49	-1.331	0.534	16.49	-5.23^	-1.190	-0.459	17.23*	-9.96^	-2.090	-1.032
PLT (x 109/L)	69	69	0.00	-3.201	4.043	69.00	0.00	-8.200	9.147	71.00	-2.90	-4.943	1.785	70.00	-1.45	-3.309	2.361
MPV (fL)	8.65	9.24	-6.82^	-1.082	-0.096	9.00	-4.05	-1.007	0.302	9.01	-4.16	-0.752	0.036	9.65*	-11.56^	-1.478	-0.531

* $p<0.05$ significance level

^ the ones with the percent change values higher than the target imprecision values when compared with the (TE_a) for biological variation

the measurements are carried out following a delay especially for the evaluations we made with the normal specimens. For other parameters; delayed processing affects stability. The delays and the possible effect of these delays on the results should be indicated in the reports. However, in case of necessity; for Coulter Gen-S analyzer; it can be concluded that it is possible to maintain the stability of specimens by refrigerated storage up to two days with some limitations.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Lawrence AC, Bevington JM, Young M. (1975) Storage of blood and the mean corpuscular volume. J Clin Pathol. 28:345-9.

[2] Gulati GL, Hyland LJ, Kocher W, Schwarting R. (2002) Changes in automated complete blood cell count and differential leukocyte count results induced by storage of blood at room temperature. Arch Pathol Lab Med. 126: 336-42.

[3] Hedberg P, Lehto T. (2009) Aging stability of complete blood count and white blood cell differential parameters analyzed by Abbott CELL-DYN Sapphire hematology analyzer. Int J Lab Hematol. 31: 87-96.

[4] Buttarello M. (2004) Quality specification in haematology: the automated blood cell count. Clin Chim Acta. 346: 45-54.

[5] International Council for Standardization of Haemotology (1993). Recommendations of the International Council for Standardization in Haemotology for ethylendiamine-tetraacetic acid anticoagulation of blood for blood cell counting and sizing. Am J Clin Pathol. 100:371-2.

[6] Picard F, Gicquel C, Marnet L, Guesnu M, Levy JP. (1999) Preliminary evaluation of the new hematology analyzer COULTER GEN-S in a university hospital. Clin Chem Lab Med. 37(6):681-6.

[7] Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M. (1999) Current databases on biologic variation: pros, cons and progress. Scand J Clin Lab Invest. 59:491-500.

[8] Wood BL, Andrews J, Miller S, Sabath DE. (1999) Refrigerated storage improves the stability of the complete blood cell count and automated differential. Am J Clin Pathol. 112(5):687-95.

[9] Warner BA, Reardon DM. (1991) A field evaluation of the Coulter STKS. Am J Clin Pathol. 95(2):207-17.