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The effect of preanalytical mechanical mixing time on complete blood cell count parameters in the emergency laboratory

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Abstract
Complete blood count (CBC) is one of the most common laboratory tests. Preanalytical factors like specimen homogenization affect the CBC test results. In the emergency laboratory, test time is a ciritcal parameter. Optimization of test procedures is of critical importance to give more accurate results in a shorter time period. This study was designed to evaluate the effects of preanalytical mechanical mixing times on CBC parameters and to optimize the mechanical mixing time for the analysis. Mechanical mixing time of 1 and 5 minutes (t= 1 and t=5 respectively) with a rotary type mixer and automatic mixing performed by the Coulter LH 780 (t=0) prior to complete blood count analysis were evaluated. Between t=0 and t=1 minutes of mechanical mixing, only a significant change in MPV values were obtained (p<0,05) but the change was not significant in terms of biological variation. In the comparison of t=0 and t=5 minutes of mechanical mixing;statistically significant changes in red blood cell (RBC), neutrophile, monocyte, and mean platelet volume (MPV) parameters were observed (p< 0,05).The changes were not significant with regard to biological variation. Mean corpsucular volume (MCV) values were different among t=0 and t=5 minutes with respect to biological variation but no statistically significant difference was observed. Automated mixing alone and additional mechanical mixing times of 1 and 5 minutes all yielded several results mostly within the biological variation limits. As shorter test completion times are desired in the emergency laboratory, we can conclude that extra mechanical mixnig times give no significant advantage in CBC analysis.

Keywords: Complete blood count, preanalytical factors, mechanical mixing, emergency laboratory

Introduction

Complete blood cell count (CBC) analysis is the most frequent test carried out in laboratories worldwide. CBC is often used as a broad screening test to determine an individual's general health status. It helps to diagnose various conditions, such as anemia, infection, inflammation, bruising, bleeding disorders or leukemia, acute hemorrhagic states, allergies and it is also crucial in monitoring the condition and/or effectiveness of treatment after a diagnosis is established [1]. A very important advantage of CBC analysis is it's short working time (approximately 10 minutes). Besides this, there is no need for fasting and a rational amount of specimen is needed fort he procedure. The widespread use of hematology analyzers (HA) has led to major improvement of cellular hematology, because of quick and accurate results found in most instances, and now preanalytical and analytical variables should be considered first within the laboratory when spurious results from the HA are found [2]. Preanalytical factors like venipuncture, collection of inadequate volumes of blood and storage conditions are among the most common factors that affect the CBC results.

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For its's quickness, accuracy and giving perspective about a large group of ilnesses, CBC is among the most ordered test especially in emergency services. In the emergency laboratory, analysis time and preanalytical delay is of much importance because delays in analysis may cause different results in both normal and pathological blood specimens.

Homogeneity of sample achieved by adequate mixing of the blood is a key factor in maintaining the quality of analytical results. Mixing of the sample upon collection is necessary to insure that the anticoaguant in the tube is dissolved well in blood to prevent formation of blood clots. Prior to analysis, blood homogenization is generally achieved in the laboratories with mechanical mixers [3].

In the present study, Beckman Coulter LH-780 (Coulter Corp, Miami,USA) hematology analyzer was used in order to compare the effect of preanalytical rolling times on normal blood specimens collected in K_2EDTA tubes with an external rotary-type mixer for 1 minute, 5 minutes and as a control, automated mixing provided by the analyzer itself, accepted as t= 0 minutes.

Materials and Methods

A total of 40 randomly selected, K_2EDTA anticoagulated blood specimens were alalyzed with Beckman Coulter LH-780 hematology analyzer at Ankara Numune Training and

Research Hospital Emergency Biochemistry Laboratory. Three specimens were collected from each patient to three seperate tubes to avoid volume changes and repetitive measurements from the same sample. No pathological specimens were included in the study. Blood specimens were analyzed after being processed in rotary type mixer for 1 minutes and 5 minutes prior to analysis. No additional mixing was applied as the control measurement with only inversion of 5 times performed by the autoanalyzer itself (t=1, t=5 and t=0 respectively). All analyses were made within the same run. Each sample was drawn into 4 mL capacity K_2EDTA BD Vacutainer tubes. analyzed at time point of < 20 minutes after collection (baseline measurement).

The Coulter LH-780 analyzer uses volume, conductivity and scatter (VCS) technology and generates 12-part CBC and a 5-part differential count. We did not carry out reticulocyte count because the device could not make a reticulocyte count. In this study we evaluated only the measured parameters, calculated parameters are not included in the evaluation. Calibration and quality control of the device were performed using S-cal and 2 levels of 5C control. External Quality Control was carried out with RIQAS system. Within subject and between subject CV values and quality specifications for total allowable error are shown in table 1 [4]. In this study we evaluated only the measured parameters, calculated parameters are not included in the evaluation.

Statistical Analysis

The groups were compared for each parameter and percent changes were calculated. All three groups were compared with each other. The ones with no additional mixing (t= 0) was accepted as base value becuase the hematology analyzer mixes the tubes by inverting 5 times prior to analysis.The percent change values obtained were compared with the individual biological variation values. Allowable total error (TE_a) was accepted as the target imprecision value. Between group differences were evaluated with paired Student's T test. $p < 0,05$ was considered to be statistically significant for all groups. Clinical variation was expressed as the percent change in gro up means.

Table 1. Within-subject and between-subject Cv values of analytes and analytical quality specifications for total error

Parameter	CV_i	CV_g	TE_a
WBC, $x10^9$/L	11.4	21.3	15.5
NE, $x10^9$/L	17.1	32.8	23.4
LY, $x10^9$/L	10.2	35.3	17.6
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	6.3	4.4
Hemoglobin, g/dL	2.9	6.8	4.2
Hematocrit, %	2.7	6.4	4
MCV, fL	1.4	4.9	2.4
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

Table 2. Mean percent changes in specimens related to rotating time

parameter	t=0	t=1	Change (%)	CI (%95) lower	CI (%95) upper	t=5	Change (%)	CI (%95) lower	CI (%95) upper
WBC, x10^9/L	7.55	7.55	0	-0,111	0,111	7.62	-0,90	-0,155	0,030
NE,x10^9/L	4,70	4,74	-0,85	-0,105	0,020	4,83*	-2,76	-0,198	0,030
LY,x10^9/L	2,17	2,14	1,38	-0,015	0,799	2,16	0,46	-0,039	0,059
MO, x10^9/L	0,53	0,51	3,90	-0,075	0,043	0,45*	15,09	0,045	0,105
EO, x10^9/L	0,18	0,16	11,1	-0,004	0,029	0,18	0	-0,013	0,013
BA,x10^9/L	0,02	0,01	50^	-0,011	0,036	0,02	0	-0,021	0,026
RBC, x10^{12}/L	4.91	4.92	-0,20	-0,231	0,001	4.93*	-0,40	-0,025	-0,023
Hemoglobin, g/dL	13.90	13.89	0,08	-0,033	0,058	13.91	-0,07	-0,064	0,040
Hematocrit, %	41.11	41.21	-0,24	-0,228	0,003	41.18	-0,17	-0,214	0,059
MCV, fL	83.85	83.84	0,01	-0,150	0,165	103.77	-23,70^	-60,393	20,554
Platelet, x10^9/L	238.38	241.5	-1,32	-6,416	0,066	240.08	0,71	-4,225	0,825
MPV, fL	8.52	8.71*	-2,2	-0,272	-0,098	8.84*	-3,7	-0,414	-0,216

t= rotating time in minutes
* $p < 0,05$ significance level
^ the ones with the percent change values higher than the target imprecision values when compared with the TE_a for biological variation

Results

The results of the 40 normal specimens with different preanalytical mixing rolling times are summarized in table 2. Among the 40 specimens included in the study, most CBC parameters appear to be similar between groups.

When the values for t=0 and t=1 groups were compared, only MPV values seemed to differ statistically ($p < 0,05$). The 95% CIs for MPV ranged between -0,272 and -0,098. In the comparisons based on biological variation, the basophile count was changed 50%, which was over the target value for imprecision, where CVi was 28 and TEa was 37.1.

In the analysis and comparison between t=0 and t=5 specimens, differences were determined in neutrophile, monocyte, RBC and MPV parameters ($p< 0,05$). The 95% Cs for neutrophiles were -0,198 and 0,030; for monocytes 0,045 and 0,105, for RBC count -0,025 and -0,023 and for MPV -0,414 and -0,216. When compared according to biological variation, the change in MCV values was -23,70%, which was over the predetermined biological imprecision limits of CV_i 1.4 and TE_a 2.4.

Discussion

Preanalytical factors are an important source of variation in clinical laboratory measurements. The process from sample collection to analysis includes many steps in each of which there can be factors affecting analytical phase. There are some preanalytical aspects which are usually neglected but they affect analytical results. For example fasting and tourniquet application times. Hematological tests are interfered from high amounts of lipids and chlymoicrons. High concentrations of triglycerides were reported to interfere with hemoglobin measuruments in Coulter instruments [5]. In hematology analysis, counts of cells and particles are performed in whole anticoagulated blood. Although it is not possible to analyse the blood samples fron fasting patients in the emergency laboratories, patients suffering from genetic or acquired hypertriglyceridemia should be noticed when evaluating hemoglobin values. Tourniquet application is another preanalytical factor affecting CBC parameters as extended tourniquet times cause elevations in hematocrit and hemoglobin counts [6]. Mixing procedure is of much importance in whole blood analysis. There are several studies examining the preanalytical mixing phase for complete blood count

analysis. In the study of Lippi et al., different mixing procedures for K2 EDTA samples on haematological testing was evaluated and revealed the data that when compared with the reference species inverted 6 times, results of unmixed specimens revealed significant decreases in RBC count, haemoglobin, hematocrit and platelet count, whereas the mean platelet volume (MPV) was significantly increased [7,8].

Prior to analysis, complete homogenization of sample is effected in mpst laboratories by using a variety of mechanical mixers. There are two types of mechanical mixers as rocking versus rotary type mixers. The disadvantage of rocking type mixers is that they need a sufficient headspace available for mixing Therefore, if the blood collection tube is completely filled, mixing to homogeneity is prevented [3]. In one study, the hemoglobin value had doubled compared to the value obtained before, ant the WBC and PLT counts were significantly reduced. But after the analysis was repeated with the same specimen several times, the values became comparable to the results obtained a week ago. This was explained by the authors as removal of aliquots from the sample for analysis caused a volume depletion and a sufficient headspace was created for the rocking mixer to perform better [9].

Rotary type mixers ara analytically more favorable than rocking type mixers. In the study of Neville, hemoglobin measures after manuel inversions by the nurse and using a rotary-type mixer in the laboratory were compared. The same Point-of-care (POC) device was used in nurses station and in the laboratory for hemoglobin detection. When the results were compared with the laboratory's automated CBC analyzer; a good correlation between autoanalyzer the POC used in the laboratory with rotary-type mixer was obtained, where as there was a poor correlation with the POC at nurses station and autoanalyzer. These results showed that only manuel inversions give non-uniform and improper mixing and rotary type mixer enhances the homogenization of samples for analysis [10].

For the hematology analyzers of Beckman Coluter inc, the analyzer itself mixes the sample by inverting it prior to analysis for 5-6 times. In our laboratory, there is a rotary type mixer which is used prior to analysis to obtained better results especially in the differential count, the usage of which for 5 minutes is recommended by Beckman Coulter technical service. But since this is the emergency laboratory, giving accurate test results in a short period of time is crucial. That's why in this study, we evaluated te mixing time of blood specimens prir to analysis if it is really necessary. We choose rotating times of 1 minute,5 minutes (which we routinely apply) and no extra mechanical mixing. In one study, K_2EDTA tubes were processed under different procedures as inverting 5 times as the gold Standard and no inversion applied. Results of the study revealed significant differences in red blood cell count and hematocrit parameters [11]. In another study by Ashenden et al.; automated mixing by the instrument or 1 minute of mechanial mixing was compared and it was shown that manual inverisons were almost as same effective as the mixing provided by the mechanical mixers and in the same study, it was declared that 15 minutes of mechaniacl mixer using is exessive [12].

In our study, we evaluated the effect of mechanical rotary-type mixer on complete blood count results. In the light of the results from previous studies; we determined the mechanical mixing times as 1 minute, 5 minutes and 0 minutes (no additional mechanical mixing). In the routine procedure of our laboratory, we apply 5 minutes of mechanical mixing prior to analysis by a rotary type mixer. But since it is an emergency laboratory, we need to shorten working time. That's why we tried 0 and 1 minutes of mechanical mixing times.

Evaluation of the results showed only significant difference in MPV parameter between 0 and 1 minutes of mechanical mixing but the change was not significant with regard to biological variation. In contrast, the difference in basophile counts were over the TE_a limit. However, the change in mean values were not statistically significant. This may be due to the low degree of basophile counts. The measured values were close to each other as 0.01 and 0.02 respectively but when the % change is calculated, it gives a result of 50 %. Other parameters did not seem to be affected by the preanalytical mixing time.

When we compared the results between t=0 and t=5 minutes; neutrophile, monocyte, RBC and MPV parameters were not much comparable and the changes were statistically significant ($p<0,05$). But when compared according to biological variation, the changes were over the acceptable limits only in MCV values. The changes in neutrophile and monocyte counts again are due to lower values as in the case of basophiles. The changes in RBC and MPV may be caused by swelling of erythrocytes. In a compararive study, it was declared that PLT undergoes a spherical change yielding to a volume increase as the particle passes through an impedence based analyzer. As MPV is the index for platelet volume, it should be quantified at fixed time from the blood drawing to allow accurate inter and intraindividual comparisons [13]. This can be also the case in our study as Beckman Coulter devices also use volume-conductivity-scatter technology.

Overall evaluation of time periods of mixing revealed that 5 minutes of additional mixing caused changes in more parameters than mixing of 1 minute, when compared to no additional mechanical mixing. Five main parameters of the complete blood count known as WBC,RBC, Hemoglobin, hematocrit and platelet counts did dot seem to differ in three groups. However, the differential count seemed to be affected from mixing of 5 minutes, as the values for t=0

and t=1 minutes were consistent with each other. MCV value was over the TEa limit with regard to biological variation in t=5 minutes. This might be due to swelling of red blood cells due to excessive rolling time. But still, the difference in MCV value were in the acceptable limits statistically. Changes in MCV and MPV values were seen similarly to be changed in the study of Turhan et al., when specimens stored at room temperature, from which we can coclude that these two parameteres have the least stability among CBC parameters [14]. The study is conducted only in normal specimens. For more accurate results, larger study groups and pathological blood specimens need to be involved for future studies.

Conclusion

Results of this study showed that most parameters had not been affected from the mechanical mixing time but still the rolling time of 5 minutes does not provide an improvement in CBC parameters. It can be concluded that it is not compulsory to use mechanical mixers in the emergency laboratories prior to CBC analysis as it will cause a delay in test completion, which is very critical in the emergency service.

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Ethical considerations: The study is registered for the ethical approval of the Ethics Committee of Ankara Numune Training and Research Hospital with the file number 993.

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