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Article

Influence of preservation length of the sample on the performance of complete blood count (CBC) in rats

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Abstract: The performance of hematological tests deteriorates with the increase in the length of sample preservation. Therefore it has been an issue to characterize the maximum permissible period spent between blood collection and measurement to have the acceptable test report. From this view point, a study was undertaken to know about the effect of preservation length on complete blood count (CBC) in rat of Long Evans strain. A total of 30 samples were collected from 10 apparently healthy rats aged between 45-48 days and the blood samples were kept in commercial test tubes treated with EDTA. The test tubes containing whole blood samples were divided into three different groups based on preservation length and were allowed to keep at 4ºC for three different lengths of time viz. 2 hours, 4 hours and 6 hours until analysis. The samples were then analyzed for their complete blood count (TEC, TLC, Hb, PCV, DLC, Absolute Leukocyte Count, Red Cell Indices, RDW-SD, RDWCV, Platelet, MPV, PCT and PDW) using Sysmex XT-1800i auto hematological analyzer. Result showed that no significant change in CBC with the variation in preservation length. Based on these findings, it can be concluded that blood samples can be preserved for as long as 6 hours to have the same report obtainable when the samples are preserved at 4ºC in refrigerated condition for 2 or 4 hours.

Keywords: complete blood count (CBC); preservation length; EDTA; rat

1. Introduction

The determination of the effects of storage on complete blood count in different anticoagulant is an aspect of quality assurance. Quality assurance involves the application of all possible means to guarantee that the results reported by the laboratory are both reliable and valid. (Tatsumi *et al*., 2002). Excessive delay in processing blood samples for hematological testing could compromise the reliability of the result. (Baker and Silvertone, 2002). It is well known that prolonged storage could compromise red blood cell (RBC) properties, in particular storage condition could lead to metabolic depletion, disturbed ion homeostasis, protein and lipid modifications (e.g., oxidation, degradation, cross-linking) accompanied by alterations in intracellular hemoglobin concentrations (Ho *et al*., 2003). It is routinely recommended that hematological determinations on blood samples are carried out immediately after blood collection, and if not possible, the samples should be refrigerated until determination to minimize artifactual changes (Wood *et al*., 1999).The components necessary for blood storage are the anticoagulant and refrigerator. In order to maintain the blood in its fluid state anticoagulant must be added, such solution also provides capability and nutrients for cellular metabolism during storage (Hess *et al*., 2000).The choice of anticoagulant and storage time is of major importance when blood samples are to be used in laboratory analysis (Faggio *et al*., 2014). Potassium and sodium salts of EDTA are

commonly used anticoagulants for routine hematology determinations because they preserve the cellular components of blood (Buttarello, 2004). Hematological sample should be refrigerated at a temperature of 4ºC. This is found to favor optimal preservation of the blood and also prevents multiplication of any bacterial which might be present (Gulatie *et al*., 2002). More recently, found that the measurement of hemoglobin concentration and RBC count are stable up to 72 h after blood collection if blood is refrigerated at around 4ºC (Robinson *et al*., 2004; Voss *et al*., 2008; Robinson *et al*., 2011; Ashenden *et al*., 2013). However, the platelet count in specimens stored at 4 °C is considered stable for up to 24 or even 72 h (Goosens *et al*., 1991).There only few researches on laboratory animal have been reported. So this research work has been selected to carry out with the following objectives to know about the effects of different storage time on various parameters of complete blood count during storage of blood at 4ºC in EDTA.

2. Materials and Methods

The study was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh.

2.1. Experimental animals

A total of 15 rats of aged between 18-20 days were purchased from International Center for Diarrheal Disease Research, Bangladesh (icddr,b), Mohakhali, Dhaka and maintained in Physiology Laboratory with proper care.

2.2. Preparation of the experimental laboratory

The laboratory was cleaned and washed with disinfectant. All necessary equipment was set properly for proper handling and care of the animals.

2.3. Management practices

Rats were housed in rectangular wooden cages (9''×11''×7'') wrapped with wire mesh and cages were cleaned regularly. Commercial rat pellet was collected from icddr,b and supplied 3 times in a day and in order to prevent spoilage, feeds were kept in poly pack. Fresh drinking water was made available for 24 hours.

2.4. Experimental design

Ten (10) rats were used for the experiment. Total 30 blood collection tubes were taken where 3 tubes for each rat. The tubes were marked as 1.a, 1.b, 1.c for tube 1, 2.a, 2.b, 2.c for tube 2 and so on. The first batches of the tube e.g. 1.a, 2.a, 3.a to 10.a were analyzed immediate after collection. The second and third batches of the samples were subjected to preservation at 4ºC and analyzed after 2 and 4 hours of preservation respectively.

2.5. Blood sample collection

After acclimatization, rats were anesthetized with diethyl ether and blood samples were collected directly from heart. Immediately after collection, each sample was separated into 3 tubes (1.a, 1.b, 1.c,) containing K_3EDTA (Vacutest Kima SrlArzergrande-Italy) and proper mixing with the anticoagulant was ensured. The time between capture and blood sampling was less than 5 min. Then each and every samples were collected as same manner and sent to laboratory for the measurement of hematological parameters viz; RBC, WBC, Platelet count, DLC, Hb %, PCV value and measurement of MCV, MCH, MCHC. The first group (group A) of test tube (with EDTA) analyzed after 2 hours of collection. The second and third group (group B and group C) was stored at 4ºC to evaluate the effect of storage time and subjected to the same analytical procedure after every 2 hours of time interval. All samples were analyzed by Sysmex XT- 1800i Automated Hematology Analyzer.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the effect of various preservation lengths on blood parameters. Bonferroni's multiple comparison tests was applied for post-hoc comparison. All statistical analyses were performed using a commercial statistical program- SPSS.

3. Results and Discussion

3.1. Effects on total erythrocyte count and erythrocyte indices

The values of different blood parameters (TEC, Hb, Hct, MCV, MCH and MCHC) placed in Table 1 showed that no significant differences among them at different length of preservation (2h, 4h and 6h) of the samples in refrigerated condition at 4ºC. The present finding is in close agreement with the work reported by Cora *et al.*

(2012). The presented result also supports the report of Buttarello (2004) who reported that these parameters show stability for 24 to 72 h at 4ºC when CBC is performed.

3.2. Effects on total leukocyte count (TLC) and absolute count of its cell component

Table 2 showed that the absolute value of WBC and its component cell viz. neutrophil, lymphocyte, monocyte, eosinophil and basophil. These parameters showed no significant differences except monocyte till the maximum length of preservation (6h) in this study. This finding closely agrees to the findings of Faggio *et al.* (2012) who reported that up to 6h these parameters show stability at 4ºC. In this study monocytes showed a significant ($P<0.05$) decrease in number at after 6h of preservation which don't similar with the findings of Queen *et al*. (2014) who reported that monocytes count increases along with the increased length of preservation.

3.3. Effects on differential leukocyte count

Differential leukocyte counts after a variable length of preservation are presented in Table 3 and are expressed in percentage. The relative counts of different parameters showed no significant variation over the period of preservation in this study. The findings shows close agreement with the findings of Cora *et al.* (2012) who reported that the neutrophil and lymphocyte percentages were unchanged for at least 48 h at either temperature.

3.4. Effects on platelet count and its indices

The platelet count and MPV do not show any significant change during the following period of preservation (Table 4) which is agreeable with the finding of Lippi *et al.* (2005) but not agreeable with Cora *et al*. (2012) who reported that there is increase in platelet count after 6h of preservation. The PCT (%) value also showed no significant difference after the whole length of preservation at 4ºC in this study (Table 4).

Table 1. Effects of various preservation lengths on total erythrocyte count (TEC) and erythrocyte indices.

Parameters	**Mean ± SD (Range)**			**Level of significance**	**P value**
	T2	**T4**	**T6**		
RBC (M/µL)	6.11 ± 0.58 (4.89-6.66)	6.59 ± 0.25 (6.09-6.89)	6.38 ± 0.35 (5.99-6.94)	NS	1.00
Hb (g/dL)	13.43 ± 0.71 (12.34-14.50)	14.27 ± 0.42 (13.70-14.80)	13.75 ± 0.65 (12.90-14.70)	NS	0.39
Hct (%)	39.85 ± 3.82 (32.30-43.10)	43.07 ± 0.97 (41.90-44.50)	41.55 ± 2.35 (38.90-45.20)	NS	0.73
MCV (fL)	65.27 ± 1.91 (63.02-68.72)	65.37 ± 1.76 (63.62-68.82)	65.07 ± 1.97 (63.02-68.82)	NS	1.00
MCH (pg)	22.12 ± 1.44 (21.22-25.24)	21.67 ± 0.51 (20.92-22.52)	21.56 ± 0.58 (21.02-22.72)	NS	1.00
MCHC (g/dL)	33.76 ± 2.10 (31.40-38.14)	33.12 ± 0.38 (32.60-33.50)	33.12 ± 0.45 (32.30-33.80)	NS	1.00

SD= Standard Deviation, NS= Non Significant, T2= Two (2) hour after collection, T4=Four (4) hour of preservation, T6=Six (6) hour of preservation.

Table 2. Effects of various preservation lengths on total leukocyte count and absolute count of its cell component.

Parameters	Mean ± SD (Range)			Level of significance	P value
	T2	T4	T6		
WBC (K/µL)	10.31 ± 2.03 (8.29-13.57)	11.62 ± 1.72 (9.15-14.4)	10.32 ± 1.62 (8.51-13.63)	NS	0.57
Neutrophil (K/µL)	2.20 ± 0.37 (1.60-2.66)	2.43 ± 0.50 (1.73-3.24)	2.06 ± 0.39 (1.65-2.71)	NS	1.00
Lymphocyte (K/µL)	7.59 ± 1.84 (5.45-11.01)	8.77 ± 1.76 (6.37-12.06)	7.57± 1.99 (5.30-11.23)	NS	0.7
Monocyte (K/µL)	0.20 ± 0.05^{ab} (0.13-0.32)	0.28 ± 0.07^{a} (0.14-0.34)	0.17 ± 0.09^{b} (0.01-0.29)	*	0.04
Eosinophil (K/µL)	0.30 ± 0.26 (0.05-0.84)	0.12 ± 0.13 (0.04-0.42)	0.49 ± 0.82 (0.05-2.35)	NS	1.00
Basophils (K/µL)	0.014± 0.005 (0.01-0.02)	0.017 ± 0.004 (0.01-0.02)	0.015 ± 0.007 (0.01-0.03)	NS	1.00

SD= Standard Deviation, T2= Two (2) hour after collection, T4=Four (4) hour of preservation, T6=Six (6) hour of preservation.
**= Significant at P<0.01 NS= Non significant
*= Significant at P<0.05

Table 3. Effects of various preservation lengths on differential leukocyte count.

Parameters	Mean ± SD (Range)			Level of significance	P value
	T2	T4	T6		
Neutrophil (%)	21.94 ± 5.13 (15.54-31.24)	21.32 ± 5.26 (13.13-28.34)	20.54 ± 5.97 (14.34-31.84)	NS	1.00
Lymphocyte (%)	73.14 ± 5.10 (65.74-81.14)	74.98 ± 4.67 (69.54-83.72)	72.76± 10.44 (53.24-82.34)	NS	1.00
Monocyte (%)	1.96 ± 0.51 (1.34-2.94)	2.45 ± 0.57 (1.54-3.24)	1.64 ± 0.80 (0.14-2.64)	NS	0.08
Eosinophil (%)	2.81 ± 2.22 (0.54-6.84)	1.10 ± 1.17 (0.44-3.74)	4.88 ± 8.29 (0.54-23.54)	NS	1.00
Basophils (%)	0.14± 0 (0.14-0.14)	0.14 ± 0 (0.14-0.14)	0.16 ± 0.04 (0.14-0.24)	NS	0.22

SD= Standard Deviation, T2= Two (2) hour after collection, T4=Four (4) hour of preservation, T6=Six (6) hour of preservation.
**= Significant at P<0.01 NS= Non significant
*= Significant at P<0.05

Table 4. Effects on platelet count and its indices.

Parameters	Mean ± SD (Range)			Level of significance	P value
	T2	T4	T6		
Platelet (K/µL)	299.14 ± 364.76 (1.00-777)	466.14 ± 312.21 (42-911)	462 ± 310.07 (21-789)	NS	1.00
MPV (fL)	5.77± 3.95 (00-8.44)	7.01 ± 3.10 (00-8.64)	5.97± 4.09 (00-8.64)	NS	1.00
PCT (%)	0.66 ± 0.74 (00-2.19)	0.37 ± 0.25 (00-0.73)	0.38 ± 0.27 (00-0.66)	NS	0.8

SD= Standard Deviation, T2= Two (2) hour after collection, T4=Four (4) hour of preservation, T6=Six (6) hour of preservation.
**= Significant at P<0.01 NS= Non significant
*= Significant at P<0.05

4. Conclusions

It was observed that blood samples preserved in anticoagulant (EDTA) for up to 6h shows no significant changes in Total Erythrocyte Count (TEC) and its indices e.g. Hb, PCV, MCV, MCH and MCHC from its baseline values. The results showed no significant variation in different length of preservation (T2, T4 and T6) in WBC count and in the counts of its component cells (neutrophil, lymphocyte, eosinophil, basophil) except in monocyte count. There was no significant change observed in differential leukocyte count (DLC).This study also showed no differences in platelet count and its indices (MPV, PCT) at different time period of preservation. The overall results of this investigation indicated that the CBC test performance of blood samples preserved for as long as 6 hours does not show any significant difference with that obtained from the samples preserved either for 2 or 4 hours at 4ºC in refrigerated condition. However, this study is merely an initiative in the field of hematological aspect of rat blood. This requires a more and detailed investigation to ascertain the confident findings and to know the effects of storage length on complete blood count in rat blood.

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Conflict of interest

None to declare.

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