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To study the effect of dilution of normal saline with EDTA – Anticoagulated blood samples on CBC parameters

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Abstract
Background: The most common pre-analytical error in hematology laboratory is inadequate & clotted blood samples. This is particularly significant in pediatric hospitals, as it is difficult to draw the venous blood from dehydrated children. Inadequate blood samples & insufficient anticoagulants according to the manufacturers data and current Clinical and Laboratory Standards Institute, results in erroneous haematology results.
Methodology: CBC results of the diluted blood samples (normal saline) & undiluted blood samples were compared. All the CBC parameters were same before & after dilution.
Conclusion- Inadequate blood samples give erroneous results. If normal saline can be added in equal parts to EDTA blood in vacutainer the CBC results are almost the same. So it can be safely concluded that instead of discarding the inadequate blood samples it can utilized by adding equal parts of normal saline which will not alter the result outcome.

Keywords: CBC, EDTA, Red cell indices, WBC, differential count, hemoglobin

1. Introduction
The most common pre-analytical error in hematology lab is inadequate & clotted blood samples. This is particularly significant in pediatric hospitals, as it is difficult to draw the venous blood from dehydrated children. Proper specimen collection is the first step in ensuring accurate and reliable result from clinical laboratory. Incorrect anticoagulant & sample volume ratio has been identified as one of the pre-analytical factors that are responsible for non-reproducible hematology analyses, especially complete blood counts (CBC) including leukocyte differential counts [1].
According to recent Clinical and Laboratory Standard Institute document, procedure for the handling and processing of blood specimens, the amount of additives placed into a tube is intended for a certain volume of blood. If less than the required blood volume is drawn, the excess amount of additives has the potential to adversely affect the accuracy of test result [2, 3].
Both standards are applied to all samples containers with different anticoagulants including ethylene diamine tetra-acetic acid (EDTA). It is important that the manufacturer instructions are precisely followed so as to ensure maximum and minimum allowable fill volumes and correct anticoagulant to specimen volume ratio [4, 5]

The main objective of this study is
- Comparative analysis of variation of CBC parameters in diluted and undiluted blood samples.
- Assess the effectiveness of using normal saline as an alternate in inadequate blood samples

To the best of authors knowledge this is probably one of the pioneering study of this unique and interesting topic.

Methodology
This study is conducted in a tertiary care referral pediatric hospital.
Sample size = 100 cases

CBC parameters included in the study are WBC, RBC, HGB, HCT, RBC INDICES (MCV, MCH, MCHC), PLT count, PLT INDICES (MPV, PDW, P-LCR),
RDW-CV & WBC Differential count
In this study undiluted EDTA-anticoagulated venous blood in a standard vacutainer is run in Sysmex -XT 2000i for CBC count. This serves as control sample.
1ml of EDTA – anticoagulated venous blood from vacutainer is taken in plain test tube + 1 ml of normal saline is mixed in the plain tube. The sample is thoroughly mixed and incubated for 10 minutes and run in Sysmex -XT 2000i for CBC. This serves as the test sample.

The 100 blood samples included all the variety of cases like normal CBC, leukocytosis, leucopenia, neutrophilias, thrombocytosis, thrombocytopenias, microcytosis, normocytosis, macrocytosis etc
The test and control samples were compared for WBC parameters

Statistical analysis of data
All data were expressed as Mean +/ - SD. Statistical analysis was done using unpaired students
t test. A level of p value <0.05 was used to indicate statistical significance in all analyses.

Comparison of CBC parameters in diluted (test) sample and undiluted (control sample)

CBC Parameters	Control Sample (undiluted)	Test Sample (diluted with NS)	Statistical Analysis
WBC (x 10^3/micro L)	11.22 ± 5.56	5.48 X 2 ± 5.44	< 0.001
Neutrophils %	51.3 ± 20.3	49.6 ± 19.5	< 0.001
Lymphocytes %	41.5 ± 16.3	39.8 ± 15.80	< 0.001
Monocytes %	8.2 ± 4.4	7.82 ± 4.28	< 0.001
Eosinophils %	4.4 ± 1.2	4.1 ± 1.38	<0.001
HGB (g/dl)	11.91 ± 1.24	5.51 X 2 ± 1.36	< 0.001
HCT %	32 ± 3.6	30.9 ± 3.42	<0.001
PLT(x 10^3/microL)	350 ±200	173.6 X2 ± 195.6	< 0.05
MPV fl	10.61± 1.41	10.2 ± 1.82	<0.001
PDW fl	13.4 ± 3.3	12.9 ± 3.15	< 0.001
P-LCR %	29.4 ± 6.6	28.1 ± 6.4	< 0.001
RBC(X 106 /microl)	4.48 ± 2.03	4.41 ± 2.01	< 0.001
MCV fl	78.3 ± 6.8	77.8 ± 6.7	< 0.001
MCH pg	25.9 ± 1.83	25.1 ± 1.71	< 0.001
MCHC g/dl	33.8 ± 1.65	31.70 ± 1.50	< 0.05
RDW-CV %	14 ± 2.43	13.12 ± 2.52	< 0.05

Discussion
The total number of 100 EDTA anticoagulated blood samples were studied for CBC parameters before and after dilution with normal saline
The WBC, HGB, PLT & absolute differential count obtained should be multiplied by 2 as correction or dilution factor.
WBC (x 10^3/micro L) in undiluted blood sample was 11.22 ± 5.56 & diluted blood sample was 5.48 x 2 ± 5.44. The p value was statistically significant
The HGB (g/dl) in undiluted blood was 11.91 ± 1.24 & diluted sample was 5.51 x 2 ± 1.36
PLT(x 10^3/micro L) in undiluted blood was 350 ±200 & in diluted blood was 173.6 x 2 ± 195.6
The mean & standard deviation of platelet count showed a wide range of variation because the samples were from normal, thrombocytopenic & thrombocytosis patients
RBC, MCV, MCH, MCHC, RDW CV, PDW, MPV, P-LCR & differential counts were almost similar before and after dilution

Conclusion
Inadequate blood samples are frequently encountered in hematology lab especially in pediatric hospital. The main aim of the study is to compare the CBC parameters in diluted and undiluted anticoagulated blood samples. This study clearly demonstrated that after the dilution of blood samples also, it will not change the CBC parameters.
So it can be safely concluded that if the blood samples are inadequate, especially in dehydrated children, then instead of rejecting the blood samples they can be diluted with normal saline & this dilution will not change the CBC parameters.

Conflict Of Interest = None Declared
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