THE EFFECT OF STORAGE ON FULL BLOOD COUNT IN DIFFERENT ANTICOAGULANTS.

Idowu Ayodeji Adewale[1*], Matthew Adeniyi Adewale[2], Philip Adewale Adeoye[3]
1. University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria.
2. APIN center, Jos University Teaching Hospital, Jos, Plateau State, Nigeria.
3. Department of Community Medicine, Jos University Teaching Hospital, Jos, Plateau State, Nigeria.

***Corresponding Author:** *Matthew Adeniyi Adewale. APIN Center, Jos University Teaching Hospital, Jos, Plateau State, Nigeria. E-mail: adewale.mathew@gmail.com*

ABSTRACT

Background: The effect of storage on full blood count in different anticoagulant was determined in view of its importance on the reliability and validity of test results as the quality of sample stored would determine the quality of results.

Methodology: A total of twenty-five samples of apparently healthy individual were assessed for their Packed cell volume (PCV), total leucocytes count (TLC), platelets count and blood cell morphology by storing 2mls each of their blood sample in Ethylenediamine tetra acetic acid (EDTA), trisodium citrate, lithium heparin, fluoride oxalate and CPDA anticoagulants for a period of 24hrs, 48hrs 72hrs, and 96hrs at 4^0C to determine any changes that would occur.

Result: The PCV record a higher result (35.18 - 40.02%) with statistically significant different PCV of sample in EDTA, CPDA and those in trisodium citrate, lithium heparin ($P< 0.05$). Fluoride oxalate and trisodium citrate had more degenerative changes on the red blood cell morphology than EDTA and CPDA, Lithium heparin showed significant changes for white blood cell count, mild thrombocytopenia was found in samples stored after 72hrs in all anticoagulant as compared with initial platelet estimate at the time of collection. On the other hand, no significant changes of white blood cell morphology occurred after their storage in all anticoagulant except fluoride oxalate. The result showed more degenerative changes in fluoride oxalate, trisodium citrate and lithium heparin than CPDA and EDTA. Samples stored for 24 hours at 4^0C would not result in significant changes in blood parameters.

Conclusion: Based on these findings EDTA is the recommended anticoagulant of choice for haematological work and storage of specimen for analysis should not be encouraged as it adversely affects full blood count. Also, haematological laboratories are advised not to keep samples beyond 24 hours at 4^0C for reliability of test results.

Keyword: Sample storage, Haematologic changes, Anticoagulants, full blood counts, Packed Cell Volume, Total Leucocytes Counts, Ethylenediamine Tetra Acetic Acid,

INTRODUCTION

Determination of the effects of storage on full blood count in different anticoagulant is an aspect of quality assurance. Quality assurance involves the application of all means possible to guarantee that the results reported by the laboratory are both reliable and valid. Reliability concerns the consistency of work. Validity concerns the degree of the test measuring what it is supposed to measure with accuracy[1] Excessive delay in processing blood samples for haematologic testing could compromise the reliability of the results [2, 3]. One of the elements that contribute to quality assurance is the nature of the specimen. Baker and Silverton in 2001, states that blood deteriorate rapidly if not under ideal conditions. Hence, it could be said that the proper storage of blood specimen is a means of guaranteeing the reliability and validity of results reported in a Haematology Laboratory[4].

Storage of blood at 4^0C for up to five days in different anticoagulants caused changes in full blood count and white cell differential results, these changes observed in most of the full blood count parameter and differential results were statistically significant. The improvements of storage anticoagulant and effective storage method have been used to monitor the viability of these erythrocytes. Amongst the changes that occur in erythrocytes during storage is the loss of its deformability property. This is as a result of membrane lipid depletion, which is an important component of the membrane and finally changes the red blood cell from the biconcave shape to spherostomatocytic cells[5].

Manufacturers of automated analysers and published literatures often states that blood specimens kept at 4^0C (refrigerated) for up to 24 hours generally revealed reliable results for full blood count[5]. However, these may not be satisfactory as the high variety of analysers used are considered. Besides, no consensus was reached about which parameters can still be reliable in delays over 24 hours when analysers were out of order due to different reasons[6].

Moreso, for the detection and investigation of certain disease condition, blood samples are collected from individuals. For these to be fully and properly investigated manually, the blood may not be completely processed immediately after collection, therefore it needs to be kept in a fluid state using an anticoagulant. Blood deteriorates rapidly if not kept under ideal laboratory condition[4] and blood which has haemolysed or been contaminated may interfere with interpretation of the results thereby leading to wrong diagnosis. Degenerative changes occur when blood was allowed to stand in the laboratory before films are made[7]. That is, when blood stay for up to 3 hours changes may be discernible and by 12 - 18 hours changes become strikingly obvious[8].

The anticoagulant recommended by the International Council for Standardization in Haematology is the dipotassium salt at a concentration of 1.50±0.25mg/ml of blood[7]. At this concentration, the tripotassium salt produces some shrinkage of red blood cells which results in a 2-3% decrease in packed cell volume (PCV) and followed by gradual mean cell volume (MCV) increase on standing whereas there are negligible changes when the dipotassium salt is used[7].

METHODS

Study location: The study was carried out among healthy individuals. All subjects were between the age of 17- and 60-years attending River State University of Science and Technology (RSUT).

Study population: Apparent healthy student attending River State University of Science and Technology (RSUT). Exclusion criteria included health challenges within the three previous months, not having TB and body mass index <18.5 or $\geq$25 kg/m2.

Safety Precautions: Blood samples were collected with care and adequate safety precautions to ensure test results are reliable, contamination of the samples was avoided and infection from blood transmissible pathogens were prevented. Protective gloves were worn when collecting and

handling blood samples. Needles, and syringes were sterile, and dry, and blood collecting materials were discarded safely to avoid injury from needles.

Blood Sample Collection: In this study, 10mls of venous blood were obtained from each subject who were apparently healthy student attending River State University of Science and Technology (RSUT) (Males and Females). The samples were collected into 2.5mg/ml of dipotassium {EDTA} anticoagulant and 2ml of the same blood into 0.5 ml of trisodium citrate inside a plastic specimen container, fluoride oxalate, CPDA, and lithium heparin, were immediately analyzed for the parameter that is day 1. The use of an electric mixer ensures proper mixing of the samples. The sample were then stored at 4^0C in the refrigerator and analyzed after 24 hours, 48hours, 72 hours, and 96 hours, the specimen were processed for analysis e.g packed cell volume (PCV), platelet count, total leucocytes count and differential white cell count.

Reagents: In this study, Turk's solution and Ammonium oxalate were used.

Procedure for Blood Sample Analysis: Standard materials and methods were employed for the analysis. Initial analysis for the packed cell volume (PCV), total leucocyte count, differential white cell count and platelet count were carried out on each sample on the day 1 after which an aliquot was prepared. The sample were then stored at 4^0C in the refrigerator and analyzed again for haematological parameters listed above after 24 hours, 48hours, 72 hours, and 96 hours. Results were compared with previous ones.

Haematocrit or Packed Cell Volume (PCV): The method used for haematocrit was that reported by Baker and Silverton (2001) which is the centrifugation method using micro-haematocrit centrifuge.

Total Leucocyte Count (WBC): This was based on the Turk's method in which acetic acid in the Turk's fluid lyses the red blood cells, leaving the leucocytes intact with their nuclei stained with gentian violet component of the fluid. A 1:20 dilution of blood was made by adding 0.02ml of blood to 0.38ml of Turk's solution in a test tube. The content was properly mixed by means of Pasteur pipette, an improved neubauer counting chamber covered with a cover slip was charged with the suspension. The white blood cells present in 4 corners $1mm^2$ area were counted and the number of WBCs per litre of blood is calculated using the first principle formula.

Platelets Counts: This is based on the Cronkite's ammonium oxalate method where the red cells where lysed and the platelets left as highly refractile particles under illumination. Before a count, a 1:20 dilution of blood was made by adding 20ul of blood to 0.38ml of diluents (ammonium oxalate). The suspension was mixed, loaded and allowed to stand for some times before the count. This is done by leaving the loaded chamber in Petri dish containing moist filter paper for 20 minutes. The platelets present in the 4 corner $0.04mm^2$ and central one (5 squares in all) were counted and the total platelet count for the whole blood sample is obtained by the first principle formula.

Differential leucocyte count: (1) the blood was mixed thoroughly before sampling; (2) a thin blood film of the blood was made on clean grease free glass slide. The slide was allowed to air dry and labelled with a lead pencil; (3) the film was flooded with the Leishman stain and allowed to fix for 2 minutes; (4) it was double diluted with equal volume of the buffered distilled water pH 6.8 and allowed to stain for 8 minutes; (5) the stain was washed off with the buffered distilled water of $_P$H 6.8; (6) the back of the slide was cleaned. The slide was drain dried at room temperature; (9) it was examined with x 10 objective for cell distribution. The differential leucocytes count was done with x 100 objective; (11) it was examined for in as many fields with x100 objective until 100 cells were counted; and (12) the counting of the various type of white cell should be performed as indicated by

the battlement method

Statistical analysis: Numerical Data was obtained from the experiment and these data were analyzed using MS--Excel and the data presented as means and standard deviations. The significant difference between means in haemalogical changes were analyzed using ANOVA and Regression analysis with a significance level of $p<0.05$

RESULTS:

Table 1: Distribution of all haematological parameter of students attending River State University of Science and Technology across the different anticoagulants after day one of sample

ANTICOAGULANT	PCV (%)	PLT (cell/mm^3)	WBC (cell/mm^3)	L(%)	N(%)	M(%)	E(%)	B(%)
EDTA	37.6±2.42	247.2±48.28	4.98±0.71	40.6±12.08	50.4±14.11	7.2±1.72	1.8±0.4	0±0
SODIUM CITRATE	36.6±4.32	241.8±45.26	4.38±0.81	38±9.45	56.6±8.85	3.6±2.15	2.2±1.17	0±0
LITHIUM HEPARIN	37.6±3.50	234.2±73.58	4.42±0.52	41±9.23	54.8±9.39	2.8±0.75	1.2±0.4	0.2±0.4
FLOURIDE OXALATE	37.8±2.79	225.8±64.12	5.48±1.11	33.2±7.08	59.8±10.11	4.6±2.8	1.8±0.4	0.2±0.4
CPDA	37.2±1.72	252±48.81	5.16±0.87	37.6±9.07	57.4±9.67	2.8±9.67	2±0.63	0.2±0.4

PCV: Packed Cell Volume; WBC: White Blood Cell Count; L: Lymphocytes; PLT: Platelets; N: Neutrophils; M: Monocytes; E: Eosinophils; B: Basophils

Table 1 (above) shows the distribution of all haematological parameter across the different anticoagulants after day one (24 hours) of sample collections.

Table 2: **Distribution of all haematological parameter of students** attending River State University of Science and Technology **across the different anticoagulants after day two of sample**

ANTICOAGULANT	PCV (%)	PLT (cell/mm^3)	WBC (cell/mm^3)	L(%)	N (%)	M(%)	E (%)	B (%)
EDTA	35.2±1.47	227.8±43.44	4.42±0.64	50.4±15.90	42.2±14.86	4.6±1.2	2.6±1.20	0.2±0.4
SODIUM CITRATE	34.4±2.50	217.2±60.49	3.74±0.66	42.6±2.06	55.2±2.64	1±0.89	1.2±0.4	0.2±0
LITHIUM HEPARIN	33.4±2.06	224.6±59.51	3.72±0.47	50.6±5.61	44.6±5.95	2.8±0.74	2±0.63	0.2±0
FLOURIDE OXALATE	35.6±2.42	220.4±50.14	3.94±0.55	48.2±2.93	46.6±4.03	5.4±1.62	2±0.63	0.2±0.4
CPDA	37±2.61	246.2±51.45	4.9±0.76	40±4.20	56±4.94	3±0.63	1.4±1.02	0±0

PCV: Packed Cell Volume ; WBC: White Blood Cell Count ; L: Lymphocytes; PLT: Platelets; N: Neutrophils; M: Monocytes; E: Eosinophils; B: Basophils

Table 2 (above) shows the distribution of all haematological parameter across the different anticoagulants after day two (48 hours) of sample collections.

Table 3: Distribution of all haematological parameter of students attending River State University of Science and Technology across the different anticoagulants after day three of sample

ANTICOAGULANT	PCV (%)	PLT (cell/mm^3)	WBC (cell/mm^3)	L(%)	N(%)	M(%)	E(%)	B(%)
EDTA	36.4±1.86	216.4±47.60	3±1.19	46.8±10.53	48.2±11.36	4.8±2.04	2.2±0.4	0.2±0.4
SODIUM CITRATE	33±3.17	219.8±50.75	3.46±0.84	44.8±6.34	49±6.54	4.2±1.94	1.8±0.4	0.2±0
LITHIUM HEPARIN	34±2.61	219±32.79	3.42±0.29	59.2±10.53	38.8±15.12	3.4±0.8	2.4±0.49	0.2±0.4
FLOURIDE OXALATE	36.4±3.26	231.2±39.63	3.3±0.41	53.8±7.22	38.6±6.47	5±1.41	2.4±0.49	0.2±0.4
CPDA	36.6±2.15	243.2±49.45	4.68±0.59	40.4±5.08	56±5.40	2.8±0.75	1.20.75	0±0

PCV: Packed Cell Volume; WBC: White Blood Cell Count; L: Lymphocytes; PLT: Platelets; N: Neutrophils; M: Monocytes; E: Eosinophils; B: Basophils

Table 3 (above) shows the distribution of all haematological parameter across the different anticoagulants after day three (72 hour) of sample collections.

Table 4: Distribution of all haematological parameter of students attending River State University of Science and Technology across the different anticoagulants after day four of sample collections

ANTICOAGULANT	PCV (%)	PLT (cell/mm^3)	WBC (cell/mm^3)	L(%)	N(%)	M(%)	E(%)	B(%)
EDTA	34.4±1.62	211±49.71	1.88±0.46	43.2±14.22	50±12.63	3.8±3.25	2.2±1.17	0.2±0.4
SODIUM CITRATE	30.8±1.17	184.8±23.85	2.76±0.42	53.4±6.41	43.4±8.54	1.4±1.74	1.8±1.17	0±0
LITHIUM HEPARIN	33.8±3.19	199.2±7.78	2.96±0.37	51.2±1.83	42.4±2.25	4.4±2.00	2±0.63	0±0
FLOURIDE OXALATE	39.6±5.31	203.8±59.54	2.4±0.30	59.6±8.84	35.6±8.75	3±1.26	1.4±0.49	0.4±0.49
CPDA	36.2±2.14	240.6±48.81	4.66±0.59	44.6±2.65	51±3.69	2.8±2.48	1.4±1.02	0.2±0.4

PCV: Packed Cell Volume; WBC: White Blood Cell Count; L: Lymphocytes; PLT: Platelets; N: Neutrophils; M: Monocytes; E: Eosinophils; B: Basophils

Table 4 (above) shows the distribution of all haematological parameter across the different anticoagulants after day four (96 hours) of sample collections.

Table 5: Distribution of all haematological parameter of students attending River State University of Science and Technology across the different anticoagulants after day five of sample collections

ANTICOAGULANT	PCV (%)	PLT (cell/mm^3)	WBC (cell/mm^3)	L(%)	N(%)	M(%)	E(%)	B(%)
EDTA	33±1.41	189.4±37.89	1.74±0.32	53.2±20.39	41±19.02	3.8±2.86	2±0.63	0.2±0.4
SODIUM CITRATE	31±0.89	171.4±16.78	2.38±0.35	56.8±10.98	40.2±7.28	2.6±0.49	2±0.63	0.4±0
LITHIUM HEPARIN	36±5.06	168.4±30.30	2.42±0.39	60.2±5.78	36.4±5.32	3.8±2.0	1.4±1.02	0.4±0.49
FLOURIDE OXALATE	40±6.36	193.8±53.02	2.04±0.52	56.8±5.27	37.2±5.84	3.4±1.62	2.4±0.49	0.2±0.4
CPDA	35±2.45	228±42.76	4.4±0.53	47±1.79	50.4±2.87	1.6±1.4	1±0.6	0±0

PCV: Packed Cell Volume; WBC: White Blood Cell Count; L: Lymphocytes; PLT: Platelets; N: Neutrophils; M: Monocytes; E: Eosinophils; B: Basophils

Table 5 (above) shows the distribution of all haematological parameter across the different anticoagulants after day four (120 hours) of sample collections.

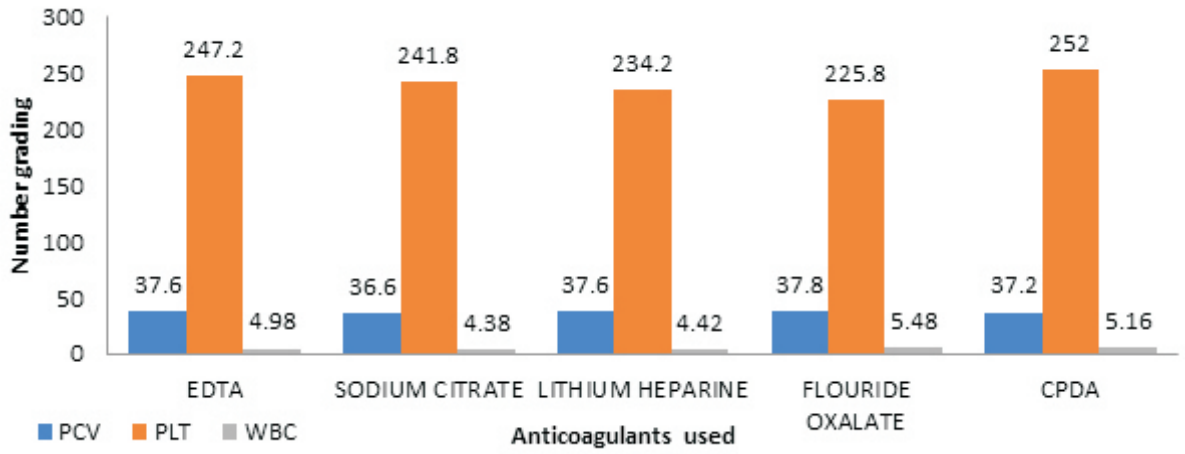

Figure 1: Bar Chart showing the comparison of the PCV (%), Platelet count (cell/mm^3) and white blood cell count (cell/mm^3) of blood sample stored in the different anticoagulant for day one

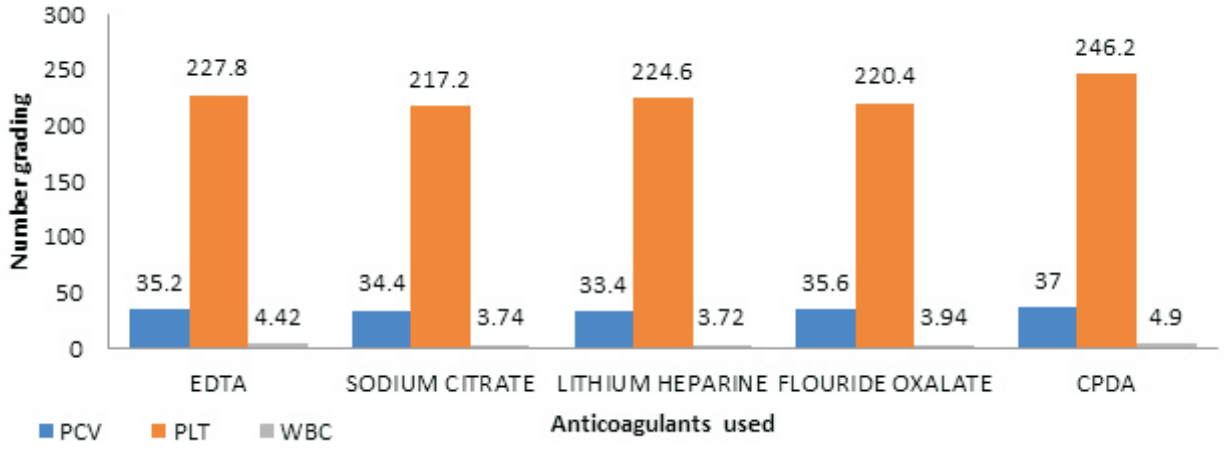

Figure 2: Bar Chart showing the comparison of the PCV (%), Platelet count (cell/mm^3) and white blood cell count (cell/mm^3) of blood sample stored in the different anticoagulant for day two

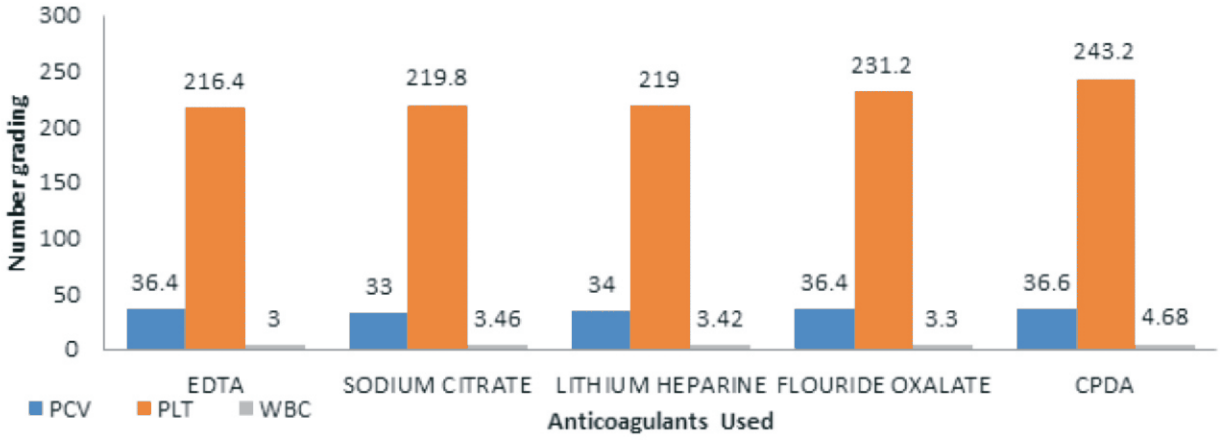

Figure 3: Bar Chart showing the comparison of the PCV (%), Platelet count (cell/mm^3) and white blood cell count (cell/mm^3) of blood sample stored in the different anticoagulant for day three

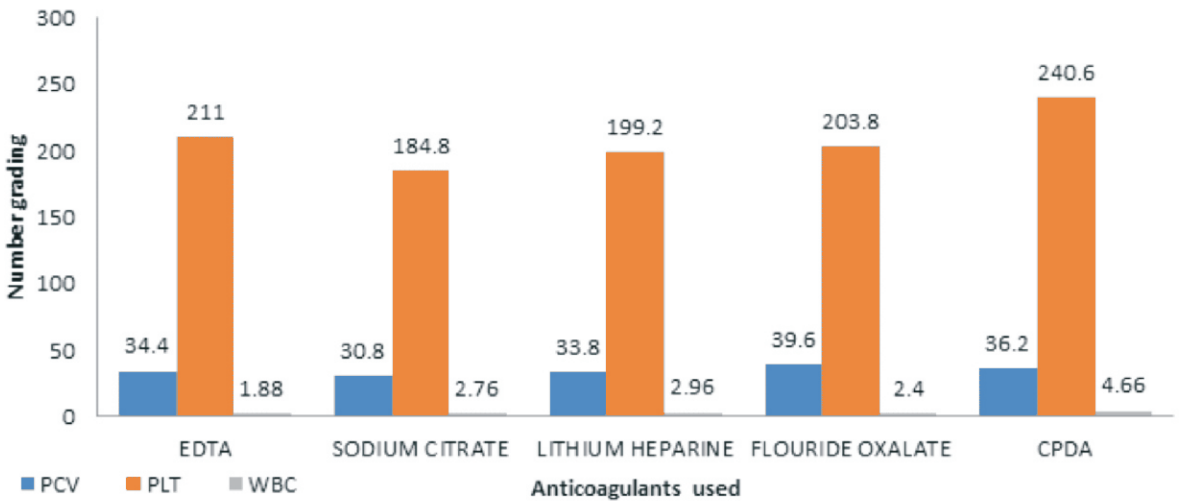

Figure 4: Bar Chart showing the comparison of the PCV (%), Platelet count (cell/mm^3) and white blood cell count (cell/mm^3) of blood sample stored in the different anticoagulant for day four.

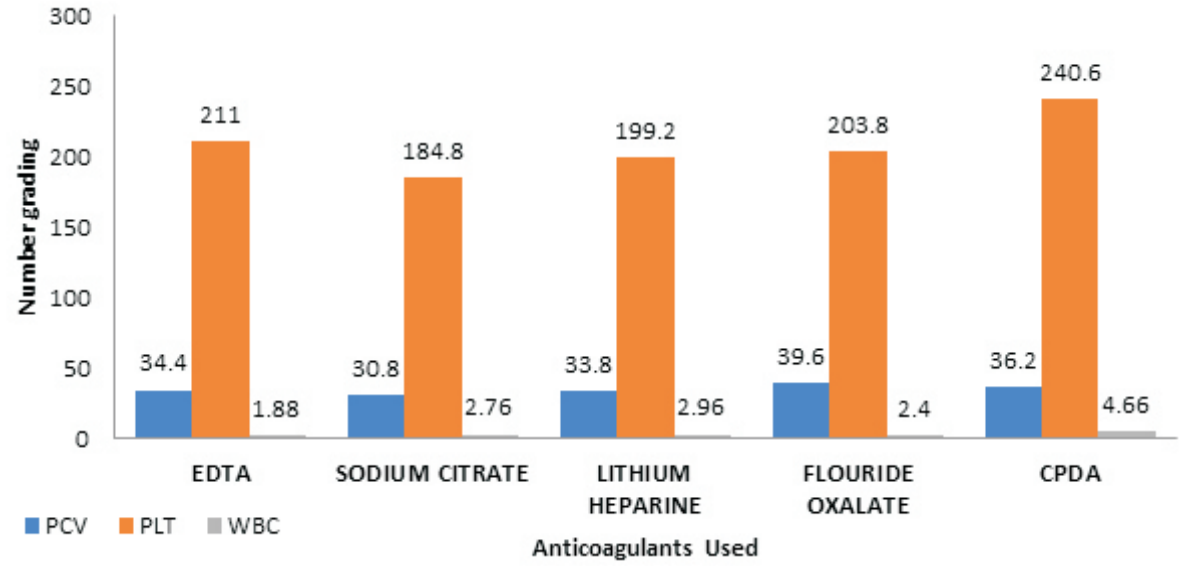

Figure 5: Bar Chart showing the comparison of the PCV (%), Platelet count (cell/mm^3) and white blood cell count (cell/mm^3) of blood sample stored in the different anticoagulant for day five.

Table 6: comparison of haematologic changes of blood samples across the differing anticoagulants.

PARAMETER	Btw day 1 and day 2	Btw day 1 and day 3	Btw day 1 and day 4	Btw day 1 and day 5
Packed Cell Volume	$P < 0.05^{bd}$	$P < 0.05^{bcd}$	$P < 0.05^{bcda}$	$P < 0.05^{abcd}$
Platelets	$P > 0.05$	$P < 0.05^{abc}$	$P < 0.05^{abcd}$	$P < 0.05^{abc}$
White Blood Cell Counts	$P < 0.05^{cd}$	$P < 0.05^{abcd}$	$P < 0.05^{abcd}$	$P < 0.05^{abcde}$
Lymphocyte	$P < 0.05^{d}$	$P < 0.05^{d}$	$P < 0.05^{d}$	$P < 0.05^{ad}$
Neutrophil	$P < 0.05^{bd}$	$P < 0.05^{bcd}$	$P < 0.05^{bcde}$	$P < 0.05^{abcde}$
Monocytes	$P > 0.05$	$P < 0.05^{ac}$	$P < 0.05^{abcde}$	$P < 0.05^{abcde}$
Eosinophil	$P < 0.05^{ab}$	$P < 0.05^{ab}$	$P < 0.05^{abd}$	$P < 0.05^{abcde}$
Basophil	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

*Comparative findings of Haematological changes in EDTA, Sodium Citrate, Lithium Heparine, Floride Oxalate and CPDA between day 1 to day 5. [a] Statistically significant for EDTA at $P < 0.05$; [b] Statistically significant for Sodium Citrate at $P < 0.05$; [c] Statistically significant for Lithium Heparine at $P < 0.05$; [d] Statistically significant for Floride Oxalate at $P < 0.05$; [e] Statistically significant for CPDA at $P < 0.05$

DISCUSSION

Storage of blood at 4^0C for up to five days in different anticoagulants caused changes in full blood count and white cell differential results, these changes was observed in most of the full blood count parameter and differential results which were found to be statistically significant.

Gulati in their work of 2002 stated that amongst the changes that occur in erythrocytes during storage is the loss of its deformability property. This is as a result of membrane lipid depletion, which is an important component of the membrane and finally changes the red blood cell from the biconcave shape to spherostomatocytic cells.

From table 4.6 samples in trisodium citrate, lithium heparin and fluoride oxalate record a significant value for the red blood cell when compared to EDTA anticoagulant for day 3 and day 5. This could be as a result of dilution effect since trisodium citrate is in liquid form and up to 0.5mls of it was used as anticoagulant for the blood sample.

When blood is stored at 4^0C, the total white blood cell count showed a significant reduction (downward trend) after 48 hours. This is also consistent with previous findings[9]. Lithium heparin show significant changes for white blood cell count in table 4.6 and is therefore not good for blood film morphology and in line with previous findings[10].

Fluoride oxalate and trisodium citrate had more degenerative changes on the red blood cell morphology than EDTA and CPDA. These changes include hypochromia and crenation of the cell. The CPDA and EDTA shows no significant value for packed cell volume, white blood cell count and platelet count and is in line with Wintrobe and Dacie findings[11,7].

More so, table 4.6 show significant value for platelets count, white blood cell count and packed cell volume with storage showing that duration of storage affects the cell morphology. These changes in packed cell volume, white blood cell count, platelets count and differential are most likely the sum effect of the loss on individual cell

characteristics and cellular degeneration that is known to occur as the cell ages[12]. Not much changes were observed in the white cell and red cell morphology when stored in EDTA and CPDA compared to that when stored in trisodium citrate, lithium heparin and fluoride oxalate, which agrees with the findings of Dacie[7]. Based on the finding of this study and the cited published report, it can be stated that even only after 24hrs, a specimen may yield unreliable result.

CONCLUSION

There are more degenerative changes observed in sample stored in trisodium citrate, lithium heparin and fluoride oxalate than those in EDTA and CPDA anticoagulant. Blood analysis (full blood count and differential) should be done immediately after collection, my study suggests that clinically reliable results may not be obtained for most full blood count and differential parameters from specimen older than one day.

REFERENCES

1. Hoffbrand, S.M Lewis, Tuddenham: Post graduate Haematology 4th Edition 2001: pp 120-163.
2. Petit, M.D Frcpa Frc Path: Director and Haematologist. Med Lab South Christchurch. New Zealand. Essentials of Haematology 1997: 3rd edition.
3. Monica Cheesbrough, District Laboratory Practice in tropical countries, part 2; 2000: Pp. 227 – 327.
4. Baker, J.F. and Silverton R.E: Introduction to Medical Laboratory Technology. Blood count Butterworths, London. 6th Edition; 2001: Pp566-588.
5. Gulati: Changes in Automated complete Blood cell count and differential leucocytes count result induced by storage of blood at room temperature. Archive of Pathol and L Medicine 2001: vol 126, No 3. Pp 336-346.
6. Roger, H and Robert, G.M: Changes in blood during storage. Medical Laboratory Haematology; Butterworths and Company, 1989: Pp 99.
7. Dacie, J.V. and Lewis, S.M: differential leucocytes count blood cells, De Gruchy's clinical hematology, 6th Edition. 2001: Pp 4- 8
8. Wood, B.L., Andrew J. Miller S., Sabath, D.E. Refrigerated storage improves stability of the complete blood cells count and automated differential. Haematopathology1999: 112: 687-695.
9. Cohle, S.D.; Abdus,S. and Mmakkaowis, D.E. Effects of the storage of Blood on the stability of haematologic parameters. American Journal of clinical pathology 1997: 76:67-69.
10. Geoffrey, M.B., George, B., Johnson, A.C. and Robert, M.E Stability of blood in commonly used Anticoagulants. American Journal of Clinical Pathology 1990: 52: 690-694.
11. Wintrobe, M.M (1996): The preservation of leukocytes in stored Blood, Clinical Haematology; Leand Febiger, Philadelphia. 6th Ed Pp 286.
12. Warner, B. A, Reardon, D.M A field Evaluation of the coulter STKS. American Journal Clinical Pathology.1999: 207-217.