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Variations in the full blood count parameters among apparently healthy humans in the Ho municipality using ethylenediamine tetraacetic acid (EDTA), sodium citrate and lithium heparin anticoagulants: A laboratory-based cross-sectional analytical study

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ABSTRACT

Background: Several studies have shown that various anticoagulants used for collection of blood samples produce varying effects on haematological analyses. Tripotassium ethylenediamine tetra-acetic acid (K_3EDTA), sodium citrate and lithium heparin remain the most used anticoagulants employed in hematological analysis. There is paucity of data on the effect of these anticoagulants on haematological parameters in humans in Ghana. We assessed the suitability of K_3EDTA, sodium citrate and lithium heparin for routine Full Blood Count (FBC) investigation.

Method: A laboratory-based analytical cross-sectional study was conducted using blood samples from 55 conveniently sampled apparently healthy tertiary students from January 2021 to October 2021. Blood samples were taken from each participant into 3 anticoagulant tubes: K_3EDTA, sodium citrate and lithium heparin and FBC parameters estimated using the Mindray automated haematology analyzer. One-way ANOVA Kruskal-Wallis test, Mann-Whitney U, Intra-class correlation coefficient (ICC) analysis, Bland-Altman's plot and Lin's concordance correlation coefficient were used where appropriate to ascertain the level of variation, consistency, and agreements among and between results. Normality testing using Shapiro-Wilk test statistic revealed non-Gaussian distribution of data, hence, were presented as median, minimum, and maximum. Data generated were analyzed using STATA v15 and MedCalc v20 where appropriate for statistical analysis. *P*-values <0.05 were considered statistically significant.

Results: The study comprised 34 males and 21 females. The median age for males (23 years: min = 20, max = 34) was statistically comparable (p = 0.2652) to that of females (22 years: min = 18, max = 34). We observed excellent consistency in the estimation of MCV (ICC = 0.94), MCH (ICC = 0.98), MCHC (ICC = 0.91), GRAN# (ICC = 0.92) and LYMPH% (ICC = 0.91) across the three anticoagulants. Heparin and K_3EDTA largely agreed on most of the FBC parameters, 50.0% (7/14) including HGB, MCV, MCH, PLT, LYMPH#, GRAN# and GRAN%. Meanwhile using K_3EDTA as a standard, heparin produced almost perfect agreement only in the assessment of RBC (CCC = 0.992) while a substantial agreement was observed in the assessment of HGB (0.971), HCT (0.958) and MCH (0.987). Citrate agreed substantially with K_3EDTA in the assessment of LYMPH % (CCC = 0.964) and moderately in the assessment of MCV (CCC = 0.948) and MCH (0.913).

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Overall, compared to K_3EDTA, heparin was highly precise and accurate in the estimation of HGB, RBC, HCT and MCH while citrate determined MCV and MCH more accurately and precisely.
Conclusion: Citrated blood consistently produced lower FBC values compared to heparin and K_3EDTA and hence suggests not reliable in the assessment of FBC among humans. Heparin agreed largely with K_3EDTA in the estimation of FBC parameters and may be used as a better alternative anticoagulant in the absence of K_3EDTA however with great caution.

1. Introduction

Blood and its components remain a vital tissue in maintaining functional homeostasis in mammals [1]. FBC parameters consisting of red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) etc. are valuable in monitoring physiologic changes and the health status of an individual [2]. The FBC is used as an index of human health status to investigate congenital abnormalities as well as to detect functional changes following exposure to potential pathological factors [2]. It is well established that blood sampling and collection, laboratory techniques, storage and the type of anticoagulant used could significantly influence the results obtained from haematological determinations [3]. The most used anticoagulants in clinical haematology include sodium and potassium salts of EDTA, salts of citric acid, heparin, and oxalates [4].

EDTA and lithium heparin are two of the most used anticoagulants in avian medicine [5]. According to studies, EDTA is a preferred anticoagulant because it preserves blood components for long and has proven highly effective in blood clot prevention [4,6]. Indeed, EDTA has been recommended for FBC by the National Committee for Clinical Laboratory Standards, principally for its cell preservation properties [7]. The mode of action of EDTA (dipotassium or tripotassium) is by chelating calcium which is the necessary mineral in the coagulation cascade and for cell-to-cell interactions [8,9]. Heparin on the other hand binds to and escalate the activity of antithrombin III to prevent the action of thrombin and other proteases needed for coagulation [5,8]. But these two anticoagulants possess inherent functional effects on various cellular constituents. In domestic animals and birds for example, K_2EDTA is the recommended anticoagulant for haematologic testing but has however been reported to cause progressive haemolysis of the red blood cells whereas heparin has also been shown to alter leukocyte morphology and does not prevent platelet aggregation [5]. Again, while it is assumed that heparin should not be used to prepare blood smears due to its erroneous counts because of leukocyte and thrombocyte clumping and blue staining background characteristic or for white blood cells estimation due to leukocyte agglutination, EDTA is also reported to be inappropriate for the assessment of erythrocytic osmotic fragility [4,5].

Studies conducted in various animal species documented varying effects of citrate on FBC parameters as against EDTA or heparin anticoagulants [10]. For instance, in appropriate blood to citrate volume ratio, a blood dilution of about ten percent is noted, reducing all cell counts by approximately 10% compared to EDTA [11]. Also, platelet activation characterized by abnormally large morphology as well as less granulation in citrated blood has been reported [12]. Results from 30 clinically healthy dogs concluded limited possibility of using sodium citrate in routine haematological analysis because of its influence on haemoglobin concentration and platelet parameters, including erythrocyte and leukocyte morphology [13].

In healthcare delivery, it is highly recommended that scientist, researchers, and medical practitioners ensure that the accuracy and reliability of test methods and procedures are investigated and for that matter validated to ensure quality patient outcome. EDTA, heparin and citrate have been widely employed in various hematological estimation of patient's samples all over the world but not many studies assessed the clinical agreements in results generated using these anticoagulants. Currently, the recommended EDTA according to the International Council for Standardization in Haematology is K_2EDTA [14] however, K_3EDTA is the most available in the Ghanaian market and the most used in the laboratories in Ghana. Further, in Ghana, only EDTA is used in FBC analysis including point of care testing (POCT). Studies to assess the utility of heparin and citrate as alternatives in FBC analysis and in POCT are lacking. Finally, variability studies in hematological results produced by EDTA, citrate and heparin have not been widely assessed within the African descents particularly when we consider the fact that these tubes are often manufactured, tested and quality controlled under condition not so comparable to that of the African continent. This study therefore assessed the clinical reliability of the three commonly used anticoagulants in haematology vis-à-vis K_3EDTA, lithium heparin and sodium citrate for the estimation of FBC of apparently healthy population in the Ho Municipality. To the best of our knowledge, this study is the first of its kind to be conducted among humans in Ghana. Most of the studies reported in literature sought to assess the effects of various anticoagulants on FBC or specific components of FBC parameters among various species of animals. This greatly hindered indebt comparative analysis between our findings and paralleled studies across Africa and the globe in humans. This study could therefore serve as a baseline study for further studies on the subject matter.

2. Materials and methods

2.1. Study design

This study was a cross-sectional laboratory-based analytical research among 55 conveniently sampled apparently healthy students from the University of Health and Allied Sciences, residing in the community of Ho Municipality, Ghana from January 2021 to October 2021. The Ho Municipality is the largest urban center of the Volta Region with a total population of about 177,281 (83,819 males and

93,462 females) representing 8.4% of the region's total population according to the 2010 Population and Housing Census (PHC).

2.2. Screening and recruitment process of study participants

This study employed a semi-structured questionnaire to collect demographic information which included participants' age, sex, and physical fitness status. Participants were also questioned on their health status to collect data on possible health conditions that may affect their FBC parameters. The choice of study participants from health exclusive institution (University of Health and Allied Sciences; website- https://www.uhas.edu.gh) was premised on the consciousness of students of their health status. Students were asked via questionnaire to state whether they had history of any chronic condition and/or infection. Because students frequently got tested for infectious pathogens, most commonly malaria, HIV/AIDS, hepatitis B and C and syphilis etc., during clinical rotations, verbal responses on their health status based on current health checks was deemed trustworthy. None of the students however reported history of any health condition that may alter their haematological parameters.

2.3. Inclusion and exclusion criteria

Students who were apparently healthy thus without history of any known chronic condition such as hypertension, diabetes, heart disease, asthma, known immune mediated conditions upon physical examination and interview as well as without any highly infectious diseases such as HIV/AIDS, hepatitis B and C etc. were recruited for this study. Only students who lived within communities in the Ho Municipality were included in this study. Students who refused to consent to this study were excluded from participating in this study. Also, students who were preparing for end of semester exams were excluded from this study due to the stress associated to long periods of studies and the irregular nutritional/diet pattern among students that may affect their FBC parameters.

2.4. Sample size determination

Using G*power statistical software version 3.1.9.4 [15] with a *t*-test for linear multiple regression, two tail, effect size of 0.15, 0.05 alpha error probability, 0.80 statistical power and a predictor value of 1, a minimum sample size of 55 was determined. Hence, a minimum of 55 participants were recruited for this study.

2.5. Blood sample collection and laboratory analysis

Five milliliters (5 ml) of whole blood sample was collected from the median cubital vein or any prominent vein from the antecubital fossa of the forearm of each participant using sterile syringe and needle. Of the 5 ml volume of whole blood collected, 1.8 ml was dispensed into sodium citrate test tube whereas 1.6 ml was dispensed into the other blood sample tubes containing K_3EDTA and lithium heparin (swiftly but gently to ensure that transfer of anticoagulant from one tube to the other was avoided) and mixed thoroughly by gently inverting the tubes containing the blood sample at least 6–8 times to ensure uniform mixture of the blood and the anticoagulants. The characteristics of the test tubes employed in this study are shown in Table 1 below. The samples were analyzed within 3–6 h after collection using Mindray BC-10 three-part automated haematology analyzer (Medsinglong Co Ltd, Guangdong, China) to estimate the participant's FBC. The haematology parameters estimated includes White Blood Cell (WBC), Haemoglobin (HGB), Red Blood Cell (RBC), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelet (PLT), Lymphocyte Absolute Count (LUMPH#), Mid-range Absolute Count (MID#), Granulocyte Absolute Count (GRAN#), Percent Lymphocyte Count (LYMPH%), Percent Mid-range Count (MID%) and Percent Granulocyte Count (GRAN%) [10].

Table 1
Characteristics of test tubes used in the laboratory assessment of FBC.

Tube details	EDTA	Heparin	Citrate
Type of tube	K_3EDTA	Lithium heparin	Sodium citrate
Dimension	13 × 75 mm	13 × 75 mm	13 × 75 mm
Storage	4–25 °C (40–77 °F)	4–25 °C (40–77 °F)	4–25 °C (40–77 °F)
Year of expiry	2023	2023	2023
Tube capacity (volume)	5 ml	4 ml	4 ml
Required volume	2.0–5.0 ml	2.0–4.0 ml	1.8 ml
Tube material	Glass	Glass	Glass
Manufacturer	Jiangsu Kangjie Medical Devices Co Ltd	BD Bristol Circle Oakville	BD Bristol Circle Oakville
Origin/country	China	India	India
Anticoagulant type & concentration	Spray-coated 5.4 mg	Spray-coated 75 USP units	Buffered sodium citrate solution 3.2%

2.6. Quality control steps

Our Mindray BC-10 three-part automated haematology analyzer was quality controlled following the manufacturer's instruction. Briefly, the manufacturer's provided quality control samples were run to ensure the accuracy and precision of the instrument performance before running the samples of the study participants. Physical examination and interview was performed to ascertain the health and fitness status of the potential participants to rule out any health conditions that may affect the outcome of the study [16].

2.7. Data analysis

Data generated were entered into Microsoft Excel 2016, checked for consistency and completeness, and then transferred into STATA version 15 and MedCalc version 20 for statistical analysis where appropriate. Test for normality was done using the Shapiro-Wilk test statistic; all continuous variables did not follow Gaussian distribution and hence were analyzed nonparametrically. One-way ANOVA Kruskal-Wallis test and Mann-Whitney *U* test were used to determine variations among and between FBC parameters respectively using K_3EDTA, sodium citrate and lithium heparin samples. Bland Altman's plot was used to determine the level of agreement between results generated by each pair of anticoagulants. No significant bias in the measurement of the parameters of interest is considered to have occurred if the line of equality was included within the 95% confidence limit of the mean difference. Linear regression between the difference and average of the FBC results of each pair of anticoagulants was determined to ascertain any proportional bias between each pair of anticoagulants in the estimation of FBC. Significant *p* values of the regression coefficient signify the occurrence of proportional bias between the measurements. If one sample *t*-test of the difference in the FBC results using two test reagents was statistically significant ($P < 0.05$), that parameter was adjudged not qualified for Bland-Altman Plot and hence excluded from Bland-Altman analysis. The Lin's concordance correlation coefficient (CCC) was employed to assess the agreement between the anticoagulants using K_3EDTA as a standard. A CCC of >0.99 signifies almost perfect agreement; >0.95–0.99 implies substantial agreement; (0.90–0.95) implies moderate agreement while <0.90 was considered poor agreement [17]. Intra-class correlation coefficient (ICC) analysis was modelled to assess consistency in results generated using the three anticoagulants. An ICC value of below 0.50 indicates poor consistency, 0.50–0.75 indicates moderate consistency, 0.75–0.90 implies good consistency and above 0.90 implies excellent consistency. *P*-value of <0.05 in all statistical comparisons was considered significant.

2.8. Ethical consideration

Ethical approval was obtained from the University of Health and Allied Sciences Research Ethics Committee (UHAS-REC) with ethical clearance certificate number UHAS REC A.10 [18] 20–21 to carry out this research. Informed written consent was also obtained from all participants before enrolling them in the study after they had been thoroughly educated on the study.

3. Results

3.1. General demographic characteristics of the study participants

This study recruited 55 patients comprising 34 males and 21 females. The participants were predominantly young adults with an average median age of 23 years (min = 20, max = 34) for males and 22 years (min = 18, max = 34) for females which were statistically comparable (p = 0.2652).

Table 2
Variations in FBC parameters using Heparin, Citrate and K_3EDTA anticoagulants.

FBC Parameters	Heparin	Citrate	EDTA	*p*
WBC (*10^9/L)	4.8 (2.8–8.6)	3.5 (1.3–7.3)	5.0 (2.7–9.5)	<0.0001
HGB (g/dL)	11.9 (8.3–14.9)	8.6 (3.5–11.6)	12.1 (7.4–15.1)	<0.0001
RBC (*10^12/L)	5.13 (3.56–6.42)	3.87 (1.66–12.12)	5.21 (3.54–6.54)	<0.0001
HCT (%)	45.7 (33.8–56.3)	33.1 (13.6–43.2)	46.1 (34.2–56.9)	<0.0001
MCV (fL)	87.7 (48.4–102.5)	85.4 (62.2–100.8)	87.4 (62.7–102.4)	**0.2546**
MCH (pg)	22.8 (15.0–26.7)	22.0 (15.0–26.1)	23.0 (15.3–26.4)	**0.0598**
MCHC (g/dL)	26.2 (24.1–28.2)	25.9 (23.1–28.1)	26.2 (23.9–28.0)	**0.0947**
PLT (*10^9/L)	157 (54–404)	156 (57–322)	253 (140–419)	<0.0001
LYMPH# (*10^9/L)	2.5 (1.4–5.1)	1.7 (0.5–3.0)	2.4 (1.3–5.7)	<0.0001
MID# (*10^9/L)	0.3 (0.1–0.8)	0.3 (0.1–0.9)	0.5 (0.3–1.3)	<0.0001
GRAN# (*10^9/L)	1.9 (0.8–4.1)	1.4 (0.3–3.4)	1.9 (0.7–4.0)	<0.0001
LYMPH%	53.9 (34.4–67.8)	51.0 (14.8–70.4)	50.1 (28.2–69.2)	**0.1307**
MID%	6.2 (2.7–15.2)	8.5 (7.8–9.3)	9.7 (5.5–24.1)	<0.0001
GRAN%	39.7 (3.5–55.2)	40.1 (18.0–72.2)	39.7 (17.9–64.0)	**0.8533**

WBC- White Blood Cell; HGB-Hemoglobin; RBC-Red Blood Cell; HCT-Hematocrit; MCV-Mean Cell Volume; MCH-Mean Cell Hemoglobin; MCHC-Mean Cell Hemoglobin Concentration; PLT-Platelet; LYMPH#-Lymphocyte Count; MID#-Mid-range Absolute Count; GRAN#-Granulocyte Absolute Count; LYMPH%-Percent Lymphocyte Count; MID%-Percent Mid-range Absolute Count; GRAN%-Percent Granulocyte Count; EDTA-Ethylenediamine Tetraacetic Acid; FBC-Full Blood Count.

Table 3
Pair-wise comparison of the variation in the estimation of FBC parameters using Heparin, Citrate and K_3EDTA anticoagulants.

FBC Parameters	Heparin	Citrate	*p*	Heparin	EDTA	*p*	Citrate	EDTA	*p*
WBC (*10^9/L)	4.8 (2.8–8.6)	3.5 (1.3–7.3)	<0.0001	4.8 (2.8–8.6)	5.0 (2.7–9.5)	**0.2474**	3.5 (1.3–7.3)	5.0 (2.7–9.5)	<0.0001
HGB (g/dL)	11.9 (8.3–14.9)	8.6 (3.5–11.6)	<0.0001	11.9 (8.3–14.9)	12.1 (7.4–15.1)	**0.7806**	8.6 (3.5–11.6)	12.1 (7.4–15.1)	<0.0001
RBC (*10^12/L)	5.13 (3.56–6.42)	3.87 (1.66–12.12)	<0.0001	5.13 (3.56–6.42)	5.21 (3.54–6.54)	**0.7020**	3.87 (1.66–12.12)	5.21 (3.54–6.54)	<0.0001
HCT (%)	45.7 (33.8–56.3)	33.1 (13.6–43.2)	<0.0001	45.7 (33.8–56.3)	46.1 (34.2–56.9)	**0.5132**	33.1 (13.6–43.2)	46.1 (34.2–56.9)	<0.0001
MCV (fL)	87.7 (48.4–102.5)	85.4 (62.2–100.8)	**0.1721**	87.7 (48.4–102.5)	87.4 (62.7–102.4)	**0.8245**	85.4 (62.2–100.8)	87.4 (62.7–102.4)	**0.1414**
MCH (pg)	22.8 (15.0–26.7)	22 (15.0–26.1)	0.0413	22.8 (15.0–26.7)	23.0 (15.3–26.4)	**0.9656**	22 (15.0–26.1)	23 (15.3–26.4)	0.0386
MCHC (g/dL)	26.2 (24.1–28.2)	25.9 (23.1–28.1)	0.0390	26.2 (24.1–28.2)	26.2 (23.9–28.0)	**0.6260**	25.9 (23.1–28.1)	26.2 (23.9–28.0)	**0.1510**
PLT (*10^9/L)	157 (54–404)	156 (57–322)	**0.9206**	157 (54–404)	253 (140–419)	<0.0001	156 (57–322)	253 (140–419)	<0.0001
LYMP# (*10^9/L)	2.5 (1.4–5.1)	1.7 (0.5–3.0)	<0.0001	2.5 (1.4–5.1)	2.4 (1.3–5.7)	**0.7283**	1.7 (0.5–3.0)	2.4 (1.3–5.7)	<0.0001
MID# (*10^9/L)	0.3 (0.1–0.8)	0.3 (0.1–0.9)	**0.0941**	0.3 (0.1–0.8)	0.5 (0.3–1.3)	<0.0001	0.3 (0.1–0.9)	0.5 (0.3–1.3)	<0.0001
GRAN# (*10^9/L)	1.9 (0.8–4.1)	1.4 (0.3–3.4)	<0.0001	1.9 (0.8–4.1)	1.9 (0.7–4.0)	**0.9941**	1.4 (0.3–3.4)	1.9 (0.7–4.0)	0.0001
LYMPH%	53.9 (34.4–67.8)	51 (14.8–70.4)	**0.0561**	53.9 (34.4–67.8)	50.1 (28.2–69.2)	**0.1304**	51.0 (14.8–70.4)	50.1 (28.2–69.2)	**0.7086**
MID%	6.2 (2.7–15.2)	8.5 (7.8–9.3)	<0.0001	6.2 (2.7–15.2)	9.7 (5.5–24.1)	<0.0001	8.5 (7.8–9.3)	9.7 (5.5–24.1)	**0.0760**
GRAN%	39.7 (3.5–55.2)	40.1 (18.0–72.2)	**0.8758**	39.7 (3.5–55.2)	39.7 (17.9–64.0)	**0.5584**	40.1 (18.0–72.2)	39.7 (17.9–64.0)	**0.7467**

WBC- White Blood Cell; HGB-Hemoglobin; RBC-Red Blood Cell; HCT-Hematocrit; MCV-Mean Cell Volume; MCH-Mean Cell Hemoglobin; MCHC-Mean Cell Hemoglobin Concentration; PLT-Platelet; LYMPH#-Lymphocyte Count; MID#-Mid-range Absolute Count; GRAN#-Granulocyte Absolute Count; LYMPH%-Percent Lymphocyte Count; MID%-Percent Mid-range Absolute Count; GRAN %-Percent Granulocyte Count; EDTA-Ethylenediamine Tetraacetic Acid; FBC-Full Blood Count.

3.2. Variations in FBC parameters using heparin, citrate and K_3EDTA anticoagulants

As shown in Table 2 below, no statistically significant variation in the median MCV, MCH, MCHC, LYMPH% and GRAN% across the three anticoagulants studied was observed. Results of the rest of the FBC parameters estimated varied significantly when compared together across the three anticoagulants studied.

3.3. Pair-wise comparison of the variation in the estimation of FBC parameters using heparin, citrate and K_3EDTA anticoagulants

Results from two anticoagulated blood i.e., heparin-citrate, heparin- K_3EDTA and citrate- K_3EDTA were compared for differences in the FBC results generated. In comparing heparin-citrate results, median MCV, PLT, MID#, LYMPH% and GRAN% produced statistically comparable results ($p > 0.05$) while the rest of the parameters varied significantly. Similar observation was made with the citrate-EDTA results which generated analogous FBC results for MCV, MCHC, LYMPH%, MID% and GRAN%. Comparison of heparin-K_3EDTA results on the other hand yielded significant variation in the PLT, MID# and MID% (Table 3).

3.4. Consistency in FBC results using heparin, citrate and K_3EDTA anticoagulants

As shown in Table 4 below, excellent consistency was demonstrated in the estimation of MCV, MCH, MCHC, GRAN# and LYMPH% across all three anticoagulants. Moderate consistency on the other hand was observed in the estimation of RBC, PLT and MID#. Poor consistency was exhibited in the estimation of MID%.

3.5. Bland Altman plot of agreement between the hematological anticoagulant performance in FBC estimation

The B-A plots in Table 5 below show heparin and citrate agreeing in the measurement of MID# and GRAN% however, significant proportional bias was observed in the measurement of MCV (linear regression coefficient = 0.279). Heparin and K_3EDTA agreed in the estimation of most of the FBC parameters including HGB, MCV, MCH, PLT, LYMPH#, GRAN# and GRAN% but with significant proportional bias in the measurement of HGB (linear regression coefficient = 0.076), MCV (linear regression coefficient = 0.230), PLT (linear regression coefficient = 0.489), and LYMPH# (linear regression coefficient = −0.358). Finally, Citrate and K_3EDTA agreed in the estimation of LYMPH%, MID% and GRAN# but with a significant level of proportional bias exhibited in the measurement of MID% (linear regression coefficient = 0.079).

3.6. Assessment of the level of agreement of heparin and citrate anticoagulants using K_3EDTA as gold standard

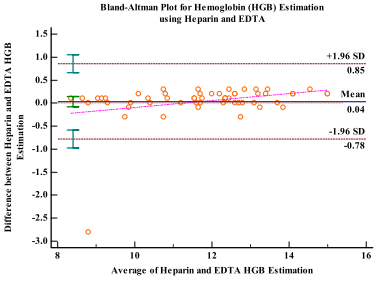
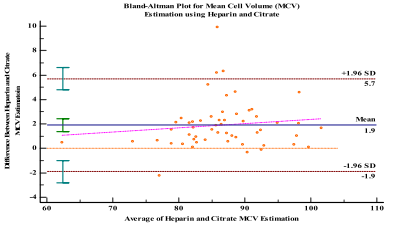
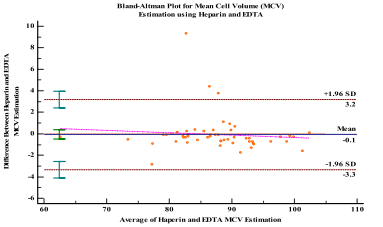
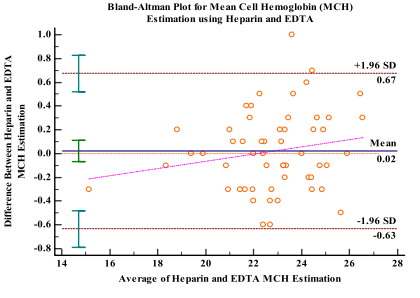
Generally, heparin agreed almost perfectly in the assessment of RBC (CCC = 0.992) but substantially agreed in the assessment of HGB (0.971), HCT (0.958) and MCH (0.987) when compared to K_3EDTA. All other measurements generated by heparin yielded poor agreements (CCC<0.90) with K_3EDTA. Citrate on the other hand agreed substantially with K_3EDTA in the assessment of LYMPH% (CCC = 0.964) but moderately in the assessment of MCV (CCC = 0.948) and MCH (0.913). When compared to K_3EDTA, heparin was highly precise and accurate in the estimation of HBG, RBC, HCT and MCH compared to only MCV and MCH by citrate (Table 6).

Table 4
Consistency in FBC results using Heparin, Citrate and K_3EDTA anticoagulants.

FBC Parameters	*ICC	95% CI	
		Lower Bound	Upper Bound
WBC (*10^9/L)	0.88	0.81	0.93
HGB (g/dL)	0.90	0.84	0.94
RBC (*10^12/L)	0.71	0.55	0.82
HCT (%)	0.87	0.80	0.92
MCV (fL)	0.94	0.91	0.97
MCH (pg)	0.99	0.98	0.99
MCHC (g/dL)	0.91	0.87	0.95
PLT (*10^9/L)	0.72	0.57	0.83
LYMPH# (*10^9/L)	0.84	0.75	0.90
MID# (*10^9/L)	0.61	0.40	0.76
GRAN# (*10^9/L)	0.92	0.87	0.95
LYMPH%	0.91	0.87	0.95
MID%	0.48	0.19	0.68
GRAN%	0.88	0.82	0.93

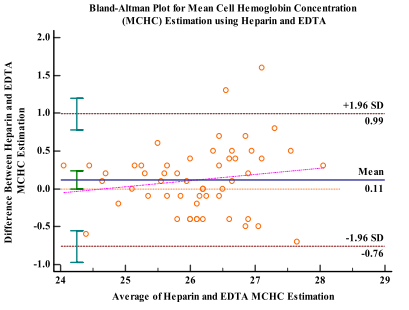
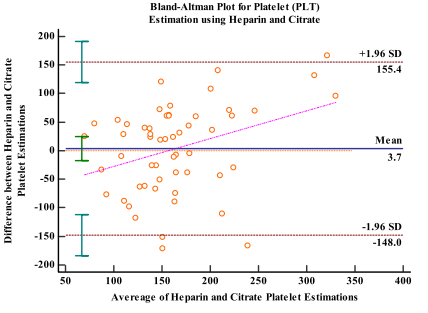
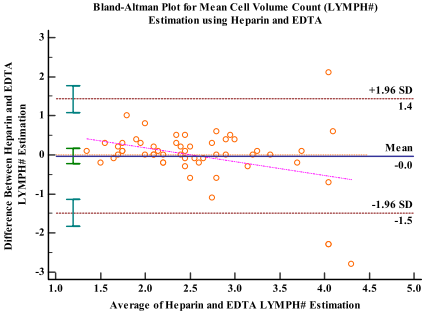
WBC- White Blood Cell; HGB-Hemoglobin; RBC-Red Blood Cell; HCT-Hematocrit; MCV-Mean Cell Volume; MCH-Mean Cell Hemoglobin; MCHC-Mean Cell Hemoglobin Concentration; PLT-Platelet; LYMPH#-Lymphocyte Count; MID#-Mid-range Absolute Count; GRAN#-Granulocyte Absolute Count; LYMPH%-Percent Lymphocyte Count; MID%-Percent Mid-range Absolute Count; GRAN%-Percent Granulocyte Count; EDTA-Ethylenediamine Tetraacetic Acid; FBC-Full Blood Count; CI-Confidence Interval. * Average reliability measure of the three anticoagulants.

Table 5
Bland Altman plot of agreement between the anticoagulant performance for FBC estimation.

FBC Variables	Heparin-Citrate	Heparin-K_3EDTA	Citrate-K_3EDTA
WBC (*10^9/L)	–	–	–
HGB (g/dL)	–	 Linear Regression coefficient = 0.076 $P = 0.0199$	–
RBC (*10^12/L)	–	–	–
HCT (%)	–	–	–
MCV (fL)	 Linear Regression coefficient = 0.279 $P = 0.0020$	 Linear Regression coefficient = 0.230 $P = 0.0163$	–
MCH (pg)	–	 Linear Regression coefficient = 0.030 $P = 0.1699$	–

(*continued on next page*)

Table 5 (*continued*)

FBC Variables	Heparin-Citrate	Heparin-K_3EDTA	Citrate-K_3EDTA
MCHC (g/dL)	–	 Linear Regression coefficient = 0.082 $P = 0.2593$	–
PLT (*10^9/L)	–	 Linear Regression coefficient = 0.489 $P = 0.0102$	–
LYMP# (*10^9/L)	–	 Linear Regression coefficient = −0.358 $P = 0.0062$	-

(*continued on next page*)

Table 5 (*continued*)

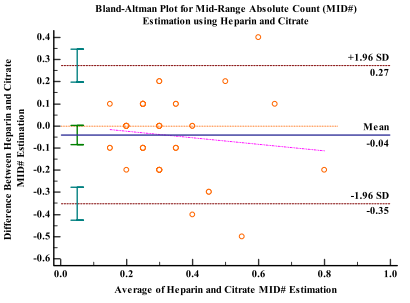
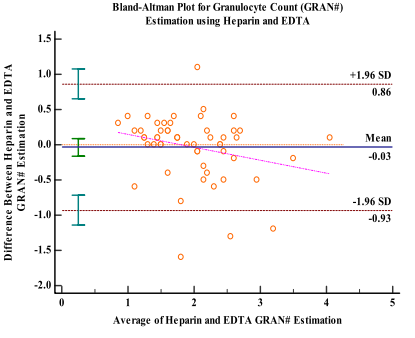
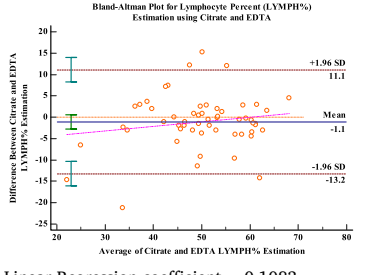
FBC Variables	Heparin-Citrate	Heparin-K_3EDTA	Citrate-K_3EDTA
MID# (*10^9/L)	 Linear Regression coefficient = −0.1445 P = 0.4138	–	–
GRAN# (*10^9/L)	–	 Linear Regression coefficient = −0.1445 P = 0.4138	–
LYMPH%	–	–	 Linear Regression coefficient = 0.1083 P = 0.2118

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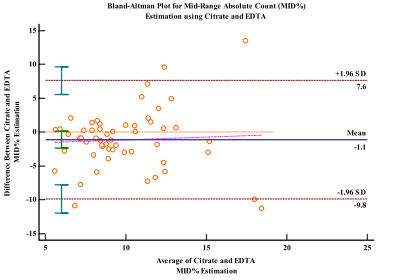
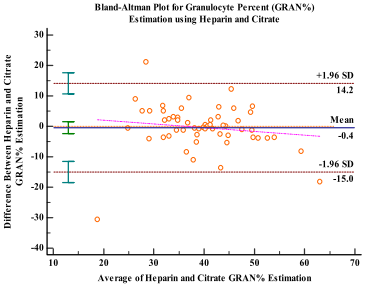
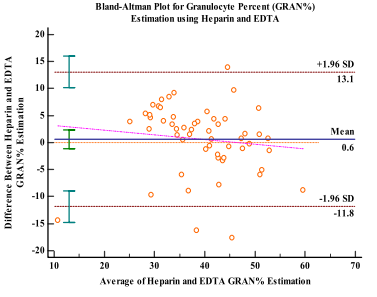
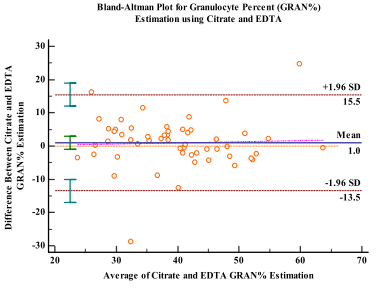
Table 5 (*continued*)

FBC Variables	Heparin-Citrate	Heparin-K_3EDTA	Citrate-K_3EDTA
MID%	–	–	 Linear Regression coefficient = 0.079 $P = 0.6980$
GRAN%	 Linear Regression coefficient = −0.1259 $P = 0.2906$	 Linear Regression coefficient = −0.08731 $P = 0.3886$	 Linear Regression coefficient = 0.03430 $P = 0.7627$

WBC- White Blood Cell; HGB-Hemoglobin; RBC-Red Blood Cell; HCT-Hematocrit; MCV-Mean Cell Volume; MCH-Mean Cell Hemoglobin; MCHC-Mean Cell Hemoglobin Concentration; PLT-Platelet; LYMPH#-Lymphocyte Count; MID#-Mid-range Absolute Count; GRAN#-Granulocyte Absolute Count; LYMPH%-Percent Lymphocyte Count; MID%-Percent Mid-range Absolute Count; GRAN %-Percent Granulocyte Count; K_3EDTA-Tripotassium Ethylenediamine Tetraacetic Acid; FBC-Full Blood Count.
Line of equality (difference = 0): Reddish-Yellow.
Regression Line: Pink.
P: p value.
-: One sample *t*-test of the difference in the FBC results using the two test reagents was statistically significant ($P < 0.05$) hence does not qualify for Bland-Altman Plot.

Table 6
Lin's concordance correlation coefficient (CCC) to assess the level of agreement of heparin and citrate anticoagulants using K_3EDTA as reference standard.

FBC Parameters	K_3EDTA-Heparin	Pearson's ρ (Precision)	Bias Corrections Factor (Accuracy)	K_3EDTA-Citrate	Pearson's ρ (Precision)	Bias Corrections Factor (Accuracy)
WBC (*10^9/L)	0.653 (0.482–0.776)	0.683	0.957	0.367 (0.236–0.485)	0.707	0.520
HGB (g/dL)	0.971 (0.951–0.982)	0.974	0.997	0.227 (0.126–0.324)	0.628	0.362
RBC (*10^12/L)	0.992 (0.986–0.995)	0.994	0.998	0.198 (0.063–0.326)	0.396	0.500
HCT (%)	0.958 (0.930–0.975)	0.966	0.992	0.189 (0.096–0.278)	0.576	0.327
MCV (fL)	0.805 (0.696–0.878)	0.824	0.977	0.948 (0.917–0.967)	0.978	0.969
MCH (pg)	0.987 (0.978–0.992)	0.987	0.999	0.913 (0.864–0.945)	0.962	0.950
MCHC (g/dL)	0.863 (0.778–0.917)	0.872	0.985	0.750 (0.613–0.843)	0.782	0.958
PLT (*10^9/L)	0.230 (0.089–0.361)	0.442	0.519	0.285 (0.173–0.391)	0.684	0.417
LYMPH# (*10^9/L)	0.611 (0.430–0.745)	0.639	0.957	0.374 (0.236–0.498)	0.668	0.560
MID# (*10^9/L)	0.116 (-0.022–0.251)	0.228	0.511	0.284 (0.117–0.435)	0.448	0.634
GRAN# (*10^9/L)	0.773 (0.645–0.858)	0.784	0.986	0.584 (0.439–0.700)	0.786	0.743
LYMPH%	0.713 (0.572–0.814)	0.773	0.923	0.964 (0.560–0.793)	0.798	0.870
MID%	0.086 (-0.043–0.212)	0.180	0.477	0.277 (0.026–0.495)	0.290	0.956
GRAN%	0.760 (0.623–0.852)	0.764	0.995	0.706 (0.545–0.817)	0.710	0.994

WBC- White Blood Cell; HGB-Hemoglobin; RBC-Red Blood Cell; HCT-Hematocrit; MCV-Mean Cell Volume; MCH-Mean Cell Hemoglobin; MCHC-Mean Cell Hemoglobin Concentration; PLT-Platelet; LYMPH#-Lymphocyte Count; MID#-Mid-range Absolute Count; GRAN#-Granulocyte Absolute Count; LYMPH%-Percent Lymphocyte Count; MID%-Percent Mid-range Absolute Count; GRAN%-Percent Granulocyte Count; K_3EDTA-Tripotassium Ethylenediamine Tetraacetic Acid; FBC-Full Blood Count.

4. Discussion

The choice of anticoagulant and its use at an appropriate concentration are important *apriori* considerations that must be made at the preanalytical stage in the haematology laboratories [19]. In this study, comparison of FBC results generated by all three anticoagulants yielded statistically comparable ($p > 0.05$) median MCV, MCH, MCHC, LYMPH% and GRAN% results. Pairwise comparison revealed similarity in the median MCV, PLT, MID#, LYMPH% and GRAN% for heparinized and citrated blood samples. Citrate and K_3EDTA produced comparable results for median MCV, MCHC, LYMPH%, MID% and GRAN% whereas Heparin and K_3EDTA on the other hand yielded similar results in 50.0% (7/14) of the FBC parameters including WBC, MCH, MCHC, PLT, LYMPH%, GRAN# and GRAN%. Largely, citrated blood produced FBC results considerably lower than those generated by heparin and K_3EDTA.

Similar observation was made by Ali and colleagues [8] in their study which assessed the performance of complete blood count in rats when different anticoagulants were used. They concluded that HCT and MCV showed a marked decrease ($P < 0.01$) in citrated sample compared to heparinized and EDTA sample. They further mentioned that the value of total leukocyte count and absolute count of neutrophil, monocyte and eosinophil showed a significant ($P < 0.01$) decrease in citrated sample than that of EDTA and heparinized sample. Concerning differential leukocyte count, they noted that the relative count of all parameters including neutrophil, lymphocyte, monocyte, and eosinophil showed a significantly ($P < 0.01$) lower values in citrated sample. They advised that heparin and EDTA were better anticoagulants than sodium citrate because samples anticoagulated with sodium citrate resulted in significant changes in blood parameters. In another study, using blood from healthy dogs, haemoglobin concentration, haematocrit and platelet counts were significantly lower in citrated blood than noted in K_3EDTA blood [13] akin to that observed in this study even though this study was conducted using human blood. Research has established the inability of haemoglobin to be oxidized in citrate for which reason lower HGB values are often recorded in citrated blood [13]; the ripple effect could be the cause of lower HCT values in citrated blood compared to heparinized and K_3EDTA anticoagulated blood observed in this study. In relation to PLT, citrate has been adjudged a strong platelet activator in sick animals, causing the spontaneous formation of PLT microaggregates which often fail recognition by modern automated haematology analyzers [20]. Though this study was conducted among apparently healthy humans, we hypothesize that this phenomenon could be a contributory factor for the low platelet count recorded in the citrated blood compared to the heparinized and K_3EDTA sample as seen in this study. While other studies however reported the widely observed EDTA-dependent

pseudothrombocytopenia (EDTA-PCTP) [21,22], we report contrary results with K_3EDTA yielding higher PLT count compared to heparin and citrate. This could be because rather than aggregative effect as observed in K_2EDTA [22], K_3EDTA causes swelling of platelets instead with minimal reduction effect on PLT count but increase in MPV [23]. It is however also possible that the significant variations observed in the preponderance of the FBC components could be because of time associated storage effects such as the morphological alterations on different component of FBC which causes them to be incorrectly counted by automated haematology analyzers [24]. Nonetheless, because the samples for this study were analyzed within a time space of 3–6 h post collection, judging from the observations made by Vives-Corrons and colleagues [24], it is possible that other factors, for example, inherent analytical faults with haematology analyzer, blood sample storage temperature [18,25] etc., may have influenced the variation observed in the FBC parameters compared to those cited earlier.

We observed excellent consistency (ICC>0.90) in the estimation of MCV, MCH, MCHC, GRAN# and LYMPH% while moderate consistency was observed in the estimation of RBC (ICC = 0.71), PLT (ICC = 0.72) and MID# (ICC = 0.61) across the three anticoagulants. This is an indication that extra care needs to be taken by a laboratory that decides to opt for any of these three anticoagulants for purposes of haematological analysis of patient's full blood count. This is particularly important because EDTA, for example at optimal concentration has been observed to initiate striking morphological changes at about 12–18 h after collection. Again, time-related and anticoagulant concentration-associated changes in PLT morphology from discoid to spherical and swelling have also been reported to occur in specimens collected in EDTA and as a result, the mean PLT volume (MPV) may not entirely be a reliable value [25]. It is therefore not too surprising to have observed in the current study that comparable PLT count was generated by heparinized and citrated blood but that produced by K_3EDTA anticoagulated blood was exceptionally high contrary to other reports [26].

This study further assessed the level of agreement in the estimation of FBC parameters using the three anticoagulants. Of the FBC parameters that qualified for the Bland-Altman plot, heparin and citrate agreed quite well in the measurement of MID# and GRAN% but with a significant proportional bias in the measurement of MCV. Citrate and EDTA anticoagulated blood agreed in the estimation of LYMPH%, MID% and GRAN#, with a significant level of proportional bias exhibited in the measurement of MID%. We observed that heparin and K_3EDTA largely agreed on most of the FBC parameters including HGB, MCV, MCH, PLT, LYMPH#, GRAN# and GRAN%. Literature is quite naïve on similar studies conducted using human blood for which reason comparative analysis becomes quite impossible. However, different levels of agreements in the estimation of hematological parameters in heparinized and EDTA samples in Hispaniolan Amazon Parrots have been reported [5]. Another study which compared K_2EDTA and K_3EDTA vacuum tubes reported clinically relevant variations for MCV, MPV, RDW, and PCT [27]. Our findings suggest that using citrate as a substitute for heparin or for K_3EDTA will most likely produce erroneous FBC results that will not agree with heparin or K_3EDTA anticoagulated blood probably because of the consistently low FBC values produced by citrated blood in majority of the FBC parameters. The citrate-mediated FBC cellular variation observed in the current study agrees in part with report by Weber and Nakashima [6] who noted that citrate as an anticoagulant induced a negative bias in PLT count which bias worsened over time and therefore advised that analysis is performed as soon as blood sample is drawn. Consequently, heparin appears to be a next choice of anticoagulant in FBC estimation in place of EDTA however, that assertion may not entirely be accurate per further observations from this study.

Considering EDTA as the most widely used anticoagulant in the estimation of FBC particularly because of its ability to irreversibly sequester ionized calcium (Ca^{2+}), thus permanently inhibiting blood coagulation and enabling a better stability of anticoagulated blood for purposes of cell enumeration and sizing [5,28,29], this study further assessed the agreement of heparin and citrate anticoagulants using K_3EDTA as the reference standard. Surprisingly, heparin produced almost perfect agreement only in the assessment of RBC while a substantial agreement was observed in the assessment of HGB, HCT and MCH. Citrate on the other hand agreed substantially with K_3EDTA in the assessment of LYMPH% and moderately in the assessment of MCV and MCH. Compared to K_3EDTA, heparin was highly precise and accurate in the estimation of HBG, RBC, HCT and MCH compared to only MCV and MCH by citrate. However, the poor level of precision in the estimation of other important FBC components such as WBC, PLT etc. renders heparin not an entirely better alternate anticoagulant to K_3EDTA in the estimation of FBC and hence, its utility must be based on thorough clinical understanding of this shortfalls.

It must be emphasized however that this study employed K_3EDTA in the estimation of the FBC parameters. This subtype of EDTA has been noted to among other things cause extensive platelet swelling over time and results in an increase in mean platelet volume (MPV) [23]. Also, since K_3EDTA is dispensed as a liquid, it causes a slight dilution of specimen resulting in all directly measured values including HGB, RBC, WBC counts and PLTs being 1–2% lower than the circulating blood values; it also affects the red blood cell size at increased concentrations and on storage than the K_2EDTA [30]. These factors informed the International Council for Standardization in Haematology in 1993 to recommend K_2EDTA as the anticoagulant of choice in specimen collection for blood cell counting and sizing [14]. The shortfalls of K_3EDTA which might have caused the results of the EDTA anticoagulated blood not to be reflective of the actual cellular counts *in-vivo* could affect the true agreement and consistencies between the anticoagulants in this study. Also, this study collected only 1.6 ml of whole blood less than the 2.0–4.0 ml required range of blood volume into K_3EDTA and lithium heparin for the analysis of FBC and this may affect the results generated for samples such as observed by Chen, Fong [31]. In K_2EDTA however, underfilling with as little as 1.0 ml of whole blood produced acceptable FBC results [32,33] making it a better anticoagulant of choice [14]. Further, the possible dilution effect of the anticoagulants may affect the results of this study, therefore further research should include a consideration to control potential dilution effects on the overall outcome of the study. This study included apparently healthy students aged 18–34 years and this may affect the outcome of the study since individuals below and above this age range were excluded (the university is largely composed of young students within the recruited age range). Finally, the study failed to employ sex stratifications to determine result variations between males and females and this may also negatively influence the outcome of the study. Further studies should hence consider sex-based stratifications to inform readers on the possible sex related variations in FBC generated using the three main anticoagulants.

5. Conclusions

This study showed significant decreases in the values of a significant number of the FBC parameters in citrated blood when compared to heparin and K_3EDTA. Furthermore, citrate exhibited poor precision and accuracy in most of the FBC parameters compared to K_3EDTA. Citrate is therefore not advisable as anticoagulant for the estimation of FBC as it produces spurious values that may affect patient's overall treatment outcome. The overall effect of heparin on FBC though not perfect is very tolerable as heparin didn't only produce comparable FBC values to that of K_3EDTA but also yielded high precision and accuracy in very important components of the FBC parameters, including the patient's hemoglobin. Nonetheless, the use of heparin as an alternate anticoagulant to K_3EDTA should be done with extreme caution.

Author contribution statement

Elliot Elikplim Akorsu: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Linda Brown Adjabeng; Maridiatu Amir Sulleymana: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Precious Kwadzokpui Kwadzokpui: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available on request.

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Additional information

Supplementary content related to this article has been published online at [URL].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17311.

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