Original Article

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Pathology Section

Underfilled K2EDTA Vacutainer on Automated Haematological Blood Cell Indices- To Reject or Reconsider?

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ABSTRACT

Introduction: The most common reason for rejection of blood samples in laboratory is because they are under-filled. Clinical and Laboratory Standards Institute (CLSI) guidelines state that blood vacutainers that contain either 10% higher or lower blood than the recommended volume must be rejected. Repeated phlebotomy has to be performed for such samples which can delay the turnaround time.

Aim: To study the effects of underfilled K2EDTA vacutainers on automated haematology indices and compare them with the standard.

Materials and Methods: This observational and comparative study was conducted at the department of pathology, Chettinad Hospital and Research Institute, Tamil Nadu for a duration of 6 months from January 2019 to June 2019. A 100 paired samples of underfilled K2EDTA vacutainers were compared with the standard for Complete Blood Count (CBC) analysis using BECKMAN COULTER LH 780 analyser. Statistical analyses was done using Statistical Package for Social Science (SPSS) software v.18 and paired student's t-test. The p-value <0.05 was considered statistically significant.

Results: All the parameters in the CBC showed comparable results between under-filled K2EDTA vacutainers of 1 mL blood volume and standard 3 mL blood volume vacutainers. There was no significant variation in statistics even when the blood volume is as low as 1 mL which is 67% less than that of the recommended volume.

Conclusion: Blood samples which are under-filled upto 67% less than recommended volume, i.e. upto 1 ml can be accepted for CBC analysis using BECKMAN COULTER LH 780 analyser. Therefore, the CBC data obtained from the under-filled vacutainers can be used for clinical diagnosis in situations where repeat blood collection is challenging or impossible.

Keywords: Automated analyser, Blood volume, Complete blood count, Ethylene diamine tetra acetic acid (dipotassium salt), Underfilling

INTRODUCTION

As per the guidelines of the Clinical and Laboratory Standards Institute [1] (CLSI, 2004) document, Procedures for the Handling and Processing of Blood Specimens, vacutainers that contain lesser or higher volumes of blood than recommended should be rejected. This was upheld by CLSI guidelines that the amount of anticoagulant in the vacutainers is designed for a given volume of blood (1.5-2 mg per mL) [2] and thus, under-filling or over-filling of vacutainers can antagonistically influence the results. Also, the difference in the blood volume for any type of vacutainer should not be more than 10% of the recommended level [2]. Underfilling of vacutainers are among the most common cause for sample rejection [3,4]. Following rejection of any blood sample, a repeat phlebotomy is required which can cause patient inconvenience especially in pediatric and elderly, delay in turnaround time, economic burden and procedure related risks and complications [5-7].

Routinely, Ethylene Di Amine Tetra Acetic Acid (EDTA) is the anticoagulant of choice for haematology samples [8]. Both dipotassium (K2EDTA, K3EDTA) and disodium salts are powerful anticoagulants but because sodium salts are less soluble in blood compared to potassium salts, potassium salts are preferred [9]. Since K3EDTA causes slight dilution, the anticoagulant of choice is K2EDTA [10]. K2EDTA has maximum chelating effect on calcium at a concentration of 1.5-2.0 mg/mL [11].

According to the available studies high concentration of liquid K3-EDTA compared to the blood volume causes increase in the ionic concentration thus making the plasma hypertonic, which in turn causes shrinkage of red cells and alter their morphology [12-15]. K3EDTA also affects WBC by causing membrane damage [13]. It can also cause platelet disintegration or abnormally enlarged platelets resulting in spurious thrombocytopenia or thrombocytosis. Hence, underfilled K3EDTA vacutainer can alter the blood parameters significantly. However, previous studies done on underfilled K2EDTA vacutainers show that there is no alteration in the haematological parameters for under-filling upto 75% of the recommended volume. However, more studies need to be carried out using other haematology analysers in other laboratories as well [9,16,17]. Also, CLSI guidelines are set based on the research done on liquid K3EDTA and not on spray dried K2EDTA vacutainers, which may yield different results. Only a few studies [9,16,17] have been conducted on underfilled K2EDTA vacutainers which has prompted us to conduct this study to know its effect on haematological parameters.

MATERIALS AND METHODS

The present study was an observational and comparative study conducted at the department of pathology, Chettinad Hospital and Research Institute, Tamil Nadu for a duration of 6 months from January 2019 to June 2019. The study was carried out after obtaining approval from the institutional human ethics committee (278/IHEC/1-19). A total of 100 paired samples including both healthy individuals and patients willing to participate in the study between the age groups of 18-65 years were included. The recommendation of Bland Altman [18] was as followed to obtain sample size of 100 for looking the level of agreement between two clinical methods. All patients except pediatric and geriatric cases for whom CBC was requested were included in the study. The volunteers were explained the purpose of this research and an

informed consent was obtained. Paediatric, geriatric and pregnant patients were excluded from the study.

Sample Collection

From each volunteer two venous blood samples were taken from a single cubital region by standard venipuncture protocol and were collected into two spray dried K2EDTA vacutainers (BD vacutainer). A total of 4 mL blood was drawn from each individual. In one vacutainer 3 mL of blood was drawn and in another vacutainer 1 mL of blood was drawn. Level of blood filling was marked externally on the vacutainers. The one marked at 1 mL was labelled as A (underfilled EDTA), other one marked at 3 mL waslabelled as B (standard). Each vacutainer was then inverted to 180° for 5-6 times to ensure proper mixing of anticoagulant and blood.

Laboratory Analysis

Samples were analysed using BECKMAN COULTER LH 780 automated analyser (Beckman coulter, India). Blood samples were analysed on the same day. All the samples were analysed only when the quality controls were within acceptable limits. Haemolysed, grossly contaminated and clotted samples were rejected. The values obtained were entered in the spreadsheet. The coulter method analyses was based on electrical impedance and Volume Conductance Scatter (VCS) technology. RBC count, WBC total count and platelet count were estimated by electrical impedance. Other RBC parameters such as MCV, MCH, MCHC, RDW were calculated. WBC differential analysis was based on VCS technology. Haemoglobin was estimated by cyanmethaemoglobin method; following WBC count the lysed WBC dilution drains into haemoglobin cuvette. A beam of white light from an incandescent lamp goes through the cuvette and through an optical filter that had a transmission wavelength of 525 nm.

STATISTICAL ANALYSIS

Collected data was statistically analysed using SPSS software v.18. Standard deviation, standard error, mean difference and 95% confidence interval were calculated. Paired student's t-test was used to analyse the results. The data from under-filled EDTA vacutainers were compared with the standard collection. The p-value <0.05 was considered statistically significant.

RESULTS

The comparison of the results between under-filled 1 mL K2EDTA vacutainers and standard 3 mL K2EDTA vacutainer are given in [Table/Fig-1].

Hundred samples of under-filled vacutainers were compared with the standard volume. RBC parameters such as RBC count, HBG, MCV, MCH, MCHC, RDW, HCT; WBC parameters such as total count and differential count; platelet count and MPV were comparable by statistical analysis between both under-filled vacutainers and standard volume [Table/Fig-1].

The result of this study shows that all the parameters in the CBC showed comparable results by statistical analysis between under-filled K2EDTA vacutainers (1 mL) and standard 3 mL vacutainers. There was no significant variation in statistics even when the blood volume was as low as 1 mL which is 67% less than that of the recommended volume.

Sl No.	Parameters with volume drawn	SD	SE	Mean difference	95% Confidence interval		p-value
					Upper bound	Lower bound	
1	**RBC(x10^{12}/l)**						
	1 mL	0.537	0.053	0.01	4.65162	4.44097	0.9884
	3 mL	0.591	0.059		4.65058	4.41861	
2	**HBG(g/dL)**						
	1 mL	2.331	0.233	0.04	12.7049	11.7910	0.9015
	3 mL	2.325	0.232		12.7447	11.8332	
3	**MCV(fl)**						
	1 mL	10.03	1.003	0.25	89.9505	81.0174	0.8624
	3 mL	9.94	0.994		84.6864	80.7875	
4	**MCH(pg)**						
	1 mL	3.93	0.39	0.02	27.6772	26.1347	0.9684
	3 mL	3.88	0.38		27.6463	26.1216	
5	**MCHC(g/dL)**						
	1 mL	1.64	0.164	0.68	32.4125	31.7694	0.5614
	3 mL	1.60	0.1600		32.5387	31.9113	
6	**RDW(%)**						
	1 mL	2.810	0.281	0.04	16.0069	14.9050	0.9225
	3 mL	2.826	0.282		15.9710	14.8629	
7	**PLT(x10^{9}/l)**						
	1 mL	81.85	8.18	11.00	294.333	262.247	0.5363
	3 mL	81.14	8.11		304.904	273.095	
8	**MPV (fl)**						
	1 mL	1.24	0.124	0.14	9.07717	8.59083	0.5265
	3 mL	1.26	0.126		8.94035	8.44364	
9	**HCT(%)**						
	1 mL	6.45	0.645	0.05	39.1012	36.5727	0.9575
	3 mL	6.41	0.641		39.0447	36.5318	
10	**WBC (x10^{9}/l)**						
	1 mL	2.82	0.282	0.12	8.96187	7.85412	0.7698
	3 mL	2.88	0.288		9.09325	7.96074	
11	**NE(%)**						
	1 mL	10.51	1.05	0.05	63.3841	59.2638	0.9743
	3 mL	10.48	1.04		63.3305	59.2214	
12	**LY(%)**						
	1 mL	8.90	0.89	0.10	29.1561	25.6639	0.9351
	3 mL	8.88	0.88		29.2539	25.7720	
13	**MO(%)**						
	1 mL	2.54	0.25	0.11	7.80745	6.80854	0.7715
	3 mL	2.48	0.24		7.90043	6.92756	
14	**EO (%)**						
	1 mL	3.99	0.399	0.06	4.13348	2.56851	0.9156
	3 mL	3.96	0.396		4.18905	2.63294	
15	**BA (%)**						
	1 mL	0.53	0.053	0.07	0.68203	0.47196	0.2858
	3 mL	0.36	0.036		0.57940	0.43459	

[Table/Fig-1]: Comparison of haematological parameters in underfilled vacutainers with standard vacutainers.
Paired student's t-test; p-value not statistically significant
RBC: Red blood cell count; HBG: Haemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT: Platelet count; MPV: Mean platelet volume; HCT: Haematocrit; WBC: White blood cell count; NE: Neutrophils; LY: Lymphocytes; MO: Monocytes; EO: Eosinophils; BA: Basophils; SD: Standard deviation; SE: Standard error

DISCUSSION

Blood samples collected in both K2- and K3EDTA can be stored at 4°C without significant alteration of blood parameters for 24 hours [11]. K2EDTA is preferred over K3EDTA because it is more hypertonic and causes erythrocyte dehydration and shrinkage [19]. Another drawback of K3 EDTA is that as it is a liquid anticoagulant it results in the dilution of the blood specimen. It lowers most of the measurements including RBC, WBC, platelet, MCV and Haemoglobin [19]. Underfilling of vacutainers leads to an excess of EDTA, which can cause platelet disintegration or abnormally enlarged platelets resulting in spurious thrombocytopenia or thrombocytosis. It can also alter RBC and WBC morphology. Previous studies done on the use of underfilled K2EDTA vacutainers for CBC analysis

showed contradicting results. ForLH 700 series, the instrument aspirates only 0.3 mL of sample in automatic aspiration mode and only 0.2 mL by manual aspiration mode. However, at least 1 mL of sample with proper proportion of anticoagulant is required for automatic haematology analysers as per guidelines [20].

Only few studies have been done on underfilled K2EDTA vacutainers and the results of our study are in line with some of the earlier studies [9,16]. In the present study, the results obtained shows that underfilling upto 1 mL does not alter the haematological parameters and showed comparable results with recommended volume.

A study by Gupta V et al., showed that underfilling of K2 EDTA vacutainers upto 1 mL did not affect the haematological parameters in healthy individuals [9]. These results are in concordance with the present study. In addition, our study population also included blood samples obtained from patients along with healthy individuals. Hence this study also proves that the CBC results obtained from underfilled K2EDTA vacutainers are comparable between healthy and diseased individuals.

Xu M et al., compared standard 4 mL to underfilled 2 mL, 1 mL and 0.5 mL K2EDTA vacutainer on healthy blood donors using Sysmex XE-2100 [16]. This study showed that upto 1 ml, all the parameters were comparable to the standard 4 mL excluding platelet count, lymphocyte percentage and automated reticulocyte count. However, when underfilling is done upto 0.5 mL, only the RDW, MCV and neutrophil percentage were comparable, all the other parameters showed significant variation. The present study showed comparable results in all parameters upto 1 mL. However, reticulocyte count and underfilling <1 mL was not included in the study.

A study by Pan L et al., compared the standard 3 mL sample to underfilled K2EDTA of 1 mL, 0.6 mL and 0.3 mL in various clinical settings [17]. This study showed that when the blood sample volume was reduced from 1 mL to 0.3 mL, there was a significant increase in the variation of haematological parameters like RBC, Hb, HCT and MCV. In contrast our study showed no significant variation between 3 mL and 1 mL sample.

Limitation(s)

The limitations in the present study include that only underfilling upto 1 mL was compared and only one type of haematology analyser was used. Also, the present study did not compare the effect of excess anticoagulant on the cell morphology. Blood samples from paediatric patients in whom underfilling of vacutainer is more commonly encountered was not included in this study.

CONCLUSION(S)

The present study did not show any significant variation in the parameters between the underfilled K2EDTA vacutainers (1 mL) and the standard volume for CBC analysis using automated analyser. Therefore, the CBC results generated from the under-filled vacutainers can be accepted for clinical diagnosis in conditions where repeat phlebotomy is a concern or impossible. This will reduce the delay in reporting the results caused due to rejection of under-filled K2-EDTA vacutainers without affecting the results. However, more studies of this type needs to be replicated in other laboratories with various other automated analysers in healthy as well as various pathological conditions in order to revise the current CLSI guidelines which are mainly based on liquid K3EDTA vacutainers.

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