RESEARCH ARTICLE

Evaluation of di-potassium and tri-potassium EDTA evacuated tubes for routine haematological testing

Rubaida Mehmood¹ | Rabia Khushi Muhammed² | Sajjid Hussain¹ | Aniq Sana³

¹PAEC, MINAR, Multan, Pakistan

²Department of Chemistry, Women University Multan, Multan, Pakistan

³Department of Chemistry, BZ University, Multan, Pakistan

Correspondence

Rubaida Mehmood, PAEC, MINAR, Multan, Pakistan.

Email: rubaidamehmood@hotmail.com

Background: The influence of blood collection devices on laboratory tests is often overlooked. We have discussed here how blood collection materials and devices can alter haematological test results, with an emphasis on blood collection tube (BCT) additives. We have compared K2 & K3-EDTA vacuum tubes of same manufacturer IMPROVACUTER[®] for CBC analysis.

Methods: Blood was collected in both tubes simultaneously. Samples were analysed immediately after collection (<15 minutes) and after 4 hours by MEK-6318J/K Haematology Analyzer. Paired student's *t*-test was applied for statistical analysis. Significant differences among results and biases were compared with current quality specifications.

Results: Significant differences were found in Neutrophils (GR) and mean platelets volume (MPV) when compared. K2-EDTA vs K3-EDTA(1st time) after < 15 minutes of sample collection, for Monocytes counts (MO), MCV, MPV, and RDW when comparing K2-EDTA (first time) vs K2-EDTA(second time, after 4 hours); and for Monocytes counts (MO), MCV, MPV, PCT, and RDW when comparing K3-EDTA(1st time) vs K3-EDTA(2nd time) in order to check the stability and reproducibility of vacutainers.

Conclusions: This study assesses the use of K2& K3-EDTA vacuum tubes whether or not they represent a bias in haematological testing (CBC). The use of K2-EDTA and K3-EDTA vacuum tubes represent a clinically relevant source of variation for MCV, MPV, RDW, and PCT.

KEYWORDS

CBC, K2-EDTA, K3- EDTA, reproducibility, stability

1 | INTRODUCTION

Pre-examination errors can be numerous (eg, inaccurate patient identification, imprecise order of draw, incorrect use of additive tubes, labeling errors, incorrect timing of collection, and clerical errors). Standard procedures and protocols are intended to prevent these problems and protect against complications.¹ The procedures involving phlebotomy, eg, time of tourniquet application²⁻⁴ and blood drawing using vacuum tubes^{5,6} are poorly studied as potential sources of errors.

Throughout the world, three different salts of the chelating agent ethylene di-amine tetra-acetic acid (EDTA: C₁₀H₁₆N₂O₈) are being used

for haematological testing: disodium, di-potassium, and tri-potassium EDTA. The potassium salt has an advantage over the sodium salt in that they are more readily soluble in the blood when present in the dry form in the collection container; however, all three salts have been shown to affect red blood cell size, especially after storage of anti-coagulated specimens for a number of hours.⁷ Di- and tri-potassium (K2 and K3) salts of ethylenediaminetetraacetic acid (EDTA) are the standard anticoagulants used for routine blood counts. K2-EDTA is mainly used in the United States, while, in Europe and Japan, K3-EDTA is the preferred preparation.⁸ The aim of this study was to evaluate K2-EDTA and K3-EDTA vacuum tubes of IMPROVACUTER[®] for routine haematological testing.

2 | MATERIALS AND METHODS

2.1 | Study design

A group of 62 apparently healthy individuals that were referred to MINAR for CBC analysis were included in this study.

2.2 | Collection of diagnostic blood specimens

The collection of all diagnostic blood specimens was performed by a single, expert Phlebotomist, following the international standard by Clinical Laboratory Standard Institute-CLSI.⁹ A batch of 15 individuals approximately was tested on a daily basis. All the individuals were relaxed prior to blood sampling on blood sampling chair. The blood was collected in IMPROVACUTER® EDTA tubes, and two amounts of blood were consecutively drawn by venipuncture with 20G straight needles (Master-A lot. no 20141110. Pakistan), directly into two different sets of vacutainers containing ethylenediaminetetraacetic acid di-potassium salt (K_2 EDTA); Tube I: 2 mL, lot no. B56003. Ethylenediaminetetraacetic acid tri-potassium salt (K_3 EDTA); Tube II: 3 mL lot no. C66006 of same company IMPROVE.

2.3 | Laboratory testing

All samples were processed for routine haematology testing immediately after collection (<15 minutes) on the MEK-6318J/K Haematology Analyzer (Manual Code No. 0614-004583F, International Div., Sales Promotion Section, Nihon Kohden Corp., Tokyo, Japan). Parameters included to test were white blood cell (WBC), red blood cell count (RBC), haematocrit (HCT), haemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCHC), RBC distribution width (RDW), counts and differential, included lymphocytes (LYMPHO), monocytes (MONO), neutrophils (NEU), platelet count (PLT), mean platelet volume (MPV), and platelet distribution width (PDW). The instrument was calibrated against appropriate proprietary reference standard material and verified with the use of proprietary controls. A multicentre evaluation of the within-run precision of the MEK-6318J/K Hematology Analyzer showed coefficients of variation (CVs) of <2.0% for WBC, for LY within 5.0%, for MO within 12.0%, for GR within 5.0%, for platelets <4.0%, for RBC <1.5%, for haemoglobin <1.5% and for MCV was <1.5%.¹⁰

2.4 | Statistical analysis

The significance of the differences between K_2 EDTA vacuum tubes and K_3 EDTA vacuum tubes of IMPROVACUTER® for collection of diagnostic blood specimens were assessed by paired Student's *t*-test after checking for normality by the D'Agostino-Pearson omnibus test.¹¹ As non-normal distribution was found for MCV, MCH, MCHC, RDW, and PDW, results were assessed by Wilcoxon ranked-pairs test. The level of statistical significance was set at $P < .05$. Finally, the biases from Tube I and Tube II in the first time (<15 minutes) and second time (after 4 hours) were compared with the current desirable quality

specifications for bias (*B*), derived from biological variation according to the formula $B < 0.25 (CV_w^2 + CV_g^2)^{1/2}$ where CV_w and CV_g are, respectively, within- and between-subject CVs, derived from biological variation.¹² The biases for each tube were calculated according to the formula: mean difference (%) = [(test tube mean - reference tube mean) / reference tube mean × 100].¹³

3 | RESULTS

The main results obtained from this study are shown in Table 1. Significant differences were recorded for, NEU and MPV when comparing Tube I vs Tube II (1st time); for MO, MCV, MPV, and RDW when comparing Tube 1 (first time vs second time) in order to check the stability of vacationers and for MO, MCV, MPV, PCT, and RDW when comparing Tube II (first time vs second time).

No significant difference ($P > .05$) was observed by repeated-measures ANOVA for WBC ($P = .97$), RBC ($P = .99$), HGB ($P = .94$), LYMPHO ($P = .78$), MONO ($P = .18$), NEU ($P = .77$), PLT ($P = .95$) and MPV ($P = .29$) as well as for, MCH ($P = .25$), MCHC ($P = .88$), PDW (.19) and RDW ($P = .68$) by the Wilcoxon ranked-pairs test. Finally, biases were calculated by comparing with the current desirable quality specifications.¹² Clinically significant variation was found for MPV when comparing between Tube 1 and Tube 11 (first time) and for MCV, MPV, and RDW between Tube 1 (first time vs second time) and for MCV, PCT, MPV, and RDW between Tube (first time vs second time).

4 | DISCUSSION

Pathology laboratories play a central role in patient care and diagnosis. Though there is lot of automation in haematology and clinical pathology labs, still there are many variables which can influence the lab results.¹⁴ Modern automated haematology systems are designed to meet the needs of high volume laboratories. As medical devices, blood collection tubes should achieve the intended performance levels during defined conditions of use.¹⁵ Even small differences in the concentration of the anticoagulant in blood collection tubes may produce appreciable differences in haematology test results.¹⁶

The results of the present study showed that MPV is influenced when comparing Tube-1 vs Tube-11. With regard to MPV in terms of clinical significance as shown in Table 1, elevated MPV levels have been identified as an independent risk factor for myocardial infarction in patients with coronary heart disease.¹⁷ Abnormally low MPV values correlate with thrombocytopenia when it is due to impaired production as in plastic anaemia. In addition, low MPV can correlate with abnormally small platelet size, sometimes a symptom of a spectrum referred to as Wiskott-Aldrich Syndrome (WAS).¹⁸

When the vacutainers were tested after 4 hours of blood collection in order to check the reproducibility and stability, it has been shown that CBC parameters remain stable for 72 hours, except mean platelet volume, which slightly increased between 48 and 72 hours, at 4°C,¹⁹ but our results indicate the differences in MCV, MPV, PCT,

TABLE 1 Variability in haematological parameters with K2-EDTA and K3-EDTA in first time (<15 min) and second time (after 4 h)

Haematological parameters (units)	Comprehensive results		Mean % difference (P value)						
	Tube 1 (K2 1st time)	Tube 2 (K3 1st time)	Tube 1 (K2 2nd time)	Tube 2 (K3 2nd time)	Tube 1 vs Tube 2 1st time	Tube 1 vs Tube 2 2nd time	Tube 1 vs Tube 2 time vs 2nd time	Tube 2 vs Tube 2 1st time vs 2nd time	Desirable Bias (%)
WBC ^a × (10 ³ /μL)	7.28 ± 1.90	7.17 ± 1.79	7.321 ± 1.88	7.224 ± 1.72	1.5 (0.05)	1.4 (0.08)	-0.5 (0.405)	-0.7 (0.45)	6.05
RBC ^a × (10 ⁶ /μL)	4.73 ± 0.70	4.71 ± 0.77	4.71 ± 0.69	4.69 ± 0.76	0.4 (0.65)	0.4 (0.53)	0.4 (0.424)	0.4 (0.81)	0.8
HGB ^a (g/dL)	12.22 ± 1.61	12.04 ± 2.25	12.22 ± 1.67	12.16 ± 2.17	1.5 (0.35)	0.5 (0.63)	0 (0.96)	-1.0 (0.62)	1.84
HCT ^a (%)	37.82 ± 4.59	37.73 ± 5.96	37.89 ± 4.55	37.73 ± 6.2	0.2 (0.82)	0.4 (0.70)	0.2 (0.56)	0 (0.99)	1.74
LYMPHO ^a × (10 ³ /μL)	2.52 ± 0.93	2.51 ± 0.90	2.38 ± 0.92	2.41 ± 0.93	0.4 (0.81)	-1.2 (0.61)	5.9 (0.01)	4.1 (0.05)	9.19
MONO ^a × (10 ³ /μL)	0.57 ± 0.29	0.60 ± 0.33	0.689 ± 0.359	0.671 ± 0.366	-5.0 (0.16)	3.0 (0.25)	-17.4 (<0.01)	-10.4 (<0.01)	13.2
NEU ^a × (10 ³ /μL)	5.91 ± 1.15	5.76 ± 1.18	5.92 ± 1.109	5.86 ± 1.11	2.6 (<0.01)	1.0 (0.18)	-0.2 (0.85)	-1.7 (0.08)	9.25
PLT ^a × (10 ³ /μL)	257.6 ± 81.17	256 ± 81.75	250 ± 77.1	252.6 ± 87.99	0.6 (0.78)	-1.0 (0.60)	3.2 (0.01)	1.3 (0.529)	5.9
MCV ^b (fL)	80.8 ± 9.39 (56.4-102)	80.85 ± 9.3 (56.7-102)	81.16 ± 9.4 (56-103)	81.11 ± 9.4 (57.5-89.9)	-0.1 (0.85)	0.1 (0.43)	-0.4 (<0.01)	-0.3 (<0.01)	1.26
MCH ^b (pg)	26.17 ± 3.54 (16.6-33.5)	26.10 ± 3.49 (16.6-33.3)	26.22 ± 3.66 (16.4-33.5)	26.16 ± 3.59 (17.1-28.3)	0.3 (0.30)	0.2 (0.17)	-0.2 (0.37)	-0.2 (0.32)	1.35
MCHC ^b (g/dL)	32.30 ± 1.18 (29.4-35.6)	32.25 ± 1.26 (29.3-35.7)	32.23 ± 1.43 (29.2-36.3)	32.18 ± 1.39 (29.7-31.1)	0.2 (0.49)	0.2 (0.42)	0.2 (0.402)	0.2 (0.33)	0.4
RDW ^b (%)	14.94 ± 2.07 (12-21.8)	14.99 ± 2.01 (12.2-21.8)	14.8 ± 2.12 (11.9-21.8)	14.81 ± 2.09 (12-22)	-0.3 (0.34)	-0.1 (0.97)	0.9 (<0.01)	1.2 (<0.01)	1.7
PCT ^a (%)	0.16 ± 0.05	0.15 ± 0.05	0.16 ± 0.05	0.17 ± 0.05	6.7 (0.06)	-5.9 (0.29)	0 (0.573)	-11.8 (<0.01)	NA
MPV ^a (fL)	6.4 ± 1.76	6.30 ± 1.71	6.82 ± 1.83	6.78 ± 1.75	1.6 (<0.01)	0.6 (0.43)	-6.2 (<0.01)	-7.1 (<0.01)	2.29
PDW ^b (%)	17.2 ± 0.89 (15.4-19.3)	17.06 ± 1.00 (15.2-20.1)	17.07 ± 0.97 (15.4-20.1)	16.94 ± 0.89 (15-19.4)	0.8 (0.12)	0.8 (0.12)	0.8 (0.14)	0.7 (0.20)	NA

NA, not available.

^aNormal distribution: the values were mean ± standard deviation; P value represents the significance by paired Student's t-test.

^bNon-normal distribution; the values were median (interquartile range).

P value represents significance by Wilcoxon Ranked-pairs test.

P < .05 regarded as significant.

and RDW as shown in Table 1. In patients with anaemia, it is the MCV measurement that allows classification as either a microcytic anaemia (MCV below normal range), normocytic anaemia (MCV within normal range), or macrocytic anaemia (MCV above normal range).²⁰ PCT is secreted by thyroid C cells. It is a bioactive peptide. It is secreted and released into the blood in infectious disease which is an indicator of inflammation and infection in clinic.²¹ RDW is also associated with mortality in patients with infectious diseases.²²

The platelet indices (MPV, PDW) have been found to be clinically useful in distinguishing immune thrombocytopenic purpura (ITP) from thrombocytopenia caused by underproduction of platelets. Ntaios G and others observed that an increased MPV and PDW were seen in immune thrombocytopenic purpura.²³ Kaito K and others similarly, reported a significantly increased MPV and PDW in ITP than in aplastic anaemia.²⁴

In this study, we compared the results of K2-EDTA Plastic tubes and K3-EDTA Glass tubes for complete blood count and white cell differentials immediately after collection of blood samples and after 4 hours of blood collection. Plastic tubes recently replaced most glass tubes following the establishment of the Occupational Safety and Health Administration (OSHA) guidelines to improve safety and reduce exposure to blood-borne pathogens.²⁵ Van Cott et al.²⁶ showed that the slight differences observed between glass tri-potassium EDTA and plastic di-potassium EDTA tubes for complete blood count, reticulocyte count, and automated white cell differential did not achieve clinical significance.

We decided to evaluate K2-EDTA and K3-EDTA tubes of IMPROVACUTER® for haematological testing because these are very frequently used in Pakistan especially in Southern Punjab, where the authors are living. Still, further studies are required for quality's laboratory managers from other countries.

REFERENCES

- Procedures for the collection of diagnostic blood specimens by venipuncture; Approved Standard—Sixth Edition. Clinical Laboratory Standards Institute CLSI H3-A6 document. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
- Lima-Oliveira G, Lippi G, Salvagno GL, et al. Transillumination: a new tool to eliminate the impact of venous stasis during the procedure for the collection of diagnostic blood specimens for routine haematological testing. *Int J Lab Hematol*. 2011;33:457-462.
- Lima-Oliveira G, Salvagno G, Lippi G, et al. Elimination of the venous stasis error for routine coagulation testing by transillumination. *Clin Chim Acta*. 2011;412:1482-1484.
- Lima-Oliveira G, Lippi G, Salvagno GL, et al. New ways to deal with known preanalytical issues: use of transilluminator instead of tourniquet for easing vein access and eliminating stasis on clinical biochemistry. *Biochem Med*. 2011;21:152-159.
- Clinical and Laboratory Standards Institute. Tubes and additives for venous blood specimen collection; Approved Standard—Fifth Edition. NCCLS H1-A5. 2003.
- Rushing J. Drawing blood with vacuum tubes. *Nursing*. 2004;34:26.
- International Council for Standardization in Haematology: Expert Panel on Cytometry. Recommendations of the International Council for Standardization in Haematology for ethylenediaminetetraacetic acid anticoagulation of blood for blood cell counting and sizing. *Am J Clin Pathol*. 1993;100:371-372.
- Goossens W, Van Duppen V, Verwilghen RL. K2- or K3-EDTA: the anticoagulant of choice in routine haematology? *Clin Lab Haematol*. 1991;13:291-295.
- Procedures for the collection of diagnostic blood specimens by venipuncture. Clinical and Laboratory Standards Institute. 2007;27.
- Celltac α Automated Hematology Analyzer Service Manual MEK-6318K by NIHON KOHDEN.
- D'Agostino RB, Stephens M. *Tests for Normal Distribution. Goodness-of-fit Techniques*. New York, NY: Macel Dekker; 1986.
- Ricos C, Alvarez V, Cava F, et al. Current databases on biological variation: pros, cons and progress. *Scand J Clin Lab Invest*. 1999;59:491-500.
- Marija Kocijancic JC, Delic-Knezevic A. Evaluation of the BD Vacutainer® RST blood collection tube for routine chemistry analytes: clinical significance of differences and stability study. *Biochem Med*. 2014;24:368-375.
- Chawla R, Goswami B, Tayal D, Mallika V. Identification of the types of preanalytical errors in the clinical chemistry laboratory: 1 year study of GB Pant Hospital. *Lab Med*. 2010;41:89-92.
- Banta HD, van Beekum WT. The regulation of medical devices and quality of medical care. *Qual Assur Health Care*. 1990;2:127.
- Sears D, Charache S, Perlstein M. Electronic blood cell counters Faulty calibration due to type and amount of anticoagulant in collection tubes. *Arch Pathol Lab Med*. 1985;109:247-249.
- Endler G, Klimesch A, Sunder-Plassmann H, et al. Mean platelet volume is an independent risk factor for myocardial infarction but not for coronary artery disease. *Br J Haematol*. 2002;117:399-404.
- Immune Deficiency Foundation. <http://primaryimmuneorg/about-primary-immunodeficiencies/specific-disease-types/wiskott-aldrich-syndrome>.
- Hedberg P, Lehto T. Aging stability of complete blood count and white blood cell differential parameters analyzed by Abbott CELL-DYN Sapphire hematology analyzer. *Int J Lab Hematol*. 2009;31:87-89.
- Schrier SL, Landaw SA. Mean corpuscular volume. <https://www.uptodate.com>. Accessed September 30, 2011.
- Hammer S, Meisner F, Dirschedl P, et al. Procalcitonin: a new marker for diagnosis of acute rejection and bacterial infection in patients after heart and lung transplantation. *Transpl Immunol*. 1998;6:235-241.
- Lee JH, Chung H, Kim K, et al. Red cell distribution width as a prognostic marker in patients with community-acquired pneumonia. *Am J Emerg Med*. 2013;31:72-79.
- Ntaios G, Papadopoulos A, Chatzinikolaou A, et al. Increased values of mean platelet volume and platelet size deviation width may provide a safe positive diagnosis of idiopathic thrombocytopenic purpura. *Acta Haematol*. 2008;119:173-177.
- Kaito K, Otsubo H, Usui N, et al. Platelet size deviation width, platelet large cell ratio, and mean platelet volume have sufficient sensitivity and specificity in the diagnosis of immune thrombocytopenia. *Br J Haematol*. 2005;128:698-702.
- Ernst DJ. Plastic collection tubes decrease risk of employee injury. *MLO Med Lab Obs*. 2001;33:44-46.
- Van Cott EM, Lewandrowski K, Patel S, et al. Comparison of glass K3 EDT A versus plastic K2 EDT A blood-drawing tubes for complete blood counts, reticulocyte counts, and white blood cell differentials. *Lab Hematol*. 2003;9:10-14.

How to cite this article: Mehmood R, Muhammed RK, Hussain S, Sana A. Evaluation of di-potassium and tri-potassium EDTA evacuated tubes for routine haematological testing. *J Clin Lab Anal*. 2018;32:e22188. <https://doi.org/10.1002/jcla.22188>