Brand of dipotassium EDTA vacuum tube as a new source of pre-analytical variability in routine haematology testing

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ABSTRACT

This study assesses the use of different dry K_2 (dipotassium) EDTA vacuum tubes and whether or not they might represent a bias in haematological testing. Blood was collected in three dipotassium EDTA vacuum tubes from different manufacturers: Venosafe, Vacuette and Vacutainer. Samples were analysed on an Advia 2120i analyser. Significant differences among results and biases were compared with current quality specifications. Significant differences were found for haematocrit (HCT), mean corpuscular volume (MCV), white blood cell count (WBC) and platelet distribution width (PDW) when comparing Venosafe *vs*. Vacuette; for MCV, WBC and PDW when comparing Venosafe *vs*. Vacutainer; and for HCT and MCV when comparing Vacuette *vs*. Vacutainer. Clinically significant variations were observed for HCT and PDW in Venosafe *vs*. Vacuette; PDW in Venosafe *vs*. Vacutainer; and HCT and MCV in Vacuette *vs*. Vacutainer. The use of dipotassium EDTA vacuum tubes from different manufacturers represent a clinically relevant source of variation for HCT, MCV and PDW.

KEY WORDS: Anticoagulants.
Blood cell count.
Potassium EDTA.
Pre-analytical phase.

Introduction

Laboratory error or interferences due to pre-analytical problems is well established in the scientific literature,[1–11] as is the importance of standardising and establishing appropriate procedures for collection of diagnostic blood specimens by venipuncture.[12–14] The procedures involving phlebotomy, such as time of tourniquet application[15–17] and the use of vacuum tubes.[18,19] have been investigated as potential sources of pre-analytical variability, but less attention has been focused on the vacuum tubes.[20,21]

There are several different brands of vacuum tube for blood collection, which are selected by laboratory or hospital managers according to technical and/or economy reasons. As regards haematological testing, the anticoagulant of choice is ethylenediaminetetraacetic acid (EDTA).[22] Three formulations of this anticoagulant can be employed (i.e., sodium [Na_2] EDTA), dipotassium [K_2] EDTA and tripotassium [K_3] EDTA). Although the choice mostly depends on the types of test to be performed, the International Council for Standardization in Haematology (ICSH) currently supports the use of dipotassium EDTA as the anticoagulant of choice for haematological testing, as this formulation is available in a spray-dried form that does not introduce dilutional effects on small sample volumes and causes a less prominent osmotic effect on blood cells.[22]

This study aims to evaluate the use of dry dipotassium EDTA vacuum tubes of different brands and how they might represent a source of pre-analytical variability in routine haematology testing.

Materials and methods

Study design

The study group included 21 healthy volunteers recruited among the personnel of the Laboratory of Clinical Biochemistry, Department of Life and Reproduction Sciences, University of Verona, Italy. This study was submitted to, and approved by, the Internal Review Board (IRB) and all participating volunteers signed to indicate their informed consent.

Collection of diagnostic blood specimens

The collection of all diagnostic blood specimens was performed by a single, expert phlebotomist, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI).[12] All volunteers remained in a seated position for 15 minutes prior to phlebotomy in order to eliminate possible interference due to posture.[23] A vein was located on the forearm by a subcutaneous tissue transilluminator device (Venoscópio IV plus, Duan do Brasil, Brazil) to prevent interference from venous stasis,[15–17] and blood was collected by venipuncture with a 20 G straight needle (Terumo Europe NV, Leuven, Belgium) directly into

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three dipotassium EDTA vacuum tubes of different brands, as follows: Tube I, 3.0 mL Venosafe (5.9 mg K_2EDTA lot 1004010; Terumo Europe, Leuven, Belgium); Tube II, 4.0 mL Vacuette (lot C100200J, K_2EDTA concentration not declared, Greiner Bio-One, Kremsmünster, Austria); Tube III, 3.0 mL Vacutainer (5.4 mg K_2EDTA lot 0033601, BD Vacutainer, Becton Dickinson Diagnostics, Plymouth, UK). To eliminate any potential interference due to the contact phase or the tissue factor, approximately 2 mL blood was first collected in a discard tube without additive (Vacuette lot A101004D, Greiner Bio-One).

Laboratory testing

All samples were processed for routine haematology testing immediately after collection (<15 min) on the same Advia 2120i haematology system (Siemens Healthcare Diagnostics, Deerfield IL, USA). Parameters tested included red blood cell count (RBC), haematocrit (HCT), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCHC), RBC distribution width (RDW), white blood cell (WBC) count and differential, including lymphocytes (LYMPHO), monocytes (MONO), neutrophils (NEU), eosinophils (EOS), basophils (BASO) and large unstained cells (LUC), 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 Advia 2120i system showed coefficients of variation (CVs) of 1.6–2.3% for WBC, 2.1–2.8% for platelets, 0.6–0.9% for RBC and <0.7% for haemoglobin, MCV and MCH.[24]

Statistical analysis

The significance of differences between the three dipotassium EDTA vacuum tubes was assessed by repeated measures ANOVA and paired Student's *t*-test after checking for normality by the D'Agostino-Pearson omnibus test.[25] As non-normal distribution was found for MCV, MCH, RDW and PDW, results were assessed by the Friedman test and Wilcoxon ranked-pairs test. The level of statistical significance was set at $P<0.05$. Finally, bias among the different tubes was compared with the current desirable quality specifications for bias (B), derived from biological variation.[26]

Results

The main results obtained from this study are shown in Table 1. Significant differences were recorded for HCT, MCV, WBC and PDW when comparing Tube I *vs*. Tube II; MCV, WBC and PDW when comparing Tube I *vs*. Tube III; HCT and MCV when comparing Tube II *vs*. Tube III. No significant difference ($P>0.05$) was observed by repeated measures ANOVA for RBC ($P=0.63$), HGB ($P=0.93$), LYMPHO ($P=0.31$), MONO ($P=0.22$), NEU ($P=0.91$), EOS ($P=0.06$), BASO ($P=0.27$), LUC ($P=0.48$), PLT ($P=0.55$) and MPV ($P=0.16$) as well as for MCHC ($P=0.88$) and RDW ($P=0.87$) by the Wilcoxon ranked-pairs test. Clinically significant variation compared with the current desirable quality specifications[26] was found for HCT and PDW between Tubes I and II, for PDW between Tubes I and III, and for HCT and MCV between Tubes II and III.

Table 1. Variability in haematological parameters with three different brands of dipotassium EDTA vacuum tube.

	Comprehensive results			Mean % difference (*P* value)			
Haematological parameter (units)	Tube I	Tube II	Tube III	Tube I *vs*. tube II	Tube I *vs*. tube III	Tube II *vs*. tube III	Desirable bias (%)[26]
RBC* (x10^6/μL)	5.11±0.40	5.12±0.40	5.12±0.41	–0.2 (0.41)	–0.2 (0.64)	0.0 (0.57)	1.7
HCT* (%)	43.6±4.4	44.6±3.2	43.8±3.2	–2.3 (0.04)	–0.5 (0.73)	1.8 (<0.01)	1.7
Hb* (g/dL)	14.34±1.14	14.34±1.18	14.33±1.17	0.0 (1.00)	0.1 (0.74)	0.1 (0.76)	1.8
MCV† (fL)	87.7 (83.1–89.2)	88.1 (83.8–90.0)	86.7 (82.4–88.4)	–0.5 (<0.01)	1.1 (<0.01)	1.6 (<0.01)	1.2
MCHC† (pg)	28.5 (26.8–29.4)	28.9 (26.6–29.4)	28.8 (26.8–29.4)	–1.4 (0.95)	–1.0 (1.00)	0.4 (0.99)	1.4
RDW† (%)	12.8 (12.3–13.6)	12.6 (12.3–13.7)	12.6 (12.3–13.6)	1.6 (0.96)	1.6 (0.93)	0.0 (1.00)	1.7
WBC* (x10^3/μL)	5.93±1.05	5.76±1.04	5.76±1.04	2.9 (0.03)	2.9 (0.03)	0.0 (0.91)	5.6
LYMPHO* (x10^3/μL)	1.91±0.65	1.90±0.66	1.87±0.65	0.5 (0.76)	2.1 (0.21)	1.6 (0.22)	7.4
MONO* (x10^3/μL)	0.33±0.12	0.33±0.11	0.34±0.11	0.0 (0.43)	–3.0 (0.25)	–3.0 (0.16)	13.2
NEU* (x10^3/μL)	3.33±0.76	3.31±0.75	3.32±0.72	0.6 (0.74)	0.3 (0.75)	–0.3 (0.97)	9.0
EOS* (x10^3/μL)	0.15±0.10	0.15±0.10	0.16±0.11	0.0 (0.39)	–6.7 (0.12)	–6.7 (0.06)	19.8
BASO* (x10^3/μL)	0.045±0.018	0.044±0.018	0.048±0.016	2.2 (0.50)	–6.7 (0.23)	–9.1 (0.20)	15.4
LUC* (x10^3/μL)	0.164±0.049	0.161±0.054	0.158±0.050	1.8 (0.62)	3.7 (0.22)	1.9 (0.49)	NA
PLT* (x10^3/μL)	291.7±60.8	292.0±59.5	294.6±60.4	–0.1 (0.91)	–1.0 (0.36)	–0.9 (0.40)	5.9
MPV* (fL)	8.35±0.64	8.24±0.56	8.31±0.62	1.3 (0.08)	0.5 (0.52)	–0.8 (0.20)	2.3
PDW† (%)	53.2 (49.2–61.0)	55.2 (49.6–58.6)	54.5 (48.1–59.6)	–3.8 (<0.01)	–2.4 (<0.01)	1.3 (0.11)	1.4

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

†Non-normal distribution: the values were median (interquartile range).

P value represents significance by Wilcoxon ranked-pairs test.

NA: not available.[26] $P<0.05$ regarded as significant.

Discussion

The reliable assessment of haematological disorders requires appropriate use of laboratory resources. Total quality in haematology testing is therefore essential to obtain reliable results and establish the most appropriate clinical decision-making.[27] Although the modern automated haematology instrumentation provides fast results, high throughput and a high degree of precision and accuracy,[28] apparently trivial extra-analytical issues such as the duration of fasting time – long considered of minor significance in routine haematology testing – can influence test results.[29] Even small differences in the concentration of the anticoagulant in blood collection tubes may produce appreciable differences in haematology test results.[30]

The results presented here support these findings, as the WBC count might reveal statistically significant differences from one manufacturer's tube to another; however, this bias does not achieve clinical significance and thereby does not represent a source of variability for the total and/or differential WBC count. Thus, it should not influence clinical decision-making. Nonetheless, the results indicate that HCT and MCV values might slightly but significantly differ when collecting blood into vacuum tubes from different manufacturers. This is important because the diagnostic approach to patients with suspected anaemia, (e.g., normochromic and normocytic anaemia) such as that due to chronic conditions[31] might be jeopardised or delayed if the laboratory manager decides to change the brand of the vacuum tubes without taking into consideration the potential changes induced by either laboratory instrumentation or primary vacuum tubes.[30]

With regard to PDW, this parameter has often been overlooked; however, several lines of evidence now indicate that PDW can be used to assess the risk of microvascular complication in diabetes patients,[32] or to distinguish thrombocytopenia in paediatric acute lymphocytic leukemia from immune thrombocytopenia.[33] The results of the present study show that PDW is also influenced when collecting blood in dipotassium EDTA vacuum tubes from different manufacturers.

In terms of the potential mechanism underlying the bias observed for HCT, MCV and PDW, three potential explanation can be suggested: i) the use of dry EDTA particles of different size, and the pathway of delivery inside the tube; ii) the final concentration of dry EDTA in the sample; and iii) the material of the tube and the stopper. Moreover, previous investigation that compared two different brands of tripotassium EDTA vacuum tube also showed clinical difference for PDW.[34]

The CLSI recommends that either dipotassium or tripotassium EDTA salts should be used in concentrations of 1.5–2.2 mg/mL, at 1.5 mg EDTA per mL blood.[18] In a previous study, Asanuma *et al*. showed that two different concentrations of dipotassium EDTA (i.e., 1.8 mg/mL versus 3.6 mg/mL) do not significantly modify the erythrocyte and platelet parameters of haemodialysis patients as measured using two different laboratory instruments (i.e., 96.8 fL versus 96.7 fL).[35] In a further study, Gari compared complete blood count parameters, WBC and flagging rates obtained with glass and plastic tubes, reporting only slight discrepancies in the results obtained between the two tube types.[36] Moreover, Van Cott *et al*. showed that the slight differences observed between glass tripotassium EDTA and plastic dipotassium EDTA tubes for complete blood count, reticulocyte count and automated white cell differential did not achieve clinical significance.[37]

As such, the different concentration of the additive as well as the composition of the tube are unlikely to be the cause of the bias observed in the present study, and therefore further investigation is required to understand the nature of this intriguing observation. □

No conflict of interest reported.

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