Incorrect order of draw of blood samples does not cause potassium EDTA sample contamination

M. P. CORNES*, R. A. SULAIMAN*, S. J. WHITEHEAD*, N. OTHONOS*, C. FORD* and R GAMA*†

Department of Clinical Chemistry, New Cross Hospital; and †Research Institute, Healthcare Sciences, University of Wolverhampton, West Midlands, UK

It is widely recommended that the order of draw of blood during phlebotomy should be as follows: blood culture/sterile tubes, then plain tubes/gel tubes, then tubes containing additives. The recommendation is based on a study, using the Becton Dickinson (BD) Vacutainer system, which reported that incorrect order of draw causes hyperkalaemia and hypocalcaemia, which are surrogate markers of *in vitro* potassium EDTA sample contamination.[1] This, however, is controversial as a similar study, also using the BD Vacutainer system, failed to confirm these findings.[2]

Development of a serum EDTA assay[3] has allowed definitive investigation into whether or not reversed order of draw of blood samples has an impact on clinical biochemistry results. Contrary to expectation, by directly measuring serum EDTA, the authors recently reported that reversed order of draw utilising the Sarstedt Safety Monovette system does not cause potassium EDTA sample contamination.[4]

It is, however, possible that *in vitro* potassium EDTA contamination due to incorrect order of draw may depend on the type of closed venesection system. Therefore, this study investigates whether reversed order of draw of blood using the BD Vacutainer system causes *in vitro* potassium EDTA contamination by measuring EDTA in biochemistry serum samples before and after collecting blood into a potassium EDTA-containing sample tube.

Eleven healthy volunteers (age range: 18–60 years) were recruited from staff at New Cross Hospital, Wolverhampton. Volunteers gave written consent to participate in the study, which was approved by the Coventry and Warwickshire Research Ethics Committee.

Each volunteer was venesected sitting in a dedicated phlebotomy chair by the same experienced phlebotomist using the BD Vacutainer system (BD, Cowley, Oxford, UK), as previously described.[4] Blood was drawn sequentially into a BD SST II gel tube, followed by a BD EDTA tube, followed by another BD SST II gel tube. Serum gel tubes were centrifuged within 30 minutes and the separated serum was frozen at –80°C until analysed in a single batch for EDTA, potassium, calcium, magnesium, zinc and alkaline phosphatase.

Serum potassium, calcium, magnesium, zinc, alkaline phosphatase and creatinine were measured using routine methodology on the Roche MODULAR analyser (Roche Diagnostics, Mannheim, Germany). Serum EDTA, which has a detection limit of 0.2 mmol/L, was also measured on the same analyser.[3] Intra-assay coefficient of variation (CV) for almost all the analytes was less than 3%, except for EDTA and zinc which showed intra-assay CV of 3.2 % at 0.25 mmol/L and 4.2% at 14.3 mmol/L, respectively.

Kolmogorov and Smirnov analysis indicated data were normally distributed. Therefore, Student's *t*-test was used to assess the significance of serum analyte differences before and after collection of the EDTA sample. Results are expressed as mean (standard deviation [SD]). Data were analysed using GraphPad Instat version 3.00 for Windows 95 (GraphPad Software, San Diego, USA).

Evidence of EDTA was undetectable (<0.2 mmol/L) in all samples. Serum potassium, calcium, magnesium, zinc and alkaline phosphatase values were similar in blood samples collected before and after collection of the EDTA blood sample (Table 1).

This report and previous studies[2,4] confirm that reversed order of draw of blood samples does not cause potassium EDTA sample contamination, irrespective of the type of closed blood collection system used. Majid *et al*. postulated[2] that the hyperkalaemia and hypocalcaemia reported by Callum and Cooper,[1] who used the BD Vacutainer system, was due to difficult venepuncture that resulted in local tissue damage, rather than due to reversed order of draw.

In vitro potassium EDTA contamination may occur with open blood collection systems by syringe needle or syringe tip contamination with potassium EDTA when delivering collected blood into EDTA sample tubes before other tubes[5] and by direct transfer of blood from potassium EDTA-containing tubes to other sample tubes. Therefore, it is likely that syringe needle or syringe tip contamination with EDTA contributes to the high prevalence of potassium EDTA contamination,[6–10] as this practice appears to be relatively common.[11] Further definitive studies may be required to confirm this proposed mechanism of potassium EDTA sample contamination in order to implement focused appropriate preventive measures.

In conclusion, this study found no evidence to support the widely accepted belief that incorrect order of draw using closed blood collection systems causes *in vitro* potassium EDTA contamination.

The authors acknowledge BD Diagnostics for providing evacuated blood collection systems.

Correspondence to: Dr Mike Cornes
Department of Clinical Chemistry, New Cross Hospital, Wednesfield Road, Wolverhampton WV10 0QP
Email: michael.cornes@nhs.net

References

1 Calam RR, Cooper MH. Recommended 'order of draw' for collecting blood specimens into additive-containing tubes. *Clin Chem* 1982; **28** (6): 1399.

2 Majid A, Heaney DC, Padmanabhan N, Spooner R. The order of draw of blood specimens into additive containing tubes does not affect potassium and calcium measurements. *J Clin Pathol* 1996; **49** (12): 1019–20.

3 Davidson D. EDTA analysis on the Roche MODULAR analyser. *Ann Clin Biochem* 2007; **44** (Pt 3): 294–6.

4 Sulaiman RA, Cornes MP, Whitehead S, Othonos N, Ford C, Gama R. Effect of order of draw of blood samples during phlebotomy on routine biochemistry results. J Clin Pathol 2011; 64 (11): 1019–20.

5 Fitzpatrick MF, Newell J, Grimes H, Egan EL. Spurious increase in plasma potassium concentration and reduction in plasma

calcium due to *in vitro* contamination with liquid potassium edetic acid at phlebotomy. *J Clin Pathol* 1987; **40** (5): 588.
6 Cornes M, Ford C, Gama R. Spurious hyperkalaemia due to EDTA contamination: common and not always easy to identify. *Ann Clin Biochem* 2008; **45** (Pt 6): 601–3.
7 Sharratt CL, Gilbert CJ, Cornes MP, Ford C, Gama R. EDTA sample contamination is common and often undetected, putting patients at unnecessary risk of harm. *Int J Clin Pract* 2009; **63** (8): 1259–62.
8 Cornes MP, Davidson DF, Darwin L *et al*. Multi-centre observational study of spurious hyperkalaemia due to EDTA contamination. *Clin Lab* 2010; **56** (11–12): 597–9.
9 Gama R, Cornes M, Ford C. Avoiding spurious hyperkalaemia. *BMJ* 2009; **339**: b4823.
10 Davidson DF. Effects of contamination of blood specimens with liquid potassium-EDTA anticoagulant. *Ann Clin Biochem* 2002; **39** (Pt 3): 273–80.
11 Berg JE, Ahee P, Berg JD. Variation in phlebotomy techniques in emergency medicine and the incidence of haemolysed samples. *Ann Clin Biochem* 2011; **48** (Pt 6): 562–5.

Table 1. Serum analyte concentrations in blood samples collected from 11 subjects before and after collection of the EDTA blood sample.

Analyte	Before EDTA	After EDTA	*P* value
EDTA (mmol/L)	<0.2	<0.2	1
Potassium (mmol/L)	4.2 (0.22)	4.2 (0.29)	0.571
Adjusted calcium (mmol/L)	2.37 (0.021)	2.39 (0.015)	0.372
Magnesium (mmol/L)	0.82 (0.052)	0.83 (0.047)	0.800
Zinc (μmol/L)	16.9 (6.23)	17.4 (6.6)	0.843
Alkaline phosphatase (IU/L)	64.2 (21.8)	65.7 (22.5)	0.872
Creatinine (μmol/L)	79 (11.0)	79 (11.2)	0.955

Results expressed as mean (SD)